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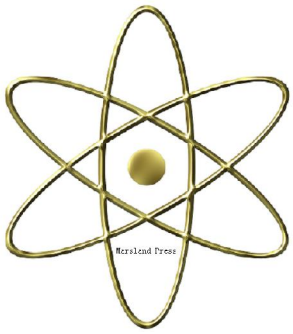

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The following manuscripts are presented as online first for peer-review, starting from March 27, 2011.
All comments are welcome: editor@sciencepub.net; sciencepub@gmail.com
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	2.11 while TiO_2 ranged from 0.01-1.46wt%. Discrimination diagrams revealed a preference for igneous fields by the granite gneiss. It is therefore deduced that this tonalitic granite gneiss has an igneous origin. **[**Adeyeye Olufemi, Ademeso Odunyemi. **The Petrography and Major Element Geochemistry of the Granite Gneiss of Arigidi area, S/W, Nigeria.** Nature and Science 2011;9(5):7-12]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: Arigidi, granite gneiss, discrimination, tonalite, igneous origin		
3	**Pollutant Dynamics And Distribution In Sediments North Of Lagos Lagoon Ecosystem.** Adeleye A. O.[1], R. O. D., Shelle[1], A. E., Akinnigbagbe[1] [1]Nigerian Institute for Oceanography and Marine Research [NIOMR] 3, Wilmot Point Road, Victoria Island, P.M.B. 12729, Lagos, Nigeria. E-mail: adedayoseun@gmail.com; Tel: +234 8030623808 ABSTRACT: Thermal pollution was investigated in the sediment North of Lagos lagoon. The result revealed the heavy metal distribution in sediment to be Fe>>>Zn>Pb>Cu. The concentration level of Cd is generally below <0.002 mg/Kg in all the stations studied. The concentration levels of heavy metal gave evidence of pollution in sediment. The results obtained confirmed that over heated water and effluent discharge was the source of pollution in this part of the lagoon. The distribution of heavy metals in sediment and safety status is discussed. [Adeleye A. O., R. O. D., Shelle, A. E., Akinnigbagbe. Pollutant Dynamics And Distribution In Sediments North Of Lagos Lagoon Ecosystem. Nature and Science 2011;9(5):13-16]. (ISSN: 1545-0740). http://www.sciencepub.net. Keywords: **Thermal pollution, Heavy metals, sediment, ecosystem, Nigeria**	Full Text	3
4	**Limonene dominates the Phytochemistry of *Trigonella foenum-graceum* in Nature** Kamal Kishore Pande [1], Lata Pande [2], Bharat Pande[3], Atul Pujari [4] Pankaj Sah [5,6] and Stuti Sah[7] [1, 4] Department of Biotechnology, Government Post Graduate College Haldwani, District Nainital, Uttarakhand State (India) [2] Food and Nutrition Laboratory (Department of Home Science) DSB Campus, Kumaun University Nainital PIN- 263003 Uttarakhand State (India) [3] Department of Chemistry, Government Post Graduate College Haldwani, District Nainital, Uttarakhand State (India) [5] Department of Botany DSB Campus, Kumaun University Nainital PIN- 263003 Uttarakhand State (India) [6] Department of Applied Sciences (Applied Biology Section), Higher College of Technology, Al-Khuwair PO Box 74, PC 133 Muscat (Sultanate of Oman) [7] Department of Microbiology, College of Basic Sciences and Humanities, G B Pant University of Agriculture Science and Technology, Pant Nagar, Uttarakhand State (India) kemscience@gmail.com, pankaj@hct.edu.om, drpankajsah1@gmail.com **Abstract:** Many plant families have some unique medicinal properties due to the presence of special chemical molecules in different parts of plants also known as “phytofoods.” Himalaya is one of the greatest mountain chains in the world which not only supports a vast variety of plants and animals but also the great diversity of traditional phytofoods. Many of these phytofoods are very vital to humans in different diseases also. In this paper, we have crucially analyzed some of the major volatile compounds and anti-microbial activities of *Trigonella foenum*-graceum. By the help of our gc-ms data, we are reporting the dominance of limonene (82.30%), d-carvone (12.97%) and n-caproaldehyde (1.87%) in this plant. [Kamal Kishore Pande, Lata Pande, Bharat Pande, Atul Pujari, Pankaj Sah and Stuti Sah. Limonene Dominates the Phytochemistry of Trigonella foenum-graceum. Nature and Science 2011;9(5):17-20]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Phytofoods, Herbal medicine, Himalaya, Limonene	Full Text	4

	Abstract: The aim of this study was to determine serum concentrations of HGF, Bcl-2 and nitric oxide (NO) in 44 patients with primary breast cancer and 15 healthy individuals as a control group using an ELISA assay for HGF and Bcl-2 while nitric oxide was determined by using colorimetric technique. The measured parameters were correlated with clinicopathological parameters that may affect the outcome of disease. In addition, ROC curve analysis was done to each parameter. The results were as follows, the mean level of HGF was 1198.79 ± 76.32 pg/ml compared with 884.67 ± 66.88 pg/ml for control (p = 0.026). The HGF levels were significantly elevated in the patients with increasing the tumor stage (p = 0.036). In addition, HGF levels were higher in negative estrogen receptor (p = 0.039). The mean level of Bcl-2 in patients was 12.83± 1.97 ng/ml compared with 5.09 ± 0.40 ng/ml for control (p = 0.027). Levels of Bcl-2 were elevated but not statistically significant in patients with GI tumors, negative nodes, ER negative tumors and postmenopausal patients (p = 0.4, 0.8, 0.7 and 0.5, respectively).The patients mean level of the nitric oxide (NO) was 63.07 ± 4.14 µmol/L compared with 43.99 ± 4.21 µmol/L for control (p = 0.014). The levels of NO were elevated but also not statistically significant in patients with tumor size I, GI tumors, ER negative tumors, positive nodes, stage II tumors and postmenopausal patients (p = 0.3, 0.6, 0.3, 0.7, 0.3 and 0.2 respectively). From the ROC curve analysis, it was observed that the area under curve for HGF, Bcl-2 and NO was 0.695, 0.842 and 0.711, respectively. This result indicates the good validity of the above markers especially Bcl-2 parameter to discriminate the positive from the negative samples. In conclusion, this study demonstrates that the serum determination of HGF, Bcl-2 or NO may help in diagnosis of the breast cancer and may aid in disease prognosis. However, larger studies with more patients are required. [Elsayed M. Mahdy, Wafaa G. Shousha, Hanaa H. Ahmed, Fathyea M. Metwally and Shimaa Sh. Ramadan. Significance of Serum HGF, Bcl-2 and Nitric Oxide in Primary Breast Cancer. Nature and Science 2011;9(5):34-41]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: HGF; Bcl-2; nitric oxide; breast cancer; diagnosis; prognosis.		
8	**Phenological episodes of *Myriophyllum spicatum* (Haloragaceae); a highly invasive species in Kashmir Himalayan aquatic ecosystems.** Shahzada Arshid* and Aijaz A Wani** Cytogenetics and Reproductive Biology Laboratory, Department of Botany, University of Kashmir, Hazratbal Srinagar-190 006, India. *shahzada194@gmail.com; **aijazbotku@gmail.com **Abstract:** Phenological behaviour of *Myriophyllum spicatum*, a highly invasive species in Kashmir Himalayan aquatic ecosystems, was studied in different standing and running water populations for a period of 12 months to monitor the various developmental stages. The plant starts its life cycle with the sprouting of rhizomes and axillary buds in standing water populations, whereas in running waters rhizomes and nodal plantlets contribute to new recruitments. In standing waters flowering phase prolongs when compared to running water populations. In standing water populations a high seed set was observed, whereas in running waters seed formation does not take place. The knowledge of time period and formation of these vegetative and sexual propagules by this invasive species is very important for its effective management and control in these ecosystems. **[**Shahzada Arshid and Aijaz A Wani. **Phenological episodes of *Myriophyllum spicatum* (Haloragaceae); a highly invasive species in Kashmir Himalayan aquatic ecosystems.** Nature and Science 2011;9(5):42-45]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** *Myriophyllum spicatum*; phenology; Kashmir Himalaya; Propagules; management	Full Text	8
9	**Antibiotic Susceptibility Profiles of Enteric Bacterial Isolates from Dumpsite Utisols and Water Sources in a Rural Community in Cross River State, Southern Nigeria.** Ikpeme Emmanuel, Nfongeh Joseph, Eja Matthew Egbebor, Etim Lawrence and Enyi-Idoh Kingsley Department of Biological Sciences, Cross River University of Technology, Calabar, Nigeria. Email: kingenyi4gold@yahoo.com mattheweja200@yahoo.com acadabuddy@yahoo.co.uk **Abstract:** A survey was conducted to establish the effects of bacterial contamination from dumpsite effluents on utisol and water and the antimicrobial susceptibility profiles of the bacterial isolates. A	Full Text	9

	total of 504 each of soil and water samples from different locations were sampled between the months of May and November, 2009. *Proteus* sp(70.24%), *Pseudomonas* sp(59.13%), *Bacillus* sp(58.33%),*Escherichia coli* (58.33%), *Campylobacter* sp(45.63%),*Klebsiella* sp(35.12%),*Shigella* sp(30.96%), *Salmonella* sp(27.98%), *Aeromonas* sp (27.98%) and *Vibrio cholera*e (10.91%) were isolated from polluted utisols, while *Bacillus* sp (86.51%), *Pseudomonas* sp (71.23%), *Escherichia coli* (60.71%), *Aeromonas* sp (52.58%), *Salmonella* sp (47.02%), *Klebsiella* (26.19%) and *Vibrio cholerae* (13.10%) were isolated from various water sources. The prevalence of the bacterial species in the two environmental sources differed significantly ($P<0.05$). All isolates were resistant to Gentamicin, Chloramphenicol and Amikacin, while low resistance values were recorded in Erythromycin (25%) and Nalidixic acid (37.50%). Adequate treatment of dumpsite effluents and the use of Erythromycin and Nalidixic acid as therapeutic measures are recommended to reduce possible health hazards. [Ikpeme Emmanuel, Nfongeh Joseph, Eja Matthew Egbebor, Etim Lawrence and Enyi-Idoh Kingsley. **Antibiotic Susceptibility Profiles of Enteric Bacterial Isolates from Dumpsite Utisols and Water Sources in a Rural Community in Cross River State, Southern Nigeria.** Nature and Science 2011;9(5):46-50]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Dumpsite effluents**,** enteric bacteria, antibiotic susceptibility, Southern Nigeria		
10	**Characteristics of rural women in developing countries** 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: abedi114@yahoo.com **Abstract:** Women's productive activities has affective role to increase revenue, rural family welfare, and its consequents is: foods status improvement, health, preventing irregular migration, literacy enhancement and development of rural family social status. Despite clearness of affective women's role at production, economy of village and country, they don't enjoy proper social base and they were deprived of educational and welfare programs especially at rural and nomadic area. Thus women and their roles should be considered particularly in order that they would find that first they are important and efficient; second they have educational needs and many technical gaps; third they shouldn't forget efforts for enabling themselves. As girls and women's discussion and solving their historical lag and restoring their social right are important and necessary, it is sensitive and accurate equally, because dominant patriarchal cultures at rural societies, put women at lower status. So that at some societies, women's duties are just upbringing and reproduction and maybe they are considered as workforce, and they are deprived of decision making and opining at family and society environment. **[**Sharareh Khodamoradi and Mohammad Abedi. **Characteristics of rural women in developing countries.** Nature and Science 2011;9(5):51-55]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** rural women, developing countries	Full Text	10
11	**Interactions between insulin like growth factor 1, thyroid hormones and blood energy metabolites in cattle with postpartum inactive ovaries** Nahed Saleh[1], Emad Mahmud, [2] Emad Waded [1] 1. Department of Clinical Pathology 2. Department of obstetrics, Fac. of Vet. Med., Menufiya Univ., El-Sadat Branch, Egypt nahedsaleh2001@yahoo.com **Abstract:** The relationship among insulin-like growth factor-1, thyroid hormones, energy metabolites and ovarian activity was investigated in cattle with postpartum inactive ovaries. The study was conducted on two groups of cows. The first group consisted of 10 cows with postpartum inactive ovaries (non-cyclic cows) based on rectal and ultrasonographic examination. The second group consisted of 8 cows in estrus (cyclic cows). The evaluated parameters included serum concentrations of variables of energy metabolites such as glucose (GLU), total lipids (TL), triglycerides (TG) and total cholesterol (TCH). Serum concentrations of total proteins (TP) were also measured. The hormones	Full Text	11

	evaluated in this study included metabolic hormones such as insulin like growth factor-1 (IGF-1), thyroxine (T4), and triiodothyronine (T3) in addition to hormonal indicators of ovarian activity as progesterone (PRO) and estradiol (E2). The results revealed that serum levels of E2 was significantly lower (P<0.05) in the non-cyclic cows compared with the cyclic group. Serum GLU concentrations showed a significant decrease (P<0.05) while TL and TCH were significantly increased (P<0.05). Metabolic hormones profile demonstrated a significant decrease (P<0.05) in IGF-1, T4 and T3 in cows with inactive ovaries compared to the cyclic cows. Correlations between the monitored variables indicated that there was a significant positive correlation (P<0.05) between GLU and E2 and a significant negative relationship between TL, TCH and E2 (P<0.05). We reported a significant positive correlation (P<0.05) between IGF-1 and E2, GLU, T4 and T3. T4 was positively correlated (P<0.05) with E2, GLU, IGF-1 and T3, while a significant negative relationship between T4 and both TL and TCH was recorded. There was also a significant positive correlation between T3 and E2, GLU, IGF-I and T4. Significant positive correlation between TL and TCH was found while serum glucose showed no strong correlations with other energy-related metabolites. These results suggest that incidence of low reproductive performance in the postpartum lactating cows is associated with a decrease of some metabolic hormones such as IGF-1, T4 and T3 and the alterations seen in these hormones could be tightly related with changing in energy metabolites suggesting that energy influences ovarian activity in postpartum lactating cows possibly through changes in secretory patterns of these metabolic hormones. [Nahed Saleh, Emad Mahmud, Emad Waded. Interactions between insulin like growth factor 1, thyroid hormones and blood energy metabolites in cattle with postpartum inactive ovaries. Nature and Science 2011;9(5):56-63]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** IGF-1; thyroid hormones; inactive ovary; energy metabolites		
12	**Nuclear Research Reactors Accidents Diagnosis Using Genetic Algorithm/Artificial Neural Networks** Abdelfattah A. Ahmed**, Nwal A. Alfishawy*, Mohamed A. Albrdini* and Imbaby I. Mahmoud** * Minufiya university, Faculty of Comp. & Inf., Minuf, Egypt. ** Atomic Energy Authority, Atomic Energy Research Center, Inshas, Egypt. **Abstract:** The Nuclear Research Reactors plants are expected to be operated with high levels of reliability, availability and safety. In order to achieve and maintain system stability and assure satisfactory and safe operation, there is increasing demand for automated systems to detect and diagnose such failures. In recent years, both Genetic algorithms and neural networks, which are inspired by computation in biological systems, are emerged as established techniques for optimization and learning. Genetic algorithms have been used in conjunction with neural networks in three major ways: First, genetic algorithms have been used to construct neural network topologies. Second, they have been used to set the weights in fixed architectures. Third, they have been used to select training data and to interpret the output behavior of neural networks. This paper is concerned with the construction of Artificial Neural Networks (ANNs) using Genetic algorithms (GAs) for the nuclear accidents diagnosis. MATLAB ANNs toolbox and GAs toolbox are employed to optimize an ANN for this purpose. When we apply the results obtained from genetic algorithms on the back-propagation algorithm, the results are similar but the design of ANNs using GAs is useful in terms of automating and optimizing the design and finding weights and biases for the suggested construction. The results obtained show the efficiency of using genetic algorithm, which can construct the high performance neural network structure for the nuclear reactor's input data. The best structure obtained is two layers ANN with correspondence values of weights and biases that are required to construct such network. [Abdelfattah A. Ahmed; Nwal Ahmed Alfishawy; Mohamed A. Albrdini, and Imbaby I. Mahmoud. Nuclear Research Reactors Accidents Diagnosis Using Genetic Algorithm/Artificial Neural Networks. Nature and Science 2011;9(5):64-74]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Genetic algorithms (GA), Artificial Neural Networks (ANN), Nuclear Reactors, Accidents Diagnosis, MATLAB	Full Text	12
13	**Yield Productivity and Energy-Saving Advantages at Applying Slow-Release Nitrogen Fertilizer in Upper Egypt**	Full Text	13

	***Khadra A.Abbady,. M.M.M Ahmed, M A Elshazely and Amer Kh A** Soils, Water and Environment Res. Instit. Agric.Res.Center. * Corresponding author: nefert60@yahoo.com **Abstract**: Two experiments have been conducted in New Valley and Assiut agriculture research stations "*Typic Torripsamments, hyperthermic and Typic Torriorthents*, coarse loamy, hyperthermic" to evaluate the fertilization of two cropping sequences of oil crops (sunflower, safflower and peanut & sesame and canola) with ureaform (UF) as a slow release nitrogen fertilizer (SRNF) comparing with ammonium nitrate (AN) as a conventional one. First sequence: the applied rates of UF-fertilizer have been 45, 67.5, 90, and 112.5 kg N.fed^{-1} added as side banding only at planting the first crop ,Sunflower, followed by safflower planting in the same previous plots and then peanut to determine the residual effect of UF-fertilizer. Ammonium nitrate (AN) has been applied in one rate of 45, 45 and 20 kg N.fed^{-1} (recommended rate) for each crop of the sequence and in the same order. It has been taken as a scale to estimate the performance of UF, in addition to no-fertilized one (Control). Second sequence: UF-fertilizer has been applied in the same rates mentioned in first sequence against the recommended rate of ammonium nitrate (45 kg N.fed^{-1} for each crop) with or without application of clay sediments and control treatment. Yield and its components, Nitrogen & energy consumption ability, net return and investment factor have been recorded. The results show that: (1) Firstly, the UF-fertilizer has almost had strong positive effect on yield and its components for both two cropping sequences. (2) Secondly, calculations of nitrogen-consumption ability have demonstrated that the UF-fertilizer has had much more efficiency at donating its nitrogen than that of AN one where their values at first cropping sequence have been (on average) 70 and 110 kg N. ton^{-1} dry matter (yield) for UF and AN respectively, as well as 92.66 and 158.72 at second cropping sequence. (3) Thirdly, calculations of Energy-consumption ability have illustrated that the saved energy with application of UF-fertilizer to produce one ton dry matter (yield) has been (on average) 36.54% for first cropping sequence and 41.6% for second cropping sequence calculated of those of AN-one. In other words, the saved energy with using UF has been (on average) 83.75 and 105.69, Liter of diesel fuel.ton^{-1} dry matter for first and second cropping sequence respectively which equivalent to 3132.25 and 3952.81 M. Joule or 0.53 and 0.67 barrel of diesel fuel or L.E. 92.13 and L.E.116.26. This would undoubtedly reduce CO_2 emissions, the first accused in global worming case. (4) Fourthly, all treatments have been almost implemented reasonable profitability (IF>3) either at first or second cropping sequences. The economic application of UF has been fulfilled when it hah been applied in high rate and then it is enough to fertilize two crops. It is also observed that added the clay has positively affected net return; however it has not given profitability. In spite of marked superiority of UF-net return value to those of AN, their IF values have been approximated. (5) Fifthly, the cost of consumed energy related to nitrogen fertilization has been reduced to about 1/2 by using UF fertilizer. [Mariam Refaat Mohamed Gad and*Mohamed Fawsy Abd-El hamid. Yield Productivity and Energy Saving Advantages at Applying Slow-Release Nitrogen Fertilizer in Upper Egypt. Nature and Science 2011;9(5):75-86]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: ureaform; slow release nitrogen fertilizer (SRNF); clay sediments; oil crops; nitrogen-consumption ability; energy-consumption ability		
14	**Response of Snap Bean *(Phaseolus vulgaris* L) Plants to Nitrogen Fertilizer and Foliar Application with Methionine and Tryptophan** **El-Awadi, M. E.[*1]; A. M. El-Bassiony[2]; Z. F. Fawzy[2] and M. A. El-Nemr[2]** [1]Botany Dept., National Research Centre, Dokki, Cairo, Egypt [2]Vegt. Res. Dept., National Research Centre, Dokki, Cairo, Egypt [*]el_awadi@yahoo.com **Abstract:** Two field experiments were carried out in two successive seasons of 2008 and2009 at the	Full Text	14

	Agricultural Experimental Station of the National Research Centre, EL-Nubaria, Elbehira Governorate, Egypt, to study the effect of different combinations of three levels of nitrogen fertilizer (100%, 65% and 35% of the recommended dose) with two levels of foliar spray methionine and tryptophan (100 and 200mgL^{-1}) on growth, pod yield, quality and some chemical constituents of snap bean plants. Results showed that fertigated snap bean plants with the highest nitrogen dose increased the vegetative growth, yield and quality. tryptophan (100mgL^{-1}) improved vegetative growth, yield and quality. Foliar application of tryptophan at both concentrations increased free amino acids content and phenolics content in the leaves. In addition, both concentrations of methionine increased free amino acid, protein percentage and nitrogen percentage in pod. It can be concluded that nitrogen fertilizer can be reduced to 65% with sprayed tryptophan amino acid (100mgL^{-1}) to obtain the highest vegetative growth, yield and quality of snap bean plants. [El-Awadi, M. E.; A. M. El-Bassiony; Z. F. Fawzy and M. A. El-Nemr. **Response of Snap Bean (*Phaseolus vulgaris* L) Plants to Nitrogen Fertilizer and Foliar Application with Methionine and Tryptophan**]. Nature and Science 2011;9(5):87-94]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Snap bean, Nitrogen fertilizers, Amino acids, Vegetative growth, Yield, Fruit quality		
15	**Medicinal Plants of submontane forest in a part of Tarai and Bhawar of Kumaun Himalaya** Bhasker Joshi Department of Botany, R. H. Govt. P. G. College, Kashipur (Kumaun University, Nainital) Uttarakhand, India-244713. E-Mail: bhaskerjoshiphd@in.com **Abstract:** The medicinal properties of forest vegetation was analyzed in a submontane forest of Tarai and Bhawar of Kumaun adjacent to Kashipur, at (29º 14-43.6)–(29º 19-50.5) E longitude and (79º 03-22.6)–(79º 04-23.2) N latitude at an elevation of 253.4–265.5 meter above the sea level, within the districts of Nainital and Udham Singh Nagar. 29 plants species belonging to 22 family, 26 genera, and 29 species were reported. Of these leaves in 19% cases, roots and whole plants in 16% cases, fruits and bark 13% cases are used. Based on life form 17 phanerophytes, 5 chamaephytes, 4 therophytes, 2 hemicryptophytes and 1 therophyte were recorded. [Bhasker Joshi. **Medicinal Plants of submontane forest in a part of Tarai and Bhawar of Kumaun Himalaya.** Nature and Science 2011;9(5):95-99]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key Words:**, Ethnomedicinal, Kumaun Himalaya, Medicinal plant, Submontane forest, Tarai and Bhawar.	Full Text	15
16	**Mechanism and Modelling for Sorption of Toxic Ion on Cement Kiln Dust** **A. El- Dakroury*, M.S. Sayed and E.EL- Sherif** Hot lab. Center and waste management, Atomic Energy Authority, P.O. 13759 Cairo Egypt *eaishaw95@yahoo.com **Abstract:** Cement manufacturing is a critically important industry in Egypt. The industrial by-product and waste materials must be managed responsibly to insure a clean and safe environment. Cement kiln dust (CKD) is a significant by-product material of the cement manufacturing process. Cement kiln dust is a waste residue composed chiefly of oxidized, anhydrous, micron – sized particles generated as a by product of the manufacture of Portland cement. The use of cement kiln dust as adsorbent in wastewater treatment has a great attention as cheap material and clay structure. This work will discuss the basic characteristics of CKD physical and chemical properties and regulatory requirements. The batch removal of Cr(VI) from aqueous solution using low-cost adsorbents such as cement kiln dust under different experimental conditions and the influences of initial Cr (VI) ion concentration (50 to 300 mg·l-1) and pH (1 to 4) were investigated in this study. Adsorption of Cr (VI) is highly pH-dependent and the results indicate that the optimum pH for the removal was found to be 1 for CKD. A comparison of kinetic models applied to the adsorption of Cr (VI) ions on the CKD was evaluated for the pseudo	Full Text	16

	first-order, the pseudo second-order, Elovich and intraparticle diffusion kinetic models, respectively. The results showed that the pseudo second-order kinetic model was found to correlate the experimental data well. [A. El- Dakroury, M.S. Sayed and E.EL- Sherif. **Mechanism and Modelling for Sorption of Toxic Ion on CementKiln Dust**]. Nature and Science 2011;9(5):100-108]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** CKD; adsorption; Cr (VI); adsorption kinetics; low-cost adsorbents		
17	**The Value of Esterman Binocular Visual Field Testing in Issuing A Driver's License for Glaucoma Patients** **Iman A. Fahmy, Fady E. Mitwally, and *Marwa A. Fouly** Glaucoma Department, Research Institute of Ophthalmology (RIO)-airo- Egypt *marwa_elfouly@yahoo.com **Abstract:** Purpose: To identify the relation between monocular visual field in glaucoma patients and binocular visual field (VF) (Esterman VF) and its effect on driving performance in different stages of glaucoma and to investigate whether Esterman disability score (EDS) is suitable for the assessment of mobility difficulty. Objective: Whether the visual efficiency scale in drivers' licensing currently adopted to determine the legal grade of visual disability associated with visual field loss is appropriate or not for the evaluation of disability regarding driving. Patients and Methods: Twenty eight patients recruited from the glaucoma clinic of the Research Institute of Ophthalmology (RIO) with different grades of glaucomatous VF affection were included in the study: mild VF affection: MD <6.00 dB, moderate VF affection: MD 6-12 dB, severe VF affection: >12 dB. Normally sighted control subjects were recruited from the outpatient clinic of the RIO. The glaucoma patients included in the study were follow-up patients of the glaucoma clinic. Detailed ophthalmological examination was performed including corrected and uncorrected visual acuity (VA) measurement using the Landolt VA chart, assessment of the angle of the anterior chamber using the Goldman contact lens for grading, examination of the optic nerve head using the 90 D indirect Volk lens, monocular visual field test using the Automated Humphery VF Analyzer 24-2 strategy and the binocular Esterman VF of the same patient on the same day. The correlation between the EDS and the monocular VF 24-2of each eye and the degree of subjective mobility difficulty was analyzed by statistical formulae. Conclusion: In addition to the currently adopted visual efficiency scale, EDS could be employed for the assessment of mobility difficulty in patients with visual field loss, also to establish new judgment criteria for issuing driver's license. [Iman A. Fahmy, Fady E. Mitwally, and *Marwa A. Fouly. **The Value of Esterman Binocular Visual Field Testing in Issuing A Driver's License for Glaucoma Patients**]. Nature and Science 2011;9(5):109-118]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Esterman Binocular Visual Field; Driver's License; Glaucoma Patient	Full Text	17
18	**Histopathological and Enzyme Changes in *Clarias gariepinus* (Burchell 1822) Exposed to Nitrite at Different Water Temperatures** Ajani, F [1]; Emikpe B. O.[2] And Adeyemo, O. K.[3] [1] Department of Animal Science and Fisheries Management,Bowen University,lwo,Nigeria [2] Department of Veterinary Pathology,University of lbadan,lbadan ,Nigeria [3] Department of Veterinary Public Health and Preventive medicine,University of lbadan,lbadan,Nigeria funmilolajani@yahoo.com; bendoctor@yahoo.com; olanikeadeyemo@hotmail.com Abstract: Nitrite is a natural component of the nitrogen cycle in ecosystems.lt is an intermediate in the oxidation of ammonium to nitrate.The elevation of ambient nitrite concentration is a potential problem for freshwater fish. This study was designed to investigate the effect of different water temperatures on	Full Text	18

	the toxic effect of nitrite in a freshwater fish. Sixty *Clarias gariepinus* (300 ± 1.30g), were exposed to nitrite at different water temperatures (27^0C and 35^0C) for 48hours. Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and total protein levels were assayed in the gill, liver and tissue (skin) of the fish. The statistical analysis was performed using the Statistical Analysis (SPSS 11.0 for Windows). Statistical differences were determined by one-way analysis of variance (ANOVA) and paired-sample t test. ALT and ALP increased significantly ($P<0.05$) in nitrite-intoxicated fish at 35^0C compared to the value obtained at 27^0C in the organs while a significant decrease ($P<0.05$) was observed for the enzyme AST at 35^0C compared to 27^0C. Protein level in all the tissues showed a significant decrease in nitrite-intoxicated fish at higher temperature. The histopathological changes observed in the gills of nitrite-treated fish at 35^0C were that of congestion and vacuolization while the liver showed generalized fatty35^0C were that of congestion and vacuolization while the liver showed generalized fatty degeneration, congestion of central veins and multifocal necrosis. Moderate hydropic degeneration of the epidermal layer was observed in the skin tissue. These results revealed that high temperature can increase the toxic action of nitrite in fish. [Ajani, F.; Emikpe B. O. And Adeyemo, O. K. Histopathological and Enzyme Changes in *Clarias gariepinus* (Burchell 1822) Exposed to Nitrite at Different Water Temperatures. Nature and Science 2011;9(5):119-124]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Nitrite , *Clarias gariepinus*, enzymes , proteins, histopathology, Temperature, toxicity.		
19	**Enzyme Mediated Amido Black Decolourization by Soil borne RS-II Strain Isolated from an Industrial Town.** Arun Kumar, Robina Sharma, Rajesh Sawhney* Bhojia Institute of Life Sciences, Budh, Baddi , Distt. Solan (H.P). 173205 India sawhneyrajesh@yahoo.com **Abstract:** Total 10 strains of microorganisms were isolated from the soils exposed to dyeing industry effluent, in and around Baddi. (HP). The isolate, RS-II, tentatively identified as *Galactomyces* sp. showed maximum amido black (azo dye) decolourization activity (72.08%), on primary screening. However, this isolate exhibited 81.43% decolourization of amido black, under optimal conditions of pH (8.0) and temperature (37^0C). The decolourization activity was found to be pH and temperature dependant, and mediated by enzymatic step. The SDS-PAGE gel electrophoresis results spotted a 66 Kd band. The purification of crude enzyme was carried out by Ion Exchange Chromatography. The activity of the pure fraction (eluted CM sepharose) was recorded as 5.5 moles/min/ml. The study highlights that RS-II has an adequate potential to decolourize the amido black dye, and the pure fraction of enzyme has even higher potential to do so. The findings could be a safe and viable solution for bioremediation of azo dye containing effluents, and could be an effective gateway to evolve more advanced and effective strategies based on the use of pure or immobilized enzymes. [Arun Kumar, Robina Sharma, Rajesh Sawhney Enzyme Mediated Amido Black Decolourization by Soil borne RS-II Strain Isolated from an Industrial Town. Nature and Science 2011;9(5):125-131]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: decolourization**,** amido black**,** azo dye, bioremediation**,** SDS-PAGE**,** *Galactomyces* sp	Full Text	19
20	**The Ends of the Earth** **(The Four Corners of the Earth)** Soleilmavis Liu Phone: +(86) 13854570873 Email: soleilmavis@yahoo.com **Abstract:** Old and New Testament Bible Stories told us The Creation Story; and God used Jews stories to teach us that we should obey God Jesus, walked the earth in the form of a man, he lived only near	Full Text	20

	Jerusalem. But God did not only live in Jerusalem. God was everywhere! The Bible mentioned "the Ends of the Earth" many times. The Queen of the South came from the Ends of the Earth (*Matthew12:42*). When The Queen of the South would rise at the Judgment, more stories of "the Ends of the Earth" would be given. This article briefly introduced where "the Ends of the Earth" were; who lived in "the Ends of the Earth"; The Brief histories and legends of "The Ends of the Earth". Indexing references were listed at the end of the paper in alphabetical order. **[**Soleilmavis Liu**. The Ends of the Earth.** Nature and Science 2011;9(5):132-139]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Bible; Creation; God; Jews; Jerusalem; End; Earth		
21	**Increase the efficiency of adult education with the proper use of learning styles** Mohammad Abedi Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran * abedi114@yahoo.com **Abstract:** Students, in fact all individuals, are most effective when they are taught in their personal learning style. In fact, there are three major types of learners: visual, auditory, and tactile/kinesthetic. While most individuals without disabilities can learn using any one of these styles, most people have one for which they show a stronger affinity. There are many tests available to help you and your students discover your best learning style. Generally speaking, however, if you are someone who is more likely to think in pictures, prefer to meet with someone in person, and are more likely to want visual diagrams when completing a project you have tendencies towards visual learning. Similarly, if you are more likely to think in terms of sounds, prefer to speak on the phone with someone, and want verbal instructions then you tend towards auditory learning. Finally, if you are more likely to think in terms of moving images like mini-movies in your mind, prefer to participate in an activity when you meet to speak with someone, and tend to jump right into a project without reading directions you tend towards tactile/kinesthetic learning. **[**Mohammad Abedi. **Increase the efficiency of adult education with the proper use of learning styles.** Nature and Science 2011;9(5):140-145]. (ISSN: 1545-0740). http://www.sciencepub.net. Keywords: learning styles, adult learning	Full Text	21
22	**Learning styles in education: with emphasis on adult education** 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: abedi114@yahoo.com **Abstract:** Though rarer today then in the past, some teachers discount the importance of learning styles. They continue to teach in their one major method without trying to vary instructional methods. This is a mistake that will lead to less learning in the classroom. On the other hand, many students and to a lesser degree some teachers make the mistake of thinking that they cannot learn using methods that are not focused on their learning style. This is also a huge mistake that in the end will result in less learning. If teachers do not help their students find ways to be successful learning information presented in any style, they are not helping them succeed in the future. The fact is that students will be faced with many different styles of teaching during the educational career. Only by finding ways to adapt and learn using other styles, will students end up succeeding. **[**Sharareh Khodamoradi and Mohammad Abedi. **Learning styles in education: with emphasis on adult education.** Nature and Science 2011;9(5):146-149]. (ISSN: 1545-0740).	Full Text	22

25	**Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs** Mehrdad Mahkam*, Nahid Poorgholy Chemistry Department, Azarbaijan University of Tarbiat Moallem. Tabriz, Iran mmahkam@yahoo.com; mahkam@azaruniv.edu **Abstract:** The aim of this work was to investigate the possibility of employing semi-covalent molecularly imprinted polymers (MIPs) as a controlled release device for ibuprofen and naproxen in biological fluids, especially gastrointestinal ones, compared to non imprinted polymers (NIPs). The carboxyl groups of ibuprofen and naproxen were converted to vinyl ester group by reacting ibuprofen and vinyl acetate as an acylating agent in the presence of catalyst. The semi-covalent molecularly imprinted polymers (MIPs) were synthesized by free radical polymerization of vinyl esters derivatives of ibuprofen and naproxen in the presence of methacrylic acid and ethyleneglycol dimethacrylate (EGDMA) as the functional monomer and cross-linker, respectively. The composition of the cross-linked three-dimensional polymers was determined by FTIR spectroscopy. The hydrolysis of drug polymer conjugates was carried out in cellophane membrane dialysis bags and the in vitro release profiles were established separately in enzyme-free simulated gastric and intestinal fluids (SGF, pH 1 and SIF, pH 7.4). Detection of hydrolysis solution by UV spectroscopy at selected intervals showed that the drug can be released by hydrolysis of the ester bond between the drug and polymer backbone in low rate. **[**Mehrdad Mahkam, Nahid Poorgholy. **Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs.** Nature and Science 2011;9(5):163-168]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words:** molecularly imprinted polymer, pH-sensitive, anti-inflammatory drugs, sustained release	Full Text	25
26	**Chemical Reaction in Tomato Plants in Response to A biotic Elicitors Treatments** [1]El-Samra, I.A.; [1]M. A. Amer; [2]M. R. Abd-El-Hamid; [3]S. S. Kabeil; and [1]A. M. El-Alwany [1]Department of Agricultural Botany, Faculty of Agriculture (Saba-Bacha), Alexandria University, P.O. Box 21531-Bolkley, Alexandria, Egypt [2]Plant Protection Institute, Agriculture Research Center, Sabahia, Alexandria, Egypt. Mubarak City for Scientific Research and Technology Applications, New Borg-El-Arab, Alexandria, Egypt bcgroup1@ymail.com **Abstract:** Early blight resistant cultivar "Tezier" and susceptible cv. "Castle rock" were tested to identification their response to *A. solani* infection on tomato seedlings pre-treated with chemical inducers: Salicylic acid (SA), Isonicotinic acid (INA), and Thiamine (vit. B_1), under greenhouse conditions. Resistant cv. "Tezier" exhibited rapid reaction represented in higher significant endogenous SA levels compared to the susceptible cv. "Castle rock" for all chemical treatments. "Tezier" endogenous SA levels surpassed "Castle Rock", 5 folds in exogenous SA, 2 fold in INA, and about 5 folds for vit. B_1 application. "Tezier" also had higher quantities in PRs accumulation (β-1, 3-glucanase, chitinase and peroxidase) in time course intervals 3, 24, 48, 72, and 96 hrs after pathogen inoculation, through increase of PRs activity which was started one day after inoculation in all the induced plants and reached maximum level after three to four days compared to "Castle rock" for all chemical inducers. Total protein content and polyphenol oxidase activity were also observed, their levels were highly significant in "Tezier". **[**El-Samra, I.A.; M. A. Amer; M. R. Abd-El-Hamid; S. S. Kabeil; and A. M. El-Alwany. **Chemical Reaction in Tomato Plants in Response to A biotic Elicitors Treatments.** Nature and Science 2011;9(5):169-185]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key Wards;** Early blight disease; Chemical inducers: Salicylic acid (SA), Isonicotinic acid (INA), and	Full Text	26

	Thiamine (vit. B_1).		
27	**Toxigenic Potential of Co-occurring Aflatoxin and Ochratoxin A Detected in Poultry feed on *Clarias gariepinus* Larvae** Ezekiel C.N.[1*], A.C. Odebode[2], S.O. Fapohunda[1], G.O. Tayo[3], O.J. Olawuyi[1], O.B. Olaoye[1], A.O. Olarinmoye[4] And O.O. Adeyemi[1] [1]Mycology Unit, Department of Biosciences & Biotechnology, Babcock University, PMB 21244, Ikeja, Lagos 100 001, Nigeria. [2]Mycology Unit, Department of Botany & Microbiology, University of Ibadan, Nigeria. [3]Animal Nutrition Unit, Department of Agriculture & Industrial Technology, Babcock University. [4]Veterinary Medicine Unit, Department of Agriculture & Industrial Technology, Babcock University. [*]Corresponding author: <chaugez@gmail.com> **Abstract:** The worldwide contamination of poultry feeds with aflatoxins and ochratoxin A (OTA) independently and in co-occurrence has been reported in several countries. However, there is paucity of information on the co-occurrence of aflatoxins and OTA and their detection by immunoassay in Nigerian poultry feed. Fourty-seven locally formulated poultry samples collected from 13 locations within Southwestern Nigeria were analyzed for total aflatoxins (TA) and ochratoxin A (OTA) using the Immunoassay method. The potential toxicities of the samples were tested by the *Clarias gariepinus* day-old larvae bioassay. Approximately 98.2% samples were positive for TA and OTA with concentrations above the limits of quantitation. The ranges of TA and OTA in the samples were <4.0 µg kg^{-1} to 575 µg kg^{-1} and <2.0 µg kg^{-1} to 14.2 µg kg^{-1} respectively. Toxicity to *C. gariepinus* larvae was concentration dependent and 17, 21 and 7 samples containing the co-occurring toxins showed high, moderate and low toxicities respectively. On the average, 88.9% and 65.5% of the total samples had concentrations above the EU permissible limits for TA in immature and mature poultry feed respectively. The overall contamination risk for TA and OTA in samples in a significant (P = 0.01) decreasing order was: chick mash, broiler finisher, layers mash, broiler starter and growers mash. **[**Ezekiel C.N., A.C. Odebode, S.O. Fapohunda, G.O. Tayo, O.J. Olawuyi, O.B. Olaoye, A.O. Olarinmoye And O.O. Adeyemi. **Toxigenic Potential of Co-occurring Aflatoxin and Ochratoxin A Detected in Poultry feed on *Clarias gariepinus* Larvae.** Nature and Science 2011;9(5):186-192]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Aflatoxin, Immunoassay, Ochratoxin A, Poultry feed, Toxicity assay	Full Text	27
28	**Estimating external demand functions for Egyptian exports of grapes In light of the current global economic variables** MohyEL-Din M. Kh. El-BeGAWY, Ezzat Awad Zaghloul, Iman Abdel-Ghafour Ahmed and Mahmoud Riad ElGebaly Agricultural Economics Department, National Research Centre, Egypt. **Abstract:** The research aim is to estimate the functions of foreign demand for Egyptian exports of grapes to the most important foreign markets, imported. These markets are the markets "the United Kingdom, the Netherlands, and Italy", considering that these markets are the main importing markets for Egyptian grapes, which absorbed about 69% of the amount of exports of Egyptian grapes during the period (2005-2009). The results showed that, an increase in export price of Egyptian grapes to the UK market by about 1% leads to a decrease in demand of about 2.412% of any commodity to be flexible in this high demand market. The cross demand elasticity's noted that the increase in the Egyptian grapes price as the main rival to Egypt market to the United Kingdom are (Spain, Germany, and the United States) is estimated at 1% lead to increased demand for Egyptian grapes about 1.521%, 1.140%, and 0.175%, respectively. These refer to the replacement relationship between grapes exported from these countries and the grapes exported from Egypt. The spending elasticity indicated that the power to	Full Text	28

	increase the true total spending import of the United Kingdom on grapes by about 1%, leading to increased spending on Egyptian grapes about 0.792%, which indicates that the Egyptian grapes is a commodity necessary within the UK market. With regard to the Netherlands market, the elasticity of demand price on Egyptian grapes showed that, the increase in the price of grapes, about 1% leads to a decrease in demand of about 1.594%, which means that a product with elastic demand in this market. The cross demand of elasticity's noted to increase in the price of exported grapes, from these states as the main rival to Egypt which are Spain, Germany, and Greece, about 1% leads to an increase of demand on Egyptian grapes about 0.481%, 0.659%, and 0.572%, respectively. These referred to the replacement relationship between Egyptian grapes from one hand and the exported grapes from those countries on the other hand. The spending elasticity power shown that to increase the real total spending import of Dutch grapes about 1%, leading to increased spending on Egyptian grapes about 0.851%, which indicates that the Egyptian grapes is a commodity necessary within the Dutch market. While, noting that the results of estimating model (ADIS) with respect to price elasticity of demand on Egyptian grapes in the Italian market, that the increase in prices by about 1% leads to a decrease in demand of about 0.469%, which means that a product with inelastic demand of the Italian market. Nevertheless, noting that cross demand's elasticity that an increase in the price of grapes, from the States as the main rival to Egypt-Italian market which are (Spain, Germany, the Netherlands, and Israel) about 1% lead to changes in demand for Egyptian grapes about 3.193%, 1.490, - 0.244%, and 1.738% respectively. These indicate the replacement relationship of exported grapes from those countries in the one hand, and the exported grapes from Egypt in the other hand (excluding the Netherlands). This relationship is complementary, in the case of high export prices of Spain, Germany, Israel, and Egypt, respectively. It is clearly seen from the spending power of elasticity that, to increase the real Italian total import spending of grapes about 1%, leading to the increased expenditure on the import Italian Egyptian grapes by 0.814%, which indicates that the Egyptian grapes is a necessary commodity in the Italian market. The study recommended that, there is a need for attention to specifications of the required quality and conformity to international standards, and develop systems to ensure quality control. As well as, working on creating a strong export institutions and export high-efficiency, to study foreign markets and their needs in terms of quantity, quality and time of export. In addition to the study of world markets, competition for Egypt in the important markets, with the need to open new markets for exports of Egyptian grapes, and not rely mainly on a single market or a limited number of markets, as it turned out coefficiency of geographic concentration of the quantity of exports of Egyptian grapes, that there is a heavy concentration in the amount of exports of Egyptian grapes, which may displays these exports to the violent tremors if was one of these markets. [MohyEL-Din M. Kh. El-BeGAWY, Ezzat Awad Zaghloul, Iman Abdel-Ghafour Ahmed and Mahmoud Riad ElGebaly. Estimating external demand functions for Egyptian exports of grapes In light of the current global economic variables. Nature and Science 2011;9(5):193-204]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key Words**: Egyptian Grapes exports, Almost Ideal Demand System (AIDS), Competitiveness capability, demand-price elasticity, cross elasticity, indicated elasticity.		
29	Light Hydrocarbons in Niger Delta Oils: Geochemical Significance of Ring Preference. Mark O. Onyema and Leo C. Osuji. Department of Pure and Industrial Chemistry, University of Port Harcourt, P.M.B 5323, Choba,Port Harcourt, Rivers State, Nigeria onyemark@yahoo.com **Abstract:** The light hydrocarbon ring preference (RP) in crude oils from the Niger Delta has been investigated. The crude oil samples were analyzed using gas chromatographic fingerprints of ring preference light hydrocarbons. The ratios of P_2^3, P_3^3 (3RP) and N_2^5 (5RP) ranged from 9.73 to 13.27%, 4.04 to 7.90% and 8.75 to 14.71% with no compositional variation of ring preference for correlation and/or differentiation. The ratio of 6RP, N_1 ranged from 38.47 to 55.17% and revealed Niger Delta	Full Text	29

	crude oils as exhibiting high 6RP. The ratio of parent P_1 separates the oils into two homologous sets. k_2 supports the grouping by P_1, compares well with RP ratio and classified EN-A4, EN-A9 (Eastern) and CE-B3, CE-C7 (Central) as marine source crude oils and WT-D5 (Western) as terrigenous source oil. Plots of ring preference further showed that Western Niger Delta oil remained distinct from the Central and Eastern oils. Gross differences observed on star plots of key ring preference parameters established that the Central and Eastern crude oils remained constrained and distinct from the Western. The ring preference appears to be reliable but must be interpreted within a complete understanding of the petroleum system under study [Mark O. Onyema and Leo C. Osuji. Light Hydrocarbons in Niger Delta Oils: Geochemical Significance of Ring Preference. Nature and Science 2011;9(5):205-210]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: Ring Preference, Light Hydrocarbons, Niger Delta, Geochemical, Star Plot.		
30	**Role of nitric acid or H_2O_2 in antioxidant defense system of *Pisum sativum* L. under drought stress** [1]Helal Ragab Moussa and [2]Mohamed Abd El-Fattah Hassan Mohamed [1]Radioisotope Department, Atomic Energy Authority, Malaeb El-Gamaa St., P.O. 12311, Dokki, Giza, Egypt. [2]Agriculture Research Center, Soils, Water and Environment Research Institute, Giza helal_moussa@hotmail.com **Abstract:** Water shortage is likely to be one of the major global environmental stresses of the 21st century. Drought is an important environmental constraint limiting the productivity of many crops worldwide. Experiments were conducted to investigate the effects of seed pretreatment by hydrogen peroxide at 70 mM or sodium nitroprusside (SNP; nitric oxide donor) at 10 µM on drought tolerance in pea seedlings. Osmotic stress was provoked by addition of polyethylene glycol to the nutrient solution at the flowering stage. H_2O_2 or SNP are active molecules involved in mediation of various biotic and abiotic stress induced physiological responses in plants. H_2O_2 or SNP pretreatment alleviate oxidative damage, accelerate proline accumulation and enhance total chlorophyll, carotenoid, photosynthetic activity ($^{14}CO_2$–fixation), and total yield/plant in leaves of pea seedlings subjected to osmotic stress. The results showed that osmotic stress induced decrease in the enzyme activities of ascorbate peroxidase, glutathione peroxidase, catalase and overproduction of $O_2^{\bullet-}$ in pea leaves, which in turn caused exacerbation of lipid peroxidation and depression of photosynthesis. Application of H_2O_2 or SNP significantly increased the enzyme activities and decrease $O_2^{\bullet-}$ production and hence inhibited lipid peroxidation. Level of H_2O_2, proline and Evan blue uptake in seedlings pretreated with H_2O_2 or SNP were markedly lower than under drought stress, indicating the operation of antioxidant system in them. Moreover, seedlings arising from H_2O_2 or SNP pretreatment enhanced the membrane stability, as revealed from greatly reduced malondialdehyde content. The present data suggest that pea seed pretreatment with H_2O_2 or SNP, a stress signal, could trigger the activation of antioxidants in seeds, which persists in the seedlings to alleviate the oxidative damage, leading to improvements in physiological attributes for the seedling growth under drought. **[**Helal Ragab Moussa and [2]Mohamed Abd El-Fattah Hassan Mohamed. **Role of nitric acid or H_2O_2 in antioxidant defense system of *Pisum sativum* L. under drought stress.** Nature and Science 2011;9(5):211-216]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Antioxidative enzymes, Drought stress, Photosynthesis, Proline, Pea	Full Text	30
31	**Protease Production and Growth Characteristics of *Aspergillus sydowii*** Arun Kumar Sharma*, Vinay Sharma and Jyoti Saxena 1. Department of Bioscience and Biotechnology, Banasthali University, 304022, Rajasthan, India.	Full Text	31

	* Corresponding Author Email: arun.k.sharma84@gmail.com **Abstract:** The present work was aimed to evaluate the optimization of medium composition for protease production by *Aspergillus sydowii*. The fungus was isolated from the garden soil of Banasthali University and was identified as *Aspergillus sydowii* on potato dextrose agar medium. It was maintained on potato dextrose agar medium for 7 days at 28^0C. Spore suspensions was prepared, inoculated in Czapek Dox broth medium supplemented with various substrates, incubated and the mycelium was separated from the culture medium by filtration and the filtrate was used to determine the protease activity. The biomass was determined after drying the mycelium at room temperature for 24 hours. Maximum growth rate of this fungus in casein as substrate was found after 8 days of incubation (7.11mgmL^{-1}) at 35^0C. But maximum protease activity was obtained after 6 days (11.95 Uh^{-1}mg^{-1}dry mycelium). The highest protease production was attained with casein, peptone and mung seedlings as nitrogen sources. The extracellular protease production and mycelial growth were influenced by the concentration of casein. Other protein sources (yeast extract) supported growth but did not induce such excellent protease synthesis and ammonia as end product repressed it, indicating catabolite repression in this microorganism. Optimal protease production was obtained at final pH 5.3. **[**Arun Kumar Sharma, Vinay Sharma and Jyoti Saxena. **Protease Production and Growth Characteristics of *Aspergillus sydowii*.** Nature and Science 2011;9(5):217-221]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words:** *Aspergillus sydowii*, Casein, Nitrogen sources, Protease.		
32	DNA fingerprinting of Rape seed (*Brassica rapa* L.) varieties of Bangladesh using SSR markers M.R. Molla[1*], M.N. Islam[2], M.M. Rohman[3], I. Ahmed[1] and L. Rahman[4] [1]Molecular Biology Laboratory, Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh [2]Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka [3]Molecular Breeding Laboratory, Plant Breeding Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh [4]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymenisingh rezwanbt@gmail.com, motiar_1@yahoo.com **Abstract:** The identification and differentiation of the varieties through DNA fingerprinting using microsatellites (SSRs) are quite effective, when the variety specific primers are used. This is gaining importance particularly when the distinguishing a variety from others using morphological traits are becoming difficult due to use of limited elite varieties for new varieties. A set of microsatellite loci (B.n.12A, B.n.38A and B.n.59A1) has been investigate to distinguish the uniqueness of nine released rape seed (*Brassica rapa* L.) varieties in Bangladesh for the purpose of obtaining distinctness of the plant variety at molecular level. In the present study a total of nine rape seed (*Brassica rapa* L.) varieties have been used to characterize those groups. Upon PCR amplification, the alleles were separated on polyacrylamide gel using a sequencing gel electrophoresis system and visualized by silver-staining method. The loci were polymorphic in all the varieties. Differences were observed in heterozygosities in the studied varieties. The mean observed heterozygosity (Ho) and expected heterozygosity (He) were 0.124 and 0.507, respectively. Varied ranges of alleles occurred might be due to mutation of di-nucleotide repeat units which could also be indicative of varietal differences. Polymorphism Information Content (PIC) values in the present study were high which ranged from 0.481 to 0.667. UPGMA dendrogram based on Nei's (1972) genetic distance indicated differentiation of nine varieties of rape seed into two main clusters: Tori-7, BARI sharisha-9 and BARI sharisha-12 grouped in cluster 2 while others in cluster 1. In cluster 1 Agrani and Sampad grouped together in sub-cluster I and with minimal genetic distance (0.000). Safal also showed nil genetic distance with SS-75 and BARI sarisha-6. The varieties Sampad and Tori-7 showed the highest genetic distance value (3.860). Nine rape seed varieties in this study showed unique and differential DNA banding patterns	Full Text	32

	across at least one and/or combination of three primers. The data obtained can be provided some levels of identity and protection against remaining and other practices are beyond ethics and rules. [Molla MR, Islam MN, Rohman MM, Ahmed I, Rahman L. DNA fingerprinting of Rape seed (*Brassica rapa* L.) varieties of Bangladesh using SSR markers. Nature and Science 2011;9(5):222-228]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords**: SSR, Genetic diversity, Polymorphism Information Content (PIC), Rape seed		

Traditional Knowledge and Utilization of Medicinal Plants of Himalayan Region

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Abstract: Primitive human societies have been depending on plants and plant products for various remedies. In certain areas these folk medical prescriptions are endemic and have survived through ages from one generation to next generation verbally. They do not exist as written knowledge. Generally these systems of medicines depend on old people's experiences. The person, prescribing these medicines has no so-called scientific knowledge about the disease. Indigenous systems of medicine are specially conditioned by heritage and myths.
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Key words: Herbal drugs, indigenous knowledge, Vaidyas, medicinal plants

1. Introduction

In the past decade, there has been renewed attention and interest in the use of traditional medicine globally (Sheldon et al., 2000). The World Health Organization (WHO) has pointed out that traditional medicine is an important contributor to its health goals. Today, according to the WHO, as many as 80% of the world's people depend on traditional medicine and in India 65% of the population in the rural area use Ayurveda and medicinal plants to help care needs (Anonumous, 1992). Thus, traditional medicine practices, conserved over decades from old civilization, can serve as an effective basis for the discovery and development of modern therapeutic drugs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases. In a report published by the World Bank, Lambert et al. (1997), pointed out that preserving and enhancing the plant knowledge and use was equivalent to rescuing a global heritage. Herbal medicines are comparatively safer than synthetic drugs. Plant- based traditional knowledge has become a recognized tool in search for new sources of drugs (Sharma and Majumdar, 2003).

The ethnobotanical survey can bring out many different clues for the development of drugs to treat human disease. Plants have been an integral part of life in many local communities for food and medicine both. India has more than 3000 years of medicinal heritage based on medicinal plants are widely used by all sections of the population either directly as folk remedies or indirectly in the preparation of modern pharmaceuticals. There has been a revival of interest in medicinal and wild plants during the last few decades among the ethnobotanists (Bhatt et al., 2000; Rajasab and Isaq2004) which is associated with an increasing desire for natural rather than synthetic medicine.

Central Himalaya is one of the biodiversity-rich zones of India in terms of vegetation and flora. Varied altitude, topography, status of soil and climatic conditions favour high species richness and support different types of forests. Deciduous and evergreen forest, semievergreen forests are the major types in the Central Himalayan region. Wide geographical and climatic diversity provides a repository of valuable medicinal and aromatic plants of the region. These plants have a valuable place in indigenous system of medicine as well as tribal dietary requirements. A review of literature revealed that ethnobotanical study in Himalayan region is limited especially the traditional knowledge. Therefore, a need was felt to document the information on medicinal drugs prepared by old people and traditional Vaidyas (one who have knowledge of herbal medicine) of the state. The Vaidyas of Uttaranchal have thus developed the medical system of therapy accordingly on the available bio-resources including wild and cultivated plant species growing in the state.

Therefore, the present investigation is an attempt to document the various herbal drugs prepared by various traditional herbal healers of Himalayan region.

2. Study Area

The present study was conducted in Uttarakhand state of central Himalaya. Uttarakhand is located in the northern region of India and span over an area of 53, 485km. The human population of the state is 8479562 of which 78% falls under rural category. The climate of the study area is referred to as monsoon warm temperate. Annual rainfall of the area is 2136 mm and mean monthly temperature varies from 7 to 27ºC during summer and 2.1 to 3.8ºC during winter. The winter is characterized by occasional snowfall. Of the

total precipitation, nearly 75% occurs during the three months of monsoon, mid-June to mid-September.

3. Methodology

Field study was carried out during the period between April 2007 and May 2008 across the various districts of Uttarakhand. The standard methods as suggested by Martin (1995) and Cotton (1999) were adopted for herbarium preparation. Plant identification was done with the help of regional and local floras. (Osmoston, 1927; Babu, 1917; Naithani, 1984; Purohit and Samant, 1995).Detailed information on wild medicinal plants were gathered through oral interviews of the local people and Vaidyas. Village people information were consulted to locate and collect these plants. Throughout the interviews the useful information on wild and medicinal plants were recorded. Semi structured questionnaire survey was conducted among knowledgeable traditional Vaidyas with a view to document the knowledge on the use of medicinal plants. The data were crosschecked by interviewing more than three Vaidyas on the use of specific plant species. Also comparison was made between the information provided by Vaidyas and available literature (Jain, 1991; Gaur, 1999; Kala, 2002). Some workshops were also organized and various groups of indigenous people including Vaidyas were invited to generate and help in documenting the indigenous knowledge of such parameters. Qualitative information so gathered was verified by cross-examination with different traditional Vaidyas.

4. Results and Discussion

The study reveals that village people in the area depend on plants for medicinal purpose. During present investigation 90 species were identified as being used for treatment of approximately 45 ailments were gathered In most of the case leaves (46%), were used for curing ailments followed by root parts (18%), fruit and seeds (14%), stem bark(12%), whole plants(8%), latex and resin (6%), flowers and other parts (4%). The drugs are prepared mainly in the form of juice, powder, decoction, paste, jam and pills.

Maximum numbers of plants were used for curing wounded parts, stomach problems, cold cough, fever, skin diseases, respiratory problems etc. It was also found that a single plant may be used for curing many ailments such as *Berberis asiatica* is used in healing ulcer, urethral discharge and jaundice. Similarly *Hedychium spicatum* is used for the treatment of many diseases (Table 1).

The results of the study indicate that most of the common species which grow in the garden and adjacent to the village areas in cultivated or forest lands are used by Vaidyas in majority of the cases for preparation of herbal drugs. Vaidyas use *Ocimum, Piper nigrum, Curcuma domestica, Brassica campastris, and Rapnhanus sativus frequently* for making various herbal drugs (Kala et al.,2005).

The Vaidyas system of medicine pursues the holistic approach and does not aim to cure only the affected organs alone but aims to find out the origin and the casual factor of the disease in order to eradicate the disease from its root (Dash, 1999).

For gathering medicinal plants from nature, the Vaidyas follow some specific guidelines. They mainly avoid collection of plants for medicinal purpose if insects, pests and or any disease have infected the plant species. The collector of plants for any medicinal use were advised not to collect plants if the plants were affected by any toxicity, sunstroke, high velocity of winds, hailstorm, fire and flood. There was restriction of collection of medicinal plants among traditional Vaidyas from cemeteries, cremation grounds, sacred places, slaughter houses, areas affected by sewer discharges or polluted water, and termite infected areas, road sides, landslide prone areas and area furrowed by rodents (Kala et al., 2005).

The plants were collected for medicinal purpose when they attain maturity and it was judged by height of plants, branching pattern, and colour and other morphological characters including right fragrance and potency. All these judgments were based on the experiences and knowledge of the collectors. There are also some guidelines for collection of different parts of the medicinal plant species. The branches are collected mainly between flowering and ripening of fruits. If the roots of trees are required for medicinal purpose, then only the bark of hard and woody roots are collected. Roots and rhizomes of annuals are collected in summer and winter after the leaf fall or when the new leaves just emerge. Milk, sap, gum, resin, latex and other liquid exudes from plants are collected in autumn yet these products are collected depending on their availability. Similarly, as per the seasonality and availability the flowers and fruits are collected (Kala *et al*., 2005).

The use of plants for treatment in India dates back to prehistoric times. This indigenous knowledge about medicinal plants and therapies was compose verbally and passed orally from generation to generation. Much later, some of this information was composed in treatise form like Rigveda, Yajuveda, Charak Samhita, Sushrut Samhita, etc. These systematized system of knowledge about medicinal plants and therapies are included under Ayurveda- the Indian Traditional Medicine System.

Despite significant development of rural health services, village people still use herbal folk medicine to a good extent for the treatment of common ailments like cough, cold, fever, headache and bodyache, constipation, dysentery, burns boils, ulcer, skin diseases,

respiratory trouble and others. The Himalayas have a wide range of herbal products as this region supports approximately 18,440 species of plants. Just like the ancient people, the Himalayan people have close relationship with nature for their basic needs like food, fuel, fodder, medicine, etc. in health care, they use their own medicine system , which is based on the ancient cultural traditions.

The cultivation and use of medicinal plants has a great potential for employment generation, particularly in rural sectors. The recent emphasis on tribal and rural development indicates that cultivation of medicinal plants can play a prominent role in this direction, if undertaken properly. Unfortunately the traditional system of herbal use in Uttarakhand is not much popular in the younger generation because they thought less opportunity in this tradition for getting immediate benefits (Kala et al., 2005).

Besides, there were several other reasons, which made to decline the tradition including less promotion of Ayurvedic medicine compared to the western medicine in the Indian education system inspite of the common belief that the pure vegetable drugs are more powerful in their efficacy than those which have under several laboratory processes (Nadkarni, 1954).

However, to meet the objective of developing the effective Ayurvedic drugs based on traditionally claimed efficacies; clinical trials coupled with extensive phytochemical investigations are required to decipher the chemical nature of biologically active compounds for more scientific utility.

Table 1: Medicinal plants used by indigenous communities of Uttarakhand

Sl	Species	Family	Part used	Diseases
1	*Aloe vera*	Liliaceae	Pulp	Fresh juice is used as cathartic and cooling. It is also used in treating fever, eye disease and joint pain
2	*Asparagus racemosus*	Liliaceae	Root	Dried root powder crushed with turmeric and the filtrate taken orally, 2 spoonful twice a day for 3 days to cure gastro intestinal disorder
3	*Asparagus odscendens*	Liliaceae	Root	Strength, vitality
4	*Asparagus curillus*	Liliaceae	Root	Gonorrhoea, piles, diabetes, rejuvenating tonic
5	*Asparagus filicinus*	Liliaceae	Root	Sexual debility, urogenital disorders
6	*Astragalus aegacanthoides*	Fabaceae	Root	Burns, boils, skin diseases
7	*Albizia lebbek*	Mimosaceae		Flowers are used to cure skin eruptions, swelling and antidote to poison
8	*Argemone mexicana*	Papaveraceae	Whole plant	Leucorrhoea, wounds
9	*Annona squamosa.*	Annonaceae	Fruit and seeds	Fruit juice is used to control dysentery. Seed paste is applied on the forehead for relief from headache.
10	*Achyranthes aspera*	Amaranthaceae	Stem, fruit, leaf, seed and whole plant	Muscular cramps, mouth blisters, snake bite, check bleeding, anti-fertility in women and gastro intestinal disorder
11	*Achyranthes bidentata*	Amaranthaceae	Whole plant	Diuretic, astringent, fever, jaundice, cough
12	*Aconitum atrox*	Ranunculaceae	Root	Rheumatism, neuralgia, paralysis, dyspepsia, phthisis, rheumatic fever, puerperal fever, asthma, snake bite
13	*Aconitum balfourii*	Ranunculaceae	Root, tuber	Septic, boils, tonsil, gastritis, leprosy
14	*Aconitum falconeri*	Ranunculaceae	Root	Paralysis, sciatica, gout, fever, rheumatism, diarrhoea
15	*Aconitum heterophyllum*	Ranunculaceae	Root , tuber	Vomiting , fever, cough, stomach ache, gastrointestinal disorders, digestive disorders, fever, piles, dysentery
16	*Aconitum voilaceum*	Ranunculaceae	Root, tuber	Stomach-ache, fever, abdominal pain, bronchitis, cough, epilepsy, headache, inflammation, neck pain, renal pain
17	*Acorus calamus*	Araceae	root	Inflammation, neck pain, asthma, gout, rheumatism, improve lost voice.
18	*Ajuga parviflora*	Lamiaceae	Leaf , seed	Leaves and seeds used to cure jaundice, ascariasis, fever, stomach-ache

19	*Ajuga bracteosa*	Lamiaceae	Leaf, root	Leaves and roots used to cure jaundice, leucorrhoea, blood purifier, fever, worm killer
20	*Abies pindrow*	Pinaceae	Leaf, resin, bark	Cough, cold, rheumatism, ulcer
21	*Abrus precatorius*	Fabaceae	Leaf, seed	Diabetes, menstruation, cough, fever, asthma
Sl	**Species**	**Family**	**Part used**	**Diseases**
22	*Aesculus indica*	Hippocastanaceae	Fruit, seed, leaf, root	Fistula, Rheumatic, leucorrhoea
23	*Ainsliaea aptera* DC.	Asteraceae	Root	Stomach-ache
24	*Bidens pilosa*	Asteraceae	Whole plant	The warm juice of the fresh plant is used to treat earache and conjunctivitis and as a styptic on wounds
25	*Boerhaavia diffusa*	Nyctaginaceae	Leaf	Leaf paste is applied on the cuts and wounds to stop bleeding
26	*Buchanania lanzan* Spreng.	Anacardiaceae	Root , leaf	The roots and leaves are pounded, mixed with buttermilk and given in diarrhoea
27	*Bergenia ciliate*	Saxifrgaceae	Root	Decoction of root is used to remove kidney stone.
28	*Berberis asiatica*		Root	Roots are used in healing ulcer, urethral discharges, in leucor, opthalmia, jaundice, fever etc.
29	*Basella alba*	Basellaceae	Leaf	Boils, blisters
30	*Bauhinia variegate*	Caesalpiniaceae	Flower, bud	Diarrhoea, dysentery, tumours, stomach disorder
31	*Butea minor*	Fabaceae	Leaf	Anthelmintic, boils, skin diseases
32	*Betula alnoides*	Betulaceae	Bark	Eye diseases
33	*Betula utilis*	Betulaceae	Bark	Cuts, burns, wounds, hysteria, jaundice, ear, pain asthma, cough, cold, internal injury, menstruation
34	*Capparis sepiaria*	Capparaaceae	Leaf	Decoction of leaf is used in cough and skin diseases
35	*Carissa carandas*	Apocynaceae	Leaf	Decoction of the leaves is given for fever
36	*Cassia angustifolia* Vahl	Caesalpiniaceae	Leaf, fruit	Leaves and fruits are used as laxative and purgative
37	*Cassia auriculata*	Caesalpiniaceae	Seeds	Seeds are ground and paste is applied to cure skin disease
38	*Cassia occidentalis*	Caesalpiniaceae	Leaf , seed	Seeds and leaves are used in skin troubles. Leaves are also used in foot and mouth disease of cattle
39	*Commelina benghalensis*	Commelinaceae	Whole plant	Whole plant is used to treat leprosy
40	*Corchorus trilocularis*	Tiliaceae	Whole plant	Plant macerated with water yields, mucilage, prescribed as a demulcent. Seeds are used in fever and for cleaning bowls
41	*Crotalaria retusa*	Fabaceae	Whole plant	Plant is used in scabies and impetigo
42	*Crotalaria verrucosa*.	Fabaceae	Leaf	The leaf decoction is given orally to cure jaundice
43	*Centella asiatica*	Apiaceae	Leaf	Painful and slow urination, Eye trouble, fever, snake bite, brain tonic, malaria, cholera
45	*Cotoneaster microphyllus*	Rosaceae	Root	Scrofula
46	*Colebrooekea oppositifolia*	Lamiaceae	Leaf, root	Cataract, epilepsy, wounds, bruises
47	*Dioscorea bulbifera*	Dioscoreaceae	Tuber	Check conception, bronchial cough ,cold
Sl	**Species**	**Family**	**Part used**	**Diseases**
48	*Dioscorea deltoidea*	Dioscoreaceae	Root	Spermatonorrhoea, piles, dysentery
49	*Dioscorea belophylla*	Dioscoreaceae	Root	Blood purifier
50	*Dioscorea kemaonensis*	Dioscoreaceae	Tuber	Arthritis, rheumatism
51	Diploknema butyracea	Sapotaceae	Seed	Skin diseases
52	*Dalbergia sissoo*	Paplionaceae	Seed`	Rheumatic pain , skin diseases, breast cancer
53	*Daphne papyraceae*	Thymeliaceae	Whole plant`	Purgative, febrifuge
54	*Datura metel*	Solanaceae	Leaf, seed, root	Fistula, gum trouble, pyorrhoea, asthma
55	*Delphinium*	Ranunculaceae	Leaf	Cut and burn

	brunonianum			
56	*Delphinium denudatum*	Ranunculaceae	Root	Contusion, ulcer, toothache, abdominal pain, respiratory disorder, ulcer
57	*Delphinium vestitum*	Ranunculaceae	Whole plant	Snake bite, cuts, wounds, fever, diarrhoea
58	*Desmodium oojeinense*	Fabaceae	Bark	Low blood pressure
59	*Desmodium heterocarpon*	Fabaceae	Whole plant	Cough, fainting, convulsion
60	*Ficus palmate*	Moraceae	Latex, root	Boils, dysentery
61	*Ficus religiosa*	Moraceae	Leaf, latex, bark	Bronchial asthma, improve female fertility,, ear trouble, snake bite
62	*Flemingia strobilifera*	Fabaceae	Whole plant	Rheumatic pain
63	*Foeniculum vulgare*	Apiaceae	Whole plant	Vomiting
64	*Fragaria vesica*	Rosaceae	Root , leaf	Headache inflammation
65	*Fritillaria roylei*	Liliaceae	Bulb	Asthma, bronchitis, burns, stomach trouble
66	*Galinsoga parviflora*	Asteraceae	Leaf	Earache
67	*Galium acutum*	Rubiaceae	Whole plant	Antiscorb, diuretic, skin diseases
68	*Geranium nepalense*	Geraniaceae	Root	Renal diseases, cuts, jaundice, toothache, ulcer, wounds, stomach disorder
69	*Geranium ocellatum*	Geraniaceae	Whole plant	Diuretic
70	*Geranium wallichiana*	Geraniaceae	Root	Dysentery, diarrhoea, astringent, ear trouble, toothache
71	*Gerbera gossypina*	Asteraceae	Root	Menstrual disorder, blood pressure, gastric
72	*Hedera nepalensis*	Araliaceae	Leaf, flower	Rheumatism
73	*Hedychium spicatum*	Zingiberaceae	Root	Gastric trouble, asthma, vomiting, blood purifier, inflammation, liver complaints, etc.
74	*Hedychium accuminatum*	Zingiberaceae	Root	Dyspepsia, piles
75	*Heracleum candicans*	Apiaceae	Root, flower	Leucoderma, menstrual disorders
Sl	**Species**	**Family**	**Part used**	**Diseases**
76	*Hypericum cernuum*	Linaceae	Flower	Wounds, boils
77	*Impatiens gigantean*	Balsaminaceae	Aerial part, seed	Wounds, scarcity, burns
78	*Inula cappa*	Asteraceae	Root	Stomach-ache, dysentery, indigestion
79	*Ipomoea carnea*	Convolvulaceae	Leaf	Rheumatism, gout, cuts, boils
80	*Iris kumaonensis*	Iridaceae	Root, leaf	Urinary, kidney disorders, fever
81	*Litsea glutinosa*	Lauraceae	Bark	Bone fracture
82	Litsea umbrosa	Lauraceae	Seed	Skin diseases, wounds
83	*Malaxis muscifera*	Orchidaceae	Bulb	Wounds, bone fracture, burns
84	*Meconopsis aculeata*	Papaveraceae	Whole plant	Fever, renal pain, colic, backache
85	*Oxalis corniculata*	Oxalidaceae	Leaf, root, seed	Cuts, wounds, swelling, insect stings, snakes bite, scorpion sting, appetite, corns, dysentery, fever, jaundice, rickets, stomach-ache
86	*Rubia cordifolia*	Rubiaceae	Whole plant	Used to increase memory
87	*Swertia chirayita* Karst.	Gentianaceae	Whole plant	Used for blood purification
88	*Saussurea obvallata*	Asteraceae	Leaf	Paste of leaf applied to treat cut and wounds
89	*Valeriana jatamansi*	Valerianaceae	Root	Roots are used in hysteria, hypochondriasis, nervous unrest, and emotional troubles, carminative, sedative etc.
90	*Thalictrum foliolosum* DC	Ranunculaceae	Leaf, root	Diuretic, purgative, tonic dyspepsia ; valued for ophthalmia
91	*Rauvolfia serpentina.*	Apocynaceae	Whole plant	To control high blood pressure
92	*Ocimum sanctum*	Lamiaceae		A handful of leaves boiled in water and the infusion taken orally till pain relief

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The Petrography and Major Element Geochemistry of the Granite Gneiss of Arigidi area, S/W, Nigeria.

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Abstract: The granite gneiss of Arigidi area, falls within the migmatite-gneiss-quartzite complex of the Nigerian basement and occurs in association with grey gneiss, granite, charnockitic rocks and pelitic gneiss lithologies. The outcrops of the rock were studied in the field, eight samples were analysed for petrographic and geochemical characteristics. In thin section, quartz, plagioclase, biotite and opaque minerals which are ubiquitous ranged from 16.3-42.2, 18.4-42.4, 11.3-28.6 and 6-10.7vol%, respectively while orthoclase, microcline, pyroxene and hornblende ranged from 0-11.1, 0-19.3, 0-12.4 and 0-16.3vol%, respectively showing that most of the samples are tonalitic in composition. Geochemically, the SiO_2 content of the granite gneiss ranged from 63.42-74.30, Al_2O_3 ranged from 11.83-15.46 while Fe_2O_3 ranged from 1.33-3.22wt%. FeO ranged from 2.13-5.83, Na_2O from 0.40-3.91, K_2O from 0.05-3.42, CaO from 0.82-5.78 and MgO from 0.42-5.47wt%. MnO ranged from 0.03-2.11 while TiO_2 ranged from 0.01-1.46wt%. Discrimination diagrams revealed a preference for igneous fields by the granite gneiss. It is therefore deduced that this tonalitic granite gneiss has an igneous origin.
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Key words: Arigidi, granite gneiss, discrimination, tonalite, igneous origin

1. Introduction

Arigidi area, falls within the migmatite-gneiss-quartzite complex of the Precambrian Basement complex of Nigeria as classified by Adekoya *et al.* (2003) and used severally (Rahaman, 2006; Dada 2006). It lies between 5°45'E to 5°49'E and 7°33'N to 7°37'N of Ikole NE sheet. Major lithologies in the area include granite gneiss, grey gneiss, biotite granite, charnockitic rocks and pelitic gneiss (Fig. 1). Metamorphism in the area is believed to have attained granulite facies grade (Rahaman and Ocan, 1988). Structurally, the occurrence of sigmoidal strike-slip shear zones (Fig 2) and centimetric strike-slip faults make the occurrence of at least three phases of deformation (D3) probable in the area (Ferre *et al.*, 1996). Ejimofor *et al.*, (1996) worked on the petrography and major element geochemistry of the basement rocks of northern Obudu area, eastern Nigeria. It was shown mineralogically that, the preference of igneous fields by the granite gneisses suggest their affinity for igneous progenitors. Elueze *et al.*, (2004) determined the petrochemistry and petrogenesis of granite gneiss from Abeokuta area, southwestern Nigeria and concluded that the abundance and variation of major and minor trace elements suggest that the protoliths of the gneisses are mainly of igneous affinity, though with probable crustal contamination.

2. Materials and methods

The area was mapped on a scale of 1:30,000. The nature of outcrop, geographical location, colour, texture, mineralogy and structures were noted on the field. Fresh samples were collected and subjected to petrographical studies using petrological microscope. Photomicrographs were captured with digital camera. AAS was used to determine Si, Al, Fe, Ca, Mg, Mn and Ti while AES was used to determine Na and K. The content of minerals was plotted on the QAP diagram for the purpose of classification while the geochemical results were plotted on the discriminatory diagrams of Middleton (1960) and Tarney (1977) to infer the petrogenesis.

3. Results

3.1 Field description

The rock occurs as low-lying outcrops, small hills and inselbergs. It is associated with augen gneiss, quartzo-feldsparthic gneiss and granite. It is fine to medium grained, weakly to strongly foliated rock and strikes predominantly in a NNW-SSE direction with a steep dip (averagely 58°) in both directions. The foliation is defined by biotite streaks which have narrow thicknesses that range between 1 and 2mm. The leucocratic quartz and K-feldspar – rich streaks have a wider thickness that range between 0.2 and 2.5cm.

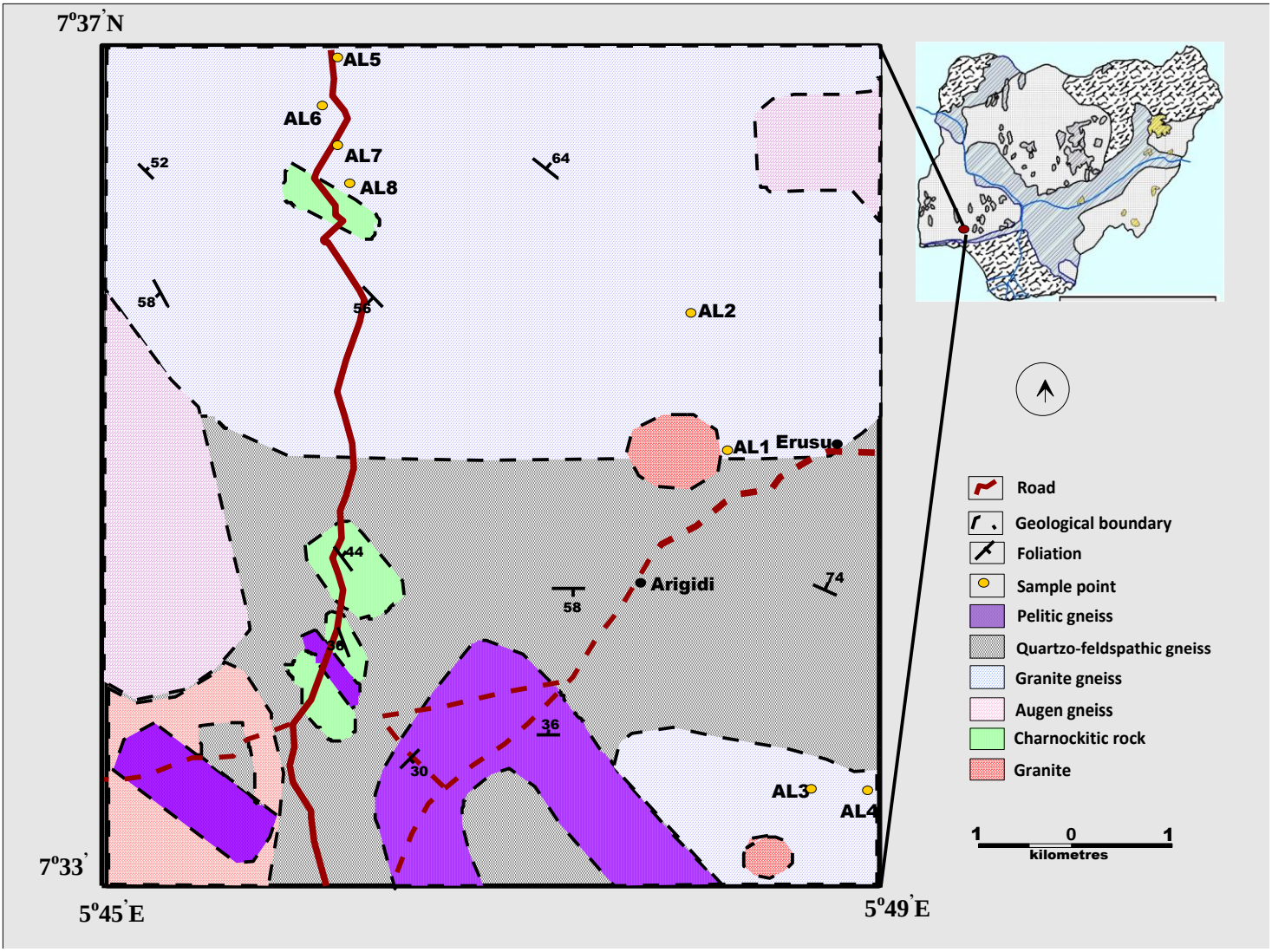

Figure 1. The Geological Map of Arigidi Area (Modified after Arigidi Independent Mapping Group, 2009)

Figure 2. Strike-Slip Sigmoidal Fault

3.2 Petrography

In thin section, the rock contains quartz which is colourless under plane polarized light and occurs as euhedral prisimatic crystals, and plagioclase with its distinguishing polysynthethic twinning according to albite law. Others minerals in the thin section are microcline typified by cross-hatched twinnings; biotite which is brownish in thin section, exhibits pleochroism and occurs as plates and laths which show preferential allignment with the foliation plane; orthoclase is colourless though it may be cloudy in contrast to quartz with twinning according to Carlsbad law as its distinguishing characteristic; pyroxene; and hornblende (Fig. 3) (Table 1). On the QAP diagram, five of the eight samples analyzed plotted as tonalites representing over 60% of the granite gneiss of Arigidi while the remaining plotted as granodiorite and granite (Fig. 4).

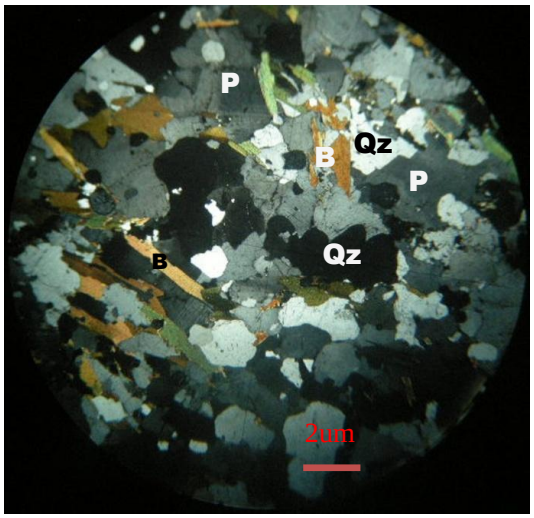

Figure 3. Photomocrograph of granite gneiss under cross nicols showing guartz (Qz), plagioclase (P), and biotite (B).

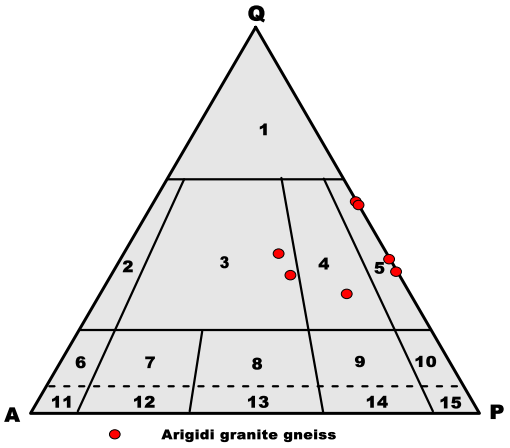

Figure 4. QAP Diagram for Arigidi Granite Gneiss (after Streckeisen, 1976).
1=Not Igneous; 2=Alkali Granite; 3=Granite; 4=Granodiorite; 5=Tonalite; 6=Alkali Quartz Syenite; 7=Quartz Syenite; 8=Quartz Monzonite; 9=Quartz Monzodiorite; 10=Quartz diorite; 11=Alkali Syenite; 12=Syenite; 13=Monzonite; 14=Monzodiorite; 15=Diorite.

3.3 Geochemistry

3.3.1 Major element data

The SiO_2 content of the granite gneiss ranges from 63.42-74.30% which corresponds to an intermediate to acid composition. Al_2O_3 ranges from 11.83-15.46% while Fe_2O_3 ranges from 1.33-3.22% and FeO varies from 2.13-5.83%. The Na_2O content ranges from 0.40-3.91%, K_2O from 0.05-3.42%, CaO from 0.82-5.78% and MgO from 0.42-5.47%. MnO ranges from 0.03-2.11% while TiO_2 ranges from 0.01-1.46% (Table 2). Geochemically, the Arigidi granite gneiss is similar to that of Southern India except for its higher FeO, Na_2O and K_2O contents and lower MnO and TiO_2 (Table 3). All other species are about the same.

4. Discussion

4.1 Petrogenesis

The geochemical data are plotted on discriminatory diagrams to establish the geochemical evolution of the rock. On the plot of K_2O versus Na_2O (after Middleton, 1960), six of the eight samples plotted outside the field of eugeosynclinal sandstones (Fig. 5) while on the TiO_2 versus SiO_2 discrimination diagram (Tarney, 1977), two of the eight samples plotted outside, one on the boundary line and the remaining five plotted in the igneous field (Fig. 6). A consensus is yet to be reached on the evolution of the Nigerian granite gneisses. Grant (1970), used $^{87}Sr/^{86}Sr$ studies to arrive at an igneous origin for the Ibadan granite gneiss. Burke *et al.* (1972), on the other hand, argued that the parent banded gneiss from which the granite gneiss was derived could have evolved by isochemical metamorphism of a shale-greywacke sequence. Rahaman and Ocan (1978), believed that the most of the granite gneisses in the Nigerian basement complex are intrusive. In the case of Onyeogocha (1984), partial melting of crustal rocks was used to explain the granite gneisses of north-central Nigeria. Rahaman (1988) stated that geochemical data available were insufficient to unequivocally distinguish between sedimentary and igneous origin for the granite gneisses.

In the face of the various schools of thought outlined above, the granite gneiss of Arigidi shows a preference for an igneous protolith as shown by the discrimination diagrams. This is further reinforced by the petrographic studies of the samples of the granite gneiss which revealed the absence of minerals, which are typical of paragneisses, like sillamanite, kyanite, staurolite or cordierite. The indication of this is that the rock is not likely to be of sedimentary origin.

In addition, Rahaman and Ocan (1988), also proposed an igneous origin for the gneisses that are associated with the pellitic gneisses of Ikare area, southwestern Nigeria of which the Arigidi granite gneiss is a part.

Finally, the tonalitic composition of the granite gneiss of Arigidi buttresses the Tonalite-Trondhjemite-Granodiorite (TTG) composition reported for the gneisses of western Nigeria (Pidgeon *et al.* 1976; Bruguier *et al.* 1994).

TABLE 1: MODAL COMPOSITION OF GRANITE GNEISSES (Vol %)

	AL1	AL2	AL3	AL4	AL5	AL6	AL7	AL8	Range
Quartz	33.2	42.2	16.3	18.2	25.2	38.1	37.3	27.2	16.3-42.2
Orthoclase	-	-	11.1	-	-	-	-	-	0-11.1
Microcline	19.3	-	-	8.3	-	-	-	-	0-19.3
Plagioclase	28.2	34.2	18.4	32.3	42.4	32.6	30.4	40.2	18.4-42.4
Biotite	13.2	14.3	28.6	18.4	22.6	21.5	23.3	11.3	11.3-28.6
Opaque Minerals	6.0	9.2	9.3	10.7	9.3	8.2	8.5	6.3	6.0-10.7
Pyroxene	-	-	-	12.4	-	-	-	14.4	0-12.4
Hornblende	-	-	-	16.2	-	-	-	-	0-16.3
Total	99.9	99.9	100.0	100.3	99.5	100.4	99.5	99.4	99.4-100.4

TABLE 2: MAIN ELEMENT GEOCHEMISTRY OF GRANITE GNEISSES (Wt %)

	AL1	AL2	AL3	AL4	AL5	AL6	AL7	AL8	Range	Mean
SiO_2	74.30	70.94	73.22	63.42	70.36	73.45	73.26	68.37	63.42-74.30	70.92
Al_2O_3	13.03	11.83	12.62	13.35	15.46	12.37	12.70	13.32	11.83-15.46	13.09
Fe_2O_3	1.33	1.42	2.11	2.72	2.29	2.52	3.22	2.74	1.33-3.22	2.32
FeO	2.42	4.14	3.22	4.10	3.20	3.84	3.36	5.83	2.13-5.83	3.76
Na_2O	2.98	3.91	3.43	0.10	1.00	0.84	0.40	0.80	0.40-3.91	1.81
K_2O	2.32	3.42	3.22	1.02	0.05	0.60	0.51	0.42	0.05-3.42	1.45
CaO	1.20	1.42	0.82	4.60	5.82	3.06	2.88	5.78	0.82-5.78	3.20
MgO	0.42	1.21	0.80	5.47	1.40	2.11	1.58	2.21	0.42-5.47	1.11
MnO	0.10	0.04	0.04	2.11	0.04	0.03	0.11	0.08	0.03-2.11	0.32
TiO_2	0.50	0.30	0.40	1.46	0.20	0.40	0.01	0.32	0.01-1.46	0.45
Total	98.62	98.6	99.88	99.26	99.82	99.42	98.03	99.87		

TABLE 3: COMPARISON OF ELEMENT OF GRANITE GNEISSES (Wt %)

	1(8)	**2(3)**	**3(4)**	**4**	**5**
SiO_2	70.92	65.48	63.46	70.36	64.60
Al_2O_3	13.09	17.94	19.87	14.42	17.00
Fe_2O_3		1.97	1.50	0.66	3.60
FeO	6.08	2.93	1.44	1.95	-
Na_2O	1.81	4.07	3.48	3.35	4.17
K_2O	1.45	1.07	5.37	5.38	3.48
CaO	3.20	3.01	5.05	2.03	3.43
MgO	1.11	-	-	0.90	1.58
MnO	0.32	0.03	0.03	-	-
TiO_2	0.45	1.75	0.64	0.32	0.54

NOTE: (n) Refers to average number of samples

1) Arigidi granite gneiss
2) Orthogneiss Vandeikya (Ejimofor *et al*., 1996)
3) Orthogneiss Ushongo (Ejimofor *et al*., 1996)
4) Granite gneiss, Jos Plateau, Nigeria (Wright, 1971)
5) Granite gneiss, S. India (Condie *et al*., 1982)

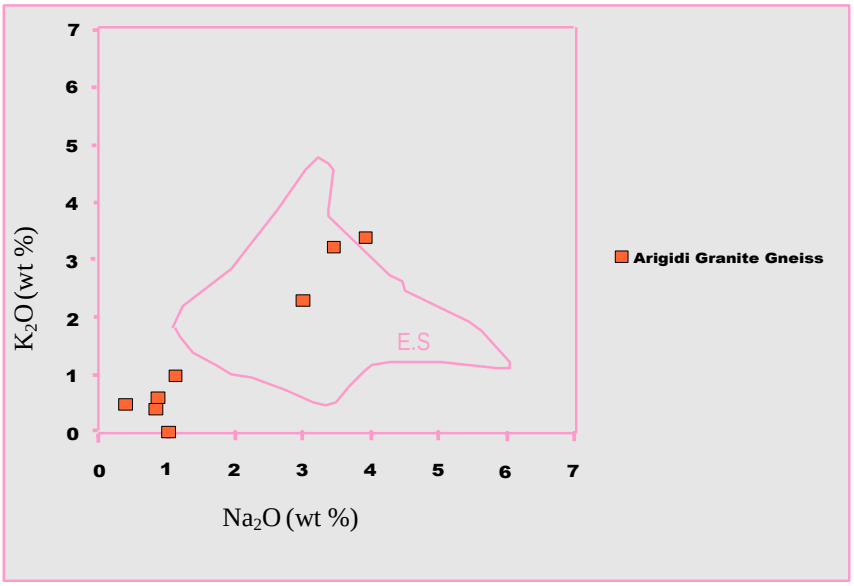

Fig. 5: K_2O versus Na_2O Discrimination Diagram (after Middleton, 1960)

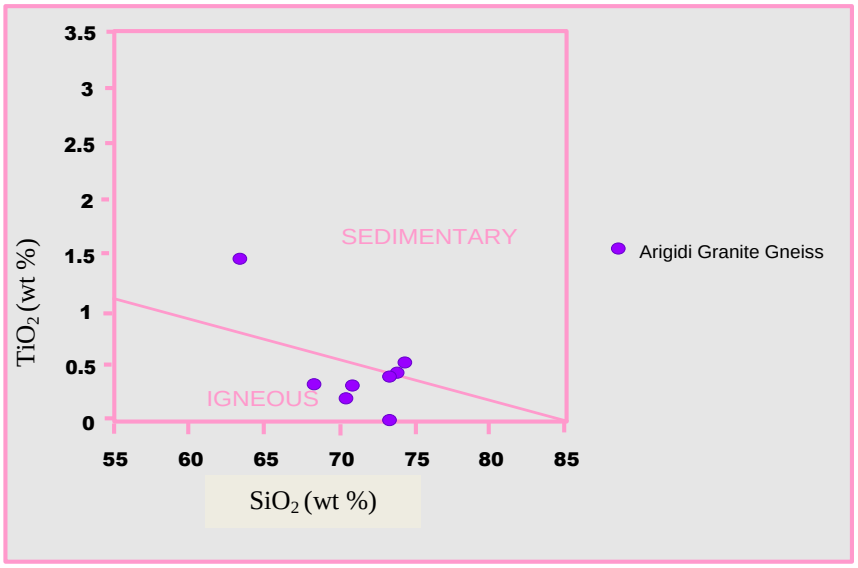

Fig. 6: TiO_2 versus SiO_2 Discrimination Diagram (after Tarney, 1977)

5. Conclusions

From the forgoing, it is therefore thought that the granite gneiss of Arigidi is an orthogneiss of tonalite composition.

6. Recommendations/Suggestion

However, it is candidly recommended that Isotope and rare earth element (REE) studies be carried out on the study area to conclusively determine the petrogenesis of the granite gneiss seeing that major element geochemistry alone cannot be used to decipher conclusively, the petrogenesis of a metamorphosed rock.

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3/1/2011

Pollutant Dynamics And Distribution In Sediments North Of Lagos Lagoon Ecosystem.

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ABSTRACT: Thermal pollution was investigated in the sediment North of Lagos lagoon. The result revealed the heavy metal distribution in sediment to be Fe>>>Zn>Pb>Cu. The concentration level of Cd is generally below <0.002 mg/Kg in all the stations studied. The concentration levels of heavy metal gave evidence of pollution in sediment. The results obtained confirmed that over heated water and effluent discharge was the source of pollution in this part of the lagoon. The distribution of heavy metals in sediment and safety status is discussed.
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Keywords: Thermal pollution, Heavy metals, sediment, ecosystem, Nigeria.

1. INTRODUCTION

Human activities have always impacted on coastal areas; it is only within the last two centuries that the effects of industrialization, intensive Agriculture, tourism and coastal engineering (dredging) have seriously begin to threaten marine life (Kakulu, S.E. and Osibanjo, O. 1988). Most of these impacts have led to environmental pollution i.e. introduction of substances or energy by man into the environment, which may put living resources and human health at risk. Many substances pollute the marine environment, but non biodegradable compounds (such as Heavy Metals) are the most dangerous due to their inmate ability to constantly remain with the ecosystem.

Heavy metals are referred to as any metallic chemicals elements that has a relatively high density and toxic, highly or poisonous at low concentrations. Heavy metals such as Cu, Zn and Pb are normal constituents of marine and estuarine environments. Additional quantities are introduced from industrial or thermal waste or sewage. They entered the biogeochemical cycle and as a result of being potentially toxic, interfere with the ecology of a particular environment. Heavy metals can be accommodated in three basic reservoirs; water, biota and sediments. Several studies have reported the accumulation of these metals in the tissue of aquatic biota from contaminated areas (Nwani et al 2010, Edem et al 2009 and Unyimadu et al 2008).

The establishment of a thermal plant in Ikorodu North axis of Lagos lagoon has resulted in increase volume of waste generated by industries and other commercial activities in the area. These wastes and effluents generated are directly or indirectly discharged into the lagoon. Toxic pollutants like Cu, Cd, As, and Cl present in heated effluent make water unsuitable for any purpose. The decrease in density, viscosity and solubility of gases increases the settling speed of suspended particles which seriously affect the food supplies of aquatic organism. (Bhatia S. C. 2009). Sediment retains most of the waste and effluent discharge into lagoon. Heavy metals presences in sediments are important indicators of pollution status of an aquatic environment.

Therefore, this study was carried out to assess the distribution of the selected heavy metals in the sediments along Egbin thermal plant situated at Ikorodu Lagos.

2. Materials And Methods

SAMPLING AREA: Lagos is a densely populated metropolitan city in Nigeria with more than 12 million people. The Lagos lagoon is the largest among other lagoon systems of the Gulf of guinea. The lagoon receives a number of important large rivers namely Yewa, Ogun, Oshun and Ona. It empties into the Atlantic Ocean at Lagos harbor. The brackish water lagoon surrounding the Lagos Island generally located between longitude 3^0 10' and 3^0 4' SE and latitude 6^0 5' and 6^0 36' N. The estimated area of the main body of the lagoon is 150.56Km^2 (Ajao *et. al* 1996). The area north of the lagoon is not well provided with road network so many communities within; traditionally rely on water as means of transportation.

The lagoon is highly polluted by urban and industrial wastes which posed a threat to the large population depending on it for potable and recreational water, as well as source of cheap and affordable protein. Six sampling stations along North of Lagos lagoon starting from Egbin thermal plant

outlet toward the West were chosen for the entire study. The stations were mapped out from the point of heated water effluent from the thermal plant toward the West. The sampling was carried out between February and April 2010.

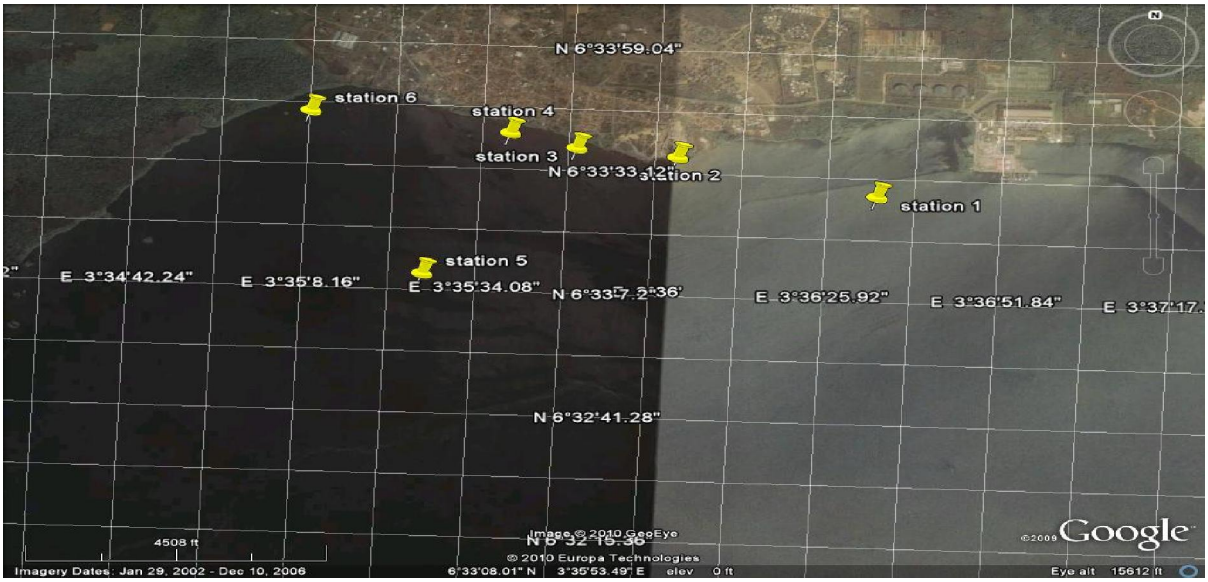


Figure 1: Satellite imagery of the study sites
Source: Google earth imageries

SAMPLE COLLECTION AND ANLYSIS:

Table 1: Positions for stations

Stations	Coordinates
Station 1	06° 33'26".7N, 03° 36' 32.6" E
Station 2	06° 33'33".9N, 03° 36' 02.6" E
Station 3	06° 33'35".3N, 03° 35' 47.5" E
Station 4	06° 33'38".1N, 03° 35' 37.5" E
Station 5	06° 33'38".0N, 03° 35' 25.4" E
Station 6	06° 33'41".7N, 03° 35' 07.6" E

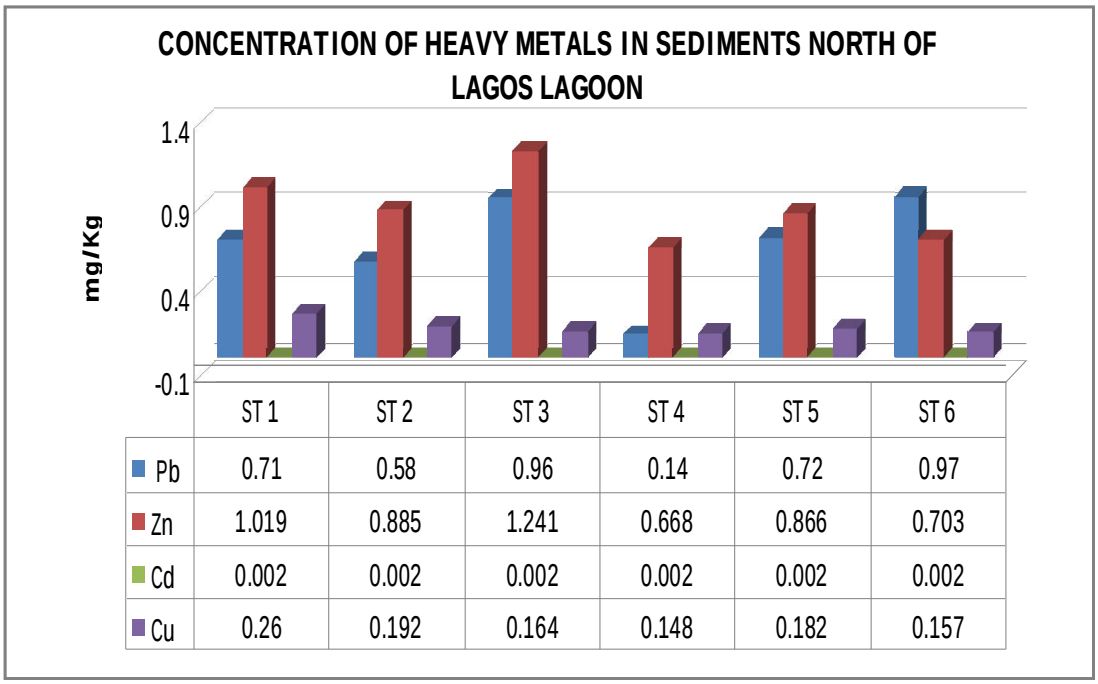
Sediments were collected with the aid of Van Veen grab at each station and stored immediately in polythene bag. Sediments collected were stored at 4° C in an ice-box and transported to the laboratory.

The entire samples were separately air dried in a laboratory. When dried, it was homogenized and sieved to remove big particulates. Homogenated sediment samples were then digested as follows: 5g of the powdered sediment samples were weighed into a 100 ml beaker. 15ml of freshly prepared mixture of HNO_3 / H_2O_2 ratio 1:1 were added to each sample and covered with a wash glass. It was allowed to stand for 30 minutes during which initial reaction subsided. . Digestion was carried out on hot plate whose temperature was allowed to rise gradually until it reached a maximum temperature of 160°C in a fume cupboard. Heating was continued for about 2 hours, reducing the volume in the beaker to about 2 - 5ml. The beaker and its contents were allowed to cool and the content was transferred with whatman filtration into a 50ml volumetric flask and made up to mark with distilled water (FAO/SIDA, 2003). The digested samples were then analyzed for Pb, Fe, Zn, Cu and Cd using a Flame Atomic Absorption Spectrophotometer model Varian SpectAA 400 plus AAS with aqueous calibration standard prepared from the stock standard solutions of the respective elements.

3. Reagents

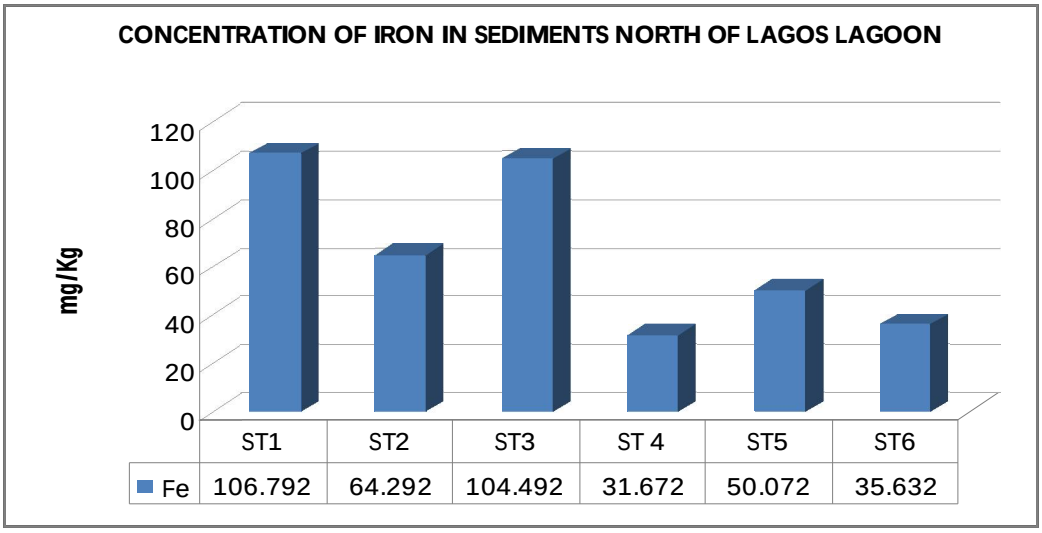
All chemicals and reagents used were of analytical grade and of highest purity possible.

Chart 2: Distribution and concentration of Heavy Metals in sediments North of Lagos Lagoon



	ST 1	ST 2	ST 3	ST 4	ST 5	ST 6
Pb	0.71	0.58	0.96	0.14	0.72	0.97
Zn	1.019	0.885	1.241	0.668	0.866	0.703
Cd	0.002	0.002	0.002	0.002	0.002	0.002
Cu	0.26	0.192	0.164	0.148	0.182	0.157

Chart 3: Distribution and concentration of Iron in sediments North of Lagos Lagoon



	ST1	ST2	ST3	ST 4	ST5	ST6
Fe	106.792	64.292	104.492	31.672	50.072	35.632

4. Result and Discussion

The heavy metal concentration in the sediments along Egbin thermal plant situated at Ikorudu, Lagos Nigeria is presented in chart 2 and 3. The concentration of the heavy metals studied was generally higher than standard set for marine environment except for Cadmium. The concentration of Fe was highest in all the stations investigated ranging from the minimum of 31.672 mg/Kg at station 4 and maximum of 106.972 mg/Kg at station 1. This is expected as previous results have shown that Fe level concentration is higher in Nigeria soil (Aderinola et al 2009). The result showed that Station 1 has the highest concentration of Fe 106.973mg/Kg and Cu 0.260mg/Kg may be as a result of receiving large volume of heated water which increase settling rate of suspended particles. It was revealed that some toxic pollutant likes Cu, Cd, As and Cl are present in heated effluents which makes water unsuitable for use. (Bhatia S. C., 2009). It was discovered that

station 4 recorded the lowest concentration of Pb, Fe, Zn, Cu, 0.140 mg/Kg, 31.672 mg/kg, 0.668 mg/kg and 0.140 mg/kg respectively. This may be due to the fact that the station is farther away from the Egbin thermal plant and other industries situated around Ikorudu axis. Cadmium was below the detection limit in any of the stations.

The result revealed the heavy metal distribution in sediment to be Fe>>>Zn>Pb>Cu. This observed trend clearly reveals sediment absorbs and accumulates toxic pollutants waste discharged into it. It has been shown that sediment permit the detection of heavy metals that may be either absent or in low concentration in the water column. The value obtained for Zn, Pb and Cu in this report is in line with the report of Aderionla (2009) who obtained 0.730±0.337 mg/kg, 0.450±0.598 mg/kg and 0.600±0.272 mg/kg respectively from Lagos lagoon Nigeria. This report is in contrary with what Nubi et al (2009) who reported Zn, Pb, Cu concentration of 77.13 mg/Kg, 19.14 mg/Kg, 11.62 mg/Kg at dumping site, Ibadan and also Unyimadu et al (2004) reported Zn, Pb concentration of 50.23±13.93, 78.76±23.23 in sediment from Lagos lagoon, Nigeria which are higher. The result showed that concentration of all the heavy metals studied were highest in station 1, followed by station 3, station 2, station 5, station 6 and station 4.

5. Conclusion

The result of this study reveals the adverse health implication the people in the study area could be exposed to, who are generally depending on this water bodies for recreational purpose, potable water and sources of cheap avoidable protein fish. Periodic monitoring of these metals in both sediment and aquatic organism to ensure safety is advocated.

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Limonene dominates the Phytochemistry of *Trigonella foenum-graceum* in Nature

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Abstract: Many plant families have some unique medicinal properties due to the presence of special chemical molecules in different parts of plants also known as "phytofoods." Himalaya is one of the greatest mountain chains in the world which not only supports a vast variety of plants and animals but also the great diversity of traditional phytofoods. Many of these phytofoods are very vital to humans in different diseases also. In this paper, we have crucially analyzed some of the major volatile compounds and anti-microbial activities of *Trigonella foenum-graceum*. By the help of our gc-ms data, we are reporting the dominance of limonene (82.30%), d-carvone (12.97%) and n-caproaldehyde (1.87%) in this plant. [Kamal Kishore Pande, Lata Pande, Bharat Pande, Atul Pujari, Pankaj Sah and Stuti Sah. Limonene Dominates the Phytochemistry of Trigonella foenum-graceum. Nature and Science 2011;9(5):17-20]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Phytofoods, Herbal medicine, Himalaya, Limonene

1. Introduction:

Many plants of the Central Himalayan region have found use as indigenous medicines. Different parts of these plants have different properties and researchers are analyzing the chemical composition of the plant part which is traditionally known to have medicinal properties and used in food in daily lives. Such edible parts of the medicinal plant are commonly known as *phytofoods*. One such important Indian Himalayan phytofood is *Trigonella foenum*-graceum.

Earlier literature reports have revealed the presence of biologically active and novel compounds in these foods. In this paper we have thoroughly analyzed the chemical composition and anti-microbial activity of volatile compounds found in *Trigonella foenum*-graceum.

2. Materials and Methods

The essential oil was obtained by steam distillation of fresh plant material (500 g) using a copper still fitted with spiral glass condensers. The distillate was saturated with NaCl and extracted with hexane. The hexane extract was dried using anhydrous sodium sulphate and the solvent was removed with a rotovap at reduced pressure and at 25 °C to yield oil. The gc-ms analysis was performed in Agilent 6890 series equipped with MSD 19091S-433 system. It is known that there is a selective loss of low boiling terpenoids by conventional hydro-distillation technique. Headspace-gc is therefore more suitable for quantification of highly volatile compounds than conventional hydro distillation technique.

For the Dynamic Headspace, 1.0 gm of sample was taken in 20 ml headspace vial. Headspace is created and these vapours are injected in GC equipped with MSD. Headspace components were identified by matching their mass spectra with those in NIST 05 MS library search and by comparing with literature reports and GC retention indices (RI).

Determination of antimicrobial activity (minimum inhibitory concentration MIC) of oil extracts by two-fold serial dilution method. The test organisms; bacterial and fungal cultures (*Staphylococcus aureus* (S.a), *Escherichia coli* (E.c), *Cndida albicans* (C.a) and *Cryptococcus neoformans* (C.n.) were obtained from Central Drug Research Institute, Lucknow and maintained as well as experimented in the Microbiology laboratory, Department of Biotechnology, M.B. Govt. P.G. College, Haldwani (Nainital).

Trigonella foenum-graecum:

Trigonella foenum-graecum seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an anti diabetic effect. The seed and leaves

are anti-cholesterolemic, anti-inflammatory, antitumor and hypoglycaemic. The main components of the oil were found to be [delta]-cadinene (27.6%), [α]-cadinol (12.1%). GC analysis of oil identified palmitic acid, linoleic acid oleic acid and stearic acid. Headspace analysis shows prominent presence of (hexanal, 2-methyl-2-butenal, 3-octen-2-one). *Trigonella foenum-graecum* was evaluated for their antifungal and antibacterial activity against *Aspergillus niger, and Staphylococcus aureus.*

3. Result and discussion:

Trigonella foenum

Present gc-ms study also reveals the presence of fatty acid. The volatile component Palmidrol (28.72%) which is an antiviral, antiinflammatory and nonsteroidal analgesic is as major bioactive constituent. Present dynamic headspace gc-ms of *trigonella foenum* claim the dominance of limonene (82.30%), d-carvone (12.97%) and n-caproaldehyde (1.87%) which are being reported first time.

Result and discussion:

Chemical Investigation

Trigonella foenum - graceum (Family:Fabacease)

GC-MS Analysis of Essential Oil

The seeds of *Trigonella foenum* yielded 0.2% by weight of *yellow* oil with a pleasant aroma. GC and GC-MS analysis of the essential oil from the seeds of *Trigonella foenum* revealed the presence of seven major compounds and all of them were identified by comparing their mass spectra with MS library. The oil is rich in Fatty acids. The fatty acids, monoterpinene hydrocarbon, pthlates and oxygenated monoterpene amounted to 53.19%, 21.92%, 15.03% and 9.85% respectively. The major constituents are 28.72% palmidrol, 24.47% octanamide n-(2-hydroxyethyl), 15.03% dioctyl phthalate, 14.58% d-limonene, 9.85% 1-carvone, 4.31% o-cymene and 3.03% γ-terpinene. **(Table 1, Figure 1).**

3.1 Dynamic Headspace GC-MS Analysis

The seeds of fresh *Trigonella foenum - graceum* were shade dried and crushed to powder. One gram of powdered material is taken for dynamic headspace gc-ms analysis. Head space (hs) gc-ms of *Trigonella foenum - graceum* revealed the presence of seven volatile organic components and all of them were identified by comparing their mass spectra with ms library. The dominant component of the oil is a monoterpene hydrocarbon. The monoterpene hydrocarbons, oxygenated monoterpenes, aliphatic aldehyde, hetrocyclic compounds and alcohols amounted to 82.57%, 13.34%, 1.87%, 1.82% and 0.4% respectively. The major constituents are 82.30% limonene, 12.97% d-carvone, 1.87% n-caproaldehyde, 1.82% 2-amylfuran and 0.40% 2,3-butanediol. **(**Table 2, Figure 2).

Table 1: Chemical composition (%) of essential oil of *Trigonella foenum – graceum* (Seed)

Pk	RT	Area%	Common Name	Method
1	12.305	4.31	o-cymene	a,b
2	12.41	14.58	d-limonene	a,b
3	13.453	3.03	γ-terpinene	a,b
4	19.299	9.85	1-carvone	a,b
5	39.222	28.72	palmidrol	a,b
6	42.725	24.47	octanamide, n-(2-hydroxyethyl)-	a,b
7	45.965	15.03	dioctyl phthalate	a,b

a = Retention Index; b = MS (GC-MS) Library

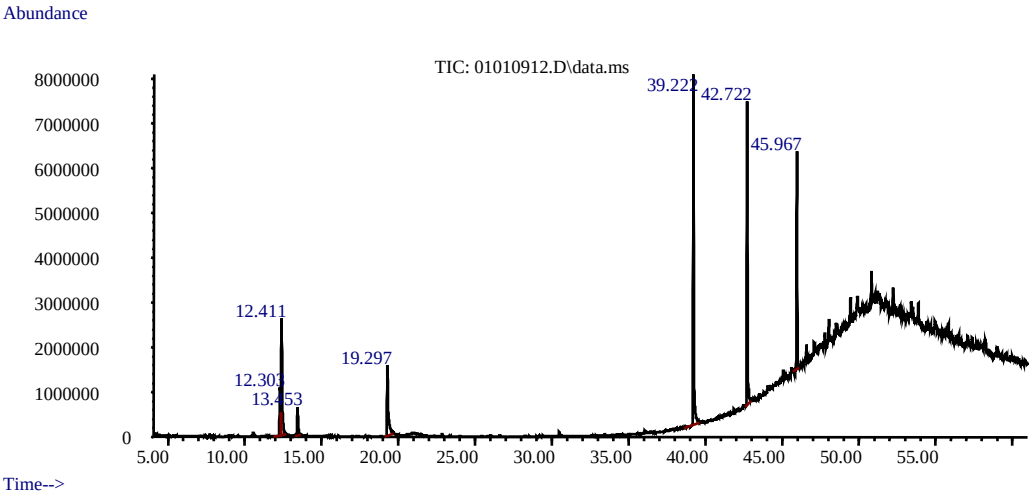

Figure 1: GC of essential oil of *Trigonella foenum – graceum* (Seed).

Table 2: Chemical composition (%) of *Trigonella foenum– graceum* (seed) by dynamic hs/gc-ms.

Pk	RT	Area %	Common Name	Method of Identification
3.	4.576	1.87	n-caproaldehyde	a,b
4.	4.966	0.40	2,3-butanediol	a,b
5.	10.725	1.82	2-amylfuran	a,b
6.	12.258	82.30	limonene	a,b
7.	12.951	0.27	γ- terpinene	a,b
8.	17.329	0.37	trans-dihydrocarvone	a,b
9.	18.838	12.97	d-carvone	a,b

a = Retention Index; b = MS (GC-MS) Library

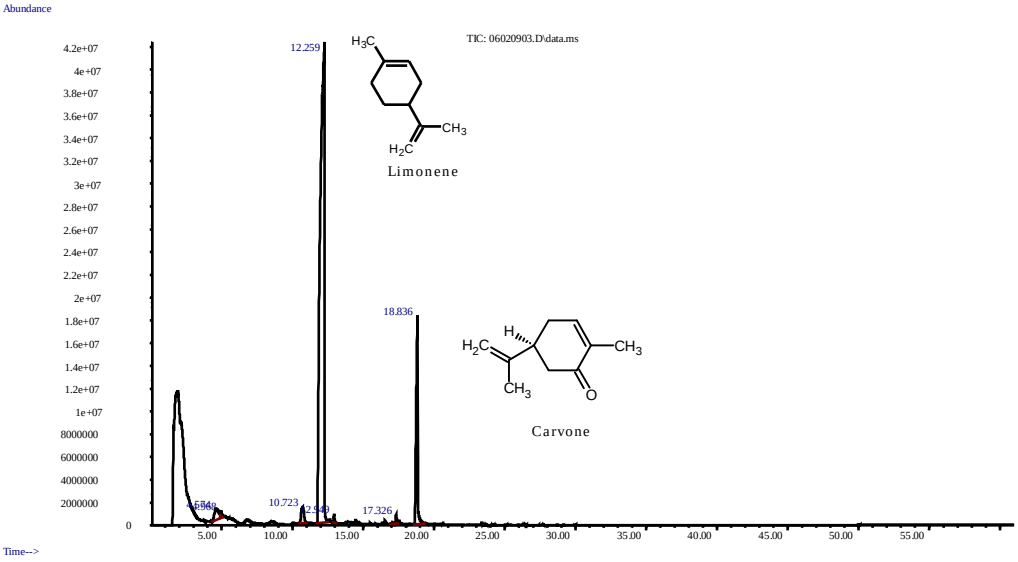

Figure 2: Dynamic headspace gc of essential oil of *Trigonella foenum – graceum* (seed).

4. Discussion

Mazza, G. *et al.* has reported over 50 volatile compounds in *Trigonella foenum* [1]. Pritee wagh observed gc analysis of oil and identified hexadecanoic acid methyl ester (palmitic acid), 9, 12-octadecadienoic acid methyl ester (linoleic acid) 9-octadecenoic acid methyl ester (oleic acid) and octadecanoic acid methyl ester (stearic acid) [2]. Present gc-ms study also reveals the presence of fatty acid. The volatile component palmidrol**,** (28.72%) which is an antiviral, anti-inflammatory and non steroidal analgesic is as major bioactive constituent. Mazza, G. *et al.* reported prominent presence of carbonyl compounds in headspace analysis [3]. In present headspace dynamic gc-ms of *Trigonella foenum* claim the dominance of limonene (82.30%), d-carvone (12.97%) and n-caproaldehyde (1.87%), which are being reported first time in the seeds of *Trigonella foenum.*

3/17/2011

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Some social factors Related to level of Environmental health Awareness in Rural Egypt

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Abstract: The research aimed to identify the impact of some social factors in age, educational level, family size, the degree of cultural openness- communication, and economic level to the level of environmental health awareness of the respondents. In addition to identifying the most important programs from which to create a clean environment conducive to increase productivity and per capita income, and then the advancement of society economically, and the achievement of social welfare for members of the rural community. The results showed that the mean scores for level of environmental health awareness by the respondents is estimated at 78.4 degrees of kidney estimated 1593 degrees, which reflects the low level of health behavior and health practices that can maintain the health of the individual and the environment. As it turns out; there is a significant correlation between the age Category, educational level, family size, level of education - communication (independent variables) and level of Environmental health awareness (dependent variable). Also found that about 62.7% of the respondents engaged in basic agriculture as a profession, while 37.3% engaged in work other than farming as a career major going about them at the side to work as an agricultural high school. The study recommended the need to work to raise the economic level and living standards of rural households, and interest in environmental health and dissemination of health education and environmental awareness among the population of the rural sector, as well as concern for the individual and the family environment and provide the necessary health to protect them from the face of dangers and diseases.

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Keywords: health awareness, cultural openness - communication, Education standard, health Education, mass Information.

Introduction:

It adopts the well-being and prosperity of the country and its recovery economically and socially to the extent to which their people health, which depends upon its production and efficiency in various fields. The environment is the most important factors affecting the health and illness of the individual. The environment is not conducive to the spread of health and other communicable disease. It is also poverty, malnutrition, housing bad, fatigue overload, psychological crises, lack of awareness, and unhealthy behavior of the most important factors leading to the occurrence of disease and mortality. In spite of the multiplicity of the views of the expert's meeting and the economy and the environment on the problems of population and the extent of the reflection of population growth on the path sustainable development, but they agreed that the population problem and its relationship to consumption and production, environment and development can not be ignored or negative stance towards it (14-1994).

The environment is an integral and important component of the health program of the community. In fact, is a program of Environmental Health Sanitation of the most important factors that lead to low prevalence of disease and reduce health problems. The environment around which to live, affected by and affects the environmental factors in this area include the following: family environment and is in the size of their resources and capabilities, the home environment and is in housing, ventilation and safe drinking water and a means of disposal of human excreta and animal litter, community environment in which they live in rights, which are represented in the provision of services, transportation, and housing health.

Research problem: represent damage caused by the environmental pollution barrier in order to achieve comprehensive development continued, as a result of wasting the wealth of natural and human, which affect the national income. There is no doubt that the individual affects the environment in which they live and influenced by them. Statistics show that 56% of the population living in the Egyptian countryside, which suffers from many problems, is reflected on the individual and society (3-1994). The community is still rural and the Egyptian village suffering from social and economic problems and much health, although directing care for this sector. Some studies suggest as to the lack of programs, and services directed to the rural sector, which increased the problems of health and the environment in this important sector (9: 1994).

The research aims: The research aims to identify:
1 - Some social factors of the respondents in terms of age, educational level, family size, openness, cultural communication, the economic level of the respondents (independent variables)
2 - Level of health awareness - the environmental category of respondents in terms of the level of healthy behavior and good health practices to maintain the health of the environment (dependent variable) with regard to the state of health to housing and health habits of respondents and their families.
3 - To study the correlation between the level of health awareness - in the category of environmental subjects and a group of independent variables.

Research method: the method involves research to clarify the concepts for each of the research variables, research, and research as well as assumptions that the research and finally a method of data collection and analysis of primary research.

First: the concepts of research:
Age of respondents:

There is a close relationship between age class of the rural population and the degree of health awareness to control environmental pollution in the environment. The age of senior farmers used to practice certain customs and traditions, which would damage health and the environment and cause pollution. It is intended that the variable in this category search age at the time of research estimate years to the nearest year.

Education Level:

There is a relationship between the degree of education and health awareness of rural families. The spread of literacy lead to the low level of health awareness and lack of attention to disease prevention or delay of treatment (9:1994). This means variable to know the educational status of quested at time of search terms were illiterate or literate or of any stage of formal education, were expressed in numeric values.

The size of the family:

The family is the basic unit to be the structure of society, and measured the strength or weakness of society as a whole strongly or weakness of the family of its constituent. The family is the field, who practiced human society in which social relationships, a tree which is planted in each of its members, traditions and customs, ideals and behavior patterns different. Family size intended in this study means all individuals which have dependents and live Category social and economic one.

The degree of cultural openness - communication: Means to communicate urban degree to an individual outside the social system (23:1969), include mass media such as radio, television, newspapers, magazines, flyers, brochures, health and used for the dissemination of culture and awareness of environmental health among the people and good practices to maintain the health of the individual and the environment. Moreover, one of the ways of health education, which also is including film and video cassette tapes. And intended openness cultural - communication over the exposure of respondents to the elements of culture, immaterial and material in terms of the extent to which respondents to the mass communication represented in radio and television, newspapers and magazines, as well as the degree of frequency of respondents in urban areas and the purpose of the frequency it has been the expression of this variable with numeric values.

Economic level - living conditions: The economic level as well as education and family size of the factors affecting the health problems (3:1994). And can be expressed in numeric values of this variable in terms of home ownership, fashionable home rations, which were acquired by Category in the home, the diversity of sources of income Category, Category average income compared with an average income of the rest of the family in the village.

Level of health awareness - Environmental: The variable's central, the ultimate objective of the research to discover factors associated with it and used as independent variables, to identify the level of behavior for good health, and health practices of the respondents to maintain the health of the environment with regard to the two-dimensional key representatives of the level of health awareness - environmental variable composite includes: health status of the dwelling, the prevailing habits, the behavior of healthy respondents and their families.

The health status of residence: Identify the health status of residence of respondents and their families and existing conditions unhealthy and practices leading to contamination of the environment within the home and attitudes in terms of availability of electricity, clean water, sanitation, ventilation home, housing density, the presence of the fold of cattle inside or outside the home, the degree of accumulation compost, ponds and marshes at the house. Prevailing customs and health behavior: It means the customs that are reflected in the behavior of the subjects and their families in some everyday situations, as well as identify the views of respondents and their attitudes to certain phrases related to personal behavior and environmental health.

With regard to the habits of boys swimming and washing utensils in the canals, and behavior, who is under examination by the injury when a family member for any of the endemic or infectious diseases or other. And to identify the causes that may be responsible for the spread of the disease in the villages, its affiliates, in terms of their point of view, the extent of the complaint from the proliferation of flies and insects, the extent of giving them vaccinations assessed for their children vaccinated subjects and his family when the spread of infectious disease, as well as the opinions of the respondents in connection with the few words in terms of preference over the use of municipal recipes, take care of cleanliness and beauty of the village, the use of rituals and other popular customs prevailing among the people of the countryside.

II: research variables

The independent variables are as follows: age of the respondents, educational level, family size, cultural openness - communication, economic level and living standard of the respondents. While the dependent variable is the level of health awareness - environmental by the respondents in terms of the level of proper health behavior and health practices of respondents to maintain health of the environment.

Third: The research hypothesis

Light of the above research suggests the existence of correlation between the level of health awareness - environmental by the respondents as the dependent variable and each of the independent variables mentioned above. Has been tested this hypothesis in zero image "that there is no relationship between the level of health awareness - environmental by the respondents as the dependent variable and each of the independent variables mentioned above.

IV: sample

The research on primary data collected through the questionnaire achieved the goal of the research. The total sample 150 Quested of the rural population was selected through a random sample of villages (Kafr Ashma, Srsena, Mitt shhala) in ELshohda center, Menofia Government.

Fifth: data collection and analysis

The research data depend on the priority that has been collected through the questionnaire, personal interviews. The questionnaire is designed to achieve the goal of the research. I have been using percentages and averages, and frequency distributions, simple correlation (R) in the data analysis to study the relationship between the dependent variable and independent variables.

Results and discussion:

Social factors, communication and economic development of the respondents

1 - age of the subjects: It is clear from Table (1) that more than half of the respondents located in the age groups representing senior age and who are between the ages of (40 - more than 60 years) 'It numbered about 89 persons whom and by an estimated 59.3% and indicates that the majority of respondents, their customs and traditions associated with Baltcassel and other customs associated with the contribution of increased pollution and Mahafezaly environment. And agree that the result with the findings of some studies in that with increasing age than control subjects in the degree of environmental pollution. Also agrees that the result is the logic of scientific terms get used to top the age of farmers on the exercise of certain customs and traditions that will bring about pollution.

The results of the search indicated that the importance of environmental awareness and related practices and healthy behavior among the rural population in general and the elderly in particular, in order to create a healthy and clean environment. The mean average age of respondents is about 57 years. The results of the estimates that there is significant correlation between age and level of health awareness - environmental by the respondents, in terms of the level of healthy behavior and good health practices to maintain the health of the environment and the estimated value of the simple correlation coefficient (t) about 0.041.

Table (1) The distribution of respondents according to the categories age

categories age	number	%
20-29	25	16.7
30-39	36	24
40-49	43	28.6
50-59	28	18.7
60>	18	12
total	150	100

Source: Compiled and calculated from field research

2 – Education Level: The increasing Educational level of the population a portlets important to extend the person's scientific knowledge related to environmental health, which reflected the absorption and adoption of practices and new ideas, as well as to create trends in personal high standard of behavior for good health, and health practices among them. As can be seen that the individual that receives a share of the education be more responsive to the changing practices of the old and bad habits and adoption of practices that help reduce environmental pollution in order to create a healthy environment clean of pathogens and vectors. It is estimated in Table (2) that more than half of the respondents was illiterate (not Inalo any premium from education), while the percentage which has completed university education to only 2% of the respondents. It is estimated that the mean level of education respondents about 2.5 degrees. As it turns out; there is no significant relationship between a level of education and level of health awareness - environmental by the respondents, with an estimated value of the simple correlation coefficient by about -0.046. The results showed in table (2) the spread of literacy among the respondents as it was found that more than half of the respondents were illiterate (52.7%). Some studies indicate a relationship between the degree of education and health awareness of the rural family, the proliferation of illiteracy leads to low awareness of health and lack of attention to disease prevention or treatment is delayed.

Table (2) The distribution of subjects, according to the educational

Level Education	number	%
illiterate	79	52.7
Reads and writes	39	26
Primary	12	8
Preparatory	9	6
Secondary	8	5.3
College	3	2
total	150	100

Source: Compiled and calculated from field research

3 - **The size of the family:** The family is the basic unit is the structure of society, and measured the strength or weakness of society as a whole strongly or weakness or weakness of its constituent families. The family is the field, who practiced human society in which social relationships, and which instilled in each of its members, traditions and customs, ideals and behavior patterns different.

These results showed the high number of children per family, where the arithmetic average of the number of children 6 son / daughter per household, while the average capacity of family 9 Items per family for a number of individuals living in the same unit of living and live a life of social and economic one, which refers to the congestion of units living to the families of the subjects and the impact on their lives and health. As shown by the presence of Altkadiwat correlation was found between the capacity of family and level of health awareness - environmental by the respondents, in terms of the level of healthy behavior and good health practices to maintain the health of the environment has reached the value of the simple correlation coefficient of about 0.071. According to some studies (3.1994), despite the presence of family planning programs, but the family size is still large and increasing number of family members affecting the food situation and the spread of infectious diseases.

The degree of cultural openness - communication of the respondents: They reflect the degree of exposure of respondents to the mass media and the degree of urban contact them.

A - The degree of exposure the subjects of mass media: The mass media in radio and television, newspapers, magazines, leaflets, booklets and other health. And use such means in the dissemination of culture and health awareness - Environmental between people and health practices to maintain the health of the environment in addition to the cinema and video as one of the ways and methods of health education. The results of table 3 indicated to increase the proportion of respondents who watch television Alpramj (28% always, 50% sometimes), and those who listen to the radio (33.3% always, 43.4% sometimes). He also noted the majority of respondents to the non-reading of newspapers and magazines, with an estimated proportion of about 64%. This may be due to the fact that 78.7% of respondents their educational, ranging from my mother (52.7%) and know how to read and write (26%), reflecting the importance of the media and visual as the main source for disseminating information and awareness of environmental health, especially in rural communities with high illiteracy rate. Therefore, you should take advantage of the

mass media to broadcast the message through scientific health at the appropriate times, either on radio or television. To submit to the listeners in the form of simplified or interesting in the form of an analog light or interesting dialogue in order to attract the largest number of listeners and viewers.

Table (3) The vulnerability of the subjects of mass media

openness communication	Always		Sometimes		Rarely		NO		Total
	N.	%	N.	%	N.	%	N.	%	
Listen to the radio	50	33.3	65	43.4	20	13.3	15	10	150
Watching TV	42	28	75	50	15	10	18	12	150
Read newspapers and magazines	15	10	22	14.7	17	11.3	96	64	150

Source: Compiled and calculated from field research

B - The degree of communication of the urban respondents: It means the degree of frequency of respondents to the nearest city to them and the purpose of the frequency; it is the degree of frequency of respondents to the nearest city to them, a city Shebin. The results showed that 22.7% of respondents attending the city daily, while 18.7% of them go once a week, while about 50.6% indicated they go to the city once a month, while about 8% said they go once a year to the city. The results also indicate the diversity of purposes for which goes respondents to the nearest city to them, it was found that 24.7% go to market crops, 20.7% for treatment, 18% to visit relatives, 12.7% for Labor, 9.3% for entertainment, 8% for the purchase of household items and the needs of the family, 4.6% for the present problems to the officials, 2% for drawn by livestock. In general, the openness of the urban areas is one important and effective factors that contribute significantly to the process of cultural friction and exposure to modern ideas and the correct behaviors to maintain health and avoid the causes of disease and environmental pollution. This has reached the arithmetic average of the degree of cultural openness - communication of respondents to 18.3 degrees. As it turns out there is significant correlation between the cultural level - the level of communication and awareness of health - environmental by the respondents, in terms of the level of healthy behavior and good health practices to maintain the health of the environment. The value of simple correlation coefficient is of about 0.097. This may be due to the fact that the Egyptian village residents are suffering the problems of poverty, ignorance and the spread of bad habits and traditions, in addition to the problems of urban planning of the countryside and the lack of infrastructure services and others. There is no doubt that the information essential role in urging the protection of the environment from pollution through the development of a media plan aimed at increasing awareness of social and environmental good behavior.

Level of Economic living standards: The economic level as well as education and family size of the factors affecting the health problems, despite the clear improvement in the economic level and the relatively high level of income in the Egyptian countryside, but that, in comparison to other developed countries is still at this level is far from the desired level, where affect household income in housing, clothing, nutrition and Edorha that affect the health of the individual. The estimates indicate that the value of the arithmetic average of the degree of the economic level of the respondents amounted to about 22.31 degrees. Also show a significant correlation between the economic level of living and level of health awareness - environmental by the respondents, in terms of the level of behavior Alasahy proper health practices to maintain the health of the environment has reached the value of the simple correlation coefficient of about 0.294. Studies show that a person who enjoys a high standard of living will be more inclined to accept the new ideas of knowledge and information. The results also showed that nearly half of respondents (51.3%) rented houses which they reside, while the percentage of those who own their homes, about 48.7%. As for the availability of devices Alcirbeiip modern has been found available homes subjects, in descending order - Radio, Color TV, TV black and white, recorder, refrigerator, washing machine - a relative importance of an estimated 92%, 66%, 29%, 30%, 26%, 3.5% respectively. As it turns out the diversity of sources of income respondents were ranked according to their relative importance in descending order as follows: Sale of agricultural crops (67.4%), the performance of occupations and the work of other non-agricultural (56.5%), paid work for others to perform some agricultural operations (23.7%), sale of dairy and products (13.4%), sale of livestock (9.4%), poultry (4.6%). He also pointed towards the 64.7% of the subjects that income levels have equal access with the rest of the families in the villages selected a sample of the study, while 30.7% pointed to low levels of income from entering the rest of the families in the same villages, while showing high levels of

entry of about 4.6% of the respondents. This results showed that about 62.7% of the respondents engaged in basic agriculture as a profession, while 37.3% engaged in work other than farming as a career major by going about their work as an agricultural high school.

Level of health awareness - environmental by the respondents: Includes the health status of residence and the prevailing habits and health behavior of the respondents and their families. The results showed a significant correlation only between the economic level of the respondents and the level of health awareness - their environmental potential level at 0.01 where the estimated value of the simple correlation coefficient of about 0.294. As it turns out non-significant correlation between the age Category, educational level, family size, level of cultural communication of the respondents (independent variables) and level of health awareness - Environmental (dependent variable), where the estimated values of simple correlation coefficient about 0.041, -0.046, -0.071, 0.097, respectively.

A - The health situation of the house: The studies on the impact of the state housing and the lack of potential for health, and private housing congestion and the lack of safe drinking water and lack of ventilation and the lack of sanitation. As experts point out that housing is health leads to many diseases Ohmaha: bronchitis, tuberculosis, rheumatic fever, heart disease, osteomalacia and rickets, infectious diseases such as meningitis, and gastroenteritis and typhoid and Albrtevodip. It is estimated availability of electricity to the homes of about 97.3% of respondents, while the available source of clean drinking water houses 68% of them, while suffering 32% of the non-availability of a source of drinking water health. As indicated 83.3% to a lack of means of sewage homes. As for the ventilation housing, he noted 83.3% to provide good ventilation their homes, while referring 16.7% that their homes and poorly ventilated. Also confirmed 30% of respondents to the presence of litter and farmyard manure (animal dung) in large quantities through the streets of the village and adjacent to their homes. As for the site barn animals has indicated 37.3% to the absence of animal shelters their homes, while only a 20% to the presence of animal pens in a building separate from the house, while referring 42.7% to the presence of animal pens inside the house and next to the living rooms. For the damage to human health, animal pens was 60.7% indicated that the presence of barn animals inside the house something normal and not harmful to health, while 39.3% pointed to the detrimental effect of the presence of animal pens inside the house. With regard to the adequacy of the number of rooms the house for a number of family members of respondents has indicated 60% as adequate, while 40% as inadequate. With regard to population density to the homes of the subjects has been shown to rise with an estimated $ 2.2 per person for room one, which shows as Houses health as well as indoor air pollution and the spread of many diseases.

B - Health habits of the respondents and their families: playing habits and traditions, an important role in the health services programs, both seeking to request these services or to refrain them and return to the folk customs in the treatment of hand, and hold on to some customs and traditions in the individual's behavior, which directly affect public health on the other. The results indicate the failure of the 97.3% of the wives or daughters of respondents wash pots in the water canals, while 93.3% said the failure of their children to practice swimming in the canals. At the same time, who pointed out the 84.7% not wash their equipment and tools for agricultural pesticide spraying canals and irrigation canals, while 15.3% have it in many cases, leading to increased pollution and disease. As for the knowledge of the subjects most prevalent diseases among the rural population of Egypt, the results showed that the most prevalent diseases in accordance with the views of respondents and their Distributions frequency in descending order as follows: schistosomiasis (87.3%), Ascaris (34.7%), conjunctivitis (18%), the common intestinal and diarrhea for children (14%), malaria (11.3%), hookworm (8.7%, anemia (7.4%), kidney disease (5.7%), arthritis (1%). and returns for these diseases to bad habits and healthy behavior is proper and non-availability of environmental awareness and adequate health care. For the views of respondents on how to obtain advice and treatment where the incidence of any disease have been ordering them and conformable to the distributions of iterative go to: a private doctor in the village (44.7%), Health Unit (32.7%), a private doctor in the city (22%) , General Hospital (20.7%), prescriptions Municipal (6.7%). As for the complaint of respondents from the large number of insects and flies, villages have indicated that, 90.7% to the spread of insects and flies, their homes and villages. Regarding the reasons for the spread of diseases in the villages of the study sample has been arranged for those reasons descending order as the following: the spread of insects and mice (70.7%), lack of hygiene and neglect (52%), lack of health awareness (26.7%), housing is not healthy (12.7%), the use of waste water for irrigation (5.3%), the spread of garbage (2%). With regard to giving children vaccinations health assessments, he pointed

to the 96.7% they vaccinate their children. also confirmed 92% of the respondents to do any vaccination against infectious disease in the case of an outbreak, and in connection with the opinions of respondents in certain phrases related to health awareness and habits, it is clear from the table (4) the approval of 99.3% of the respondents on the need for attention to cleanliness of the village and beauty, while noting 54.7% to their washing hands before eating, while stressing 90.7% on their approval of the proverbial "man doctor himself," as noted by 33.3% to preference use recipes municipality to go to the doctor, which may be due to a culture of success in non-attendance at places of treatment for regular check-ups to make sure the integrity of health, so do not go to the health unit or doctor If your only intensified by the disease.

Table (4) The views of respondents in some of the phrases associated with health awareness and habits

Phrases related to health awareness and habits of the subjects	OK		sometimes		not OK	
	N.	%	N.	%	N.	%
- Needed care and cleanliness of the village beauty	149	99.3	1	0.7	-	-
- Must wash hands before eating	68	45.3	82	54.7	-	-
- Man is himself a doctor	136	90.7	5	3.3	9	6
- Better to use the recipes for the municipality to go to a doctor	50	33.3	30	20	70	46.7
- Do you think that Zar alleviate some diseases	11	7.3	5	3.4	134	89.3
- Is a remedy for every disease	138	92	5	3.3	7	4.7
- Must wash hands / mouth after eating	68	45.3	82	54.7	-	-
- To be washing dishes and cooking Unni after eating immediately with soap and water	106	70.7	44	29.3	-	-

Source: Compiled and calculated from field research

Conclusion: The results of research to the low level of health behavior and health practices that can maintain the health of the individual and the environment, and to achieve the advancement of society economically and socially. Therefore, the research recommends the need to work to raise the economic level and living standards of rural families through the establishment of small-scale production and to encourage projects of productive families and attention to health, the environment and the dissemination of health education and environmental awareness among the population of the rural sector, as well as concern for the individual and the family and provide a healthy environment - clean water, clean housing, garbage disposal - necessary to protect them from the face of dangers and diseases, and work to increase awareness of the behavioral to the public about hygiene and environmental improvement health, in addition to the establishment of rural industries, especially related to rotate the waste.

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Cultural control of elm bark beetle, *Scolytus kashmirensis* Schedl (Coleoptera: Scolytidae) infesting elm trees (*Ulmus* spp.) in Kashmir

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Abstract- Cultural control was carried out against the *Scolytus kashmirensis*, the shot-hole borer and the fungal vector (*Ophiostoma ulmi*) of Dutch elm disease of elm trees (*Ulmus* spp.) in Kashmir. Seasonal pruning, sanitation and removal of brood trees was done to assess the effectiveness of the cultural control in relation to the borer, *Scolytus kashmirensis*. Seasonal pruning reduced infestation of *S. kashmirensis* significantly; spring and autumn pruning reduced it by 2.33% and 63.67% respectively. Sanitation reduced the borer infestation rate by 61.02% and 63.49% in two treated elm plots. Removal of brood trees reduced the infestation rate by 42.41%. *Ulmus villosa* though prone to their attack showed slight resistance as compared to the *U. Wallichiana*. Experiments assessing the significance of these processes are presented in this paper.
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Keywords: *Scolytus kashmirensis*; *Ophiostoma ulmi*; *Ulmus* spp; Pruning; Sanitation; Brood trees.

Introduction:

Bark beetles (Coleoptera :Scolytidae) are of great economic importance to forestry and horticulture in the temperate climatic zones including the Valley of Kashmir. Elm trees in poor physiological conditions are often attacked by species of the genus *Scolytus* (Coleoptera: Scolytidae) which, although they are secondary pests, are a major cause of trees decay. Dutch Elm Disease (DED) (Gibbs and Brasier, 1973), caused by the fungus *Ophiostoma ulmi* (Schwarz) Nannfeltd [=*Ceratocystis ulmi* (Buisman) C. Moreau], is one of the most destructive plant diseases to affect elm trees (Brasier, 1991).

Ulmus wallichiana is the host of elm bark beetle, *Scolytus kashmirensis* Schedl (Schedl, 1957). *S. kashmirensis* is the common shot-hole borer on elm trees in Kashmir Valley. It acts as a vector of *O. himal ulmi*, the Dutch elm disease pathogen only in the Himalayas (Brasier and Mehrotra, 1995).

Although the disease may be transmitted in several ways (Schwarz, 1922., Smucker, 1935), insects are the best fungal vectors (Marchel, 1927; Jacot, 1934; 1936; Collins *et al.*, 1936). In particular, the elm bark beetles belonging to the genus *Scolytus* Geoffroy (Coleoptera: Scolytidae) have been demonstrated to be the most efficient vector of the fungal spores (Gibbs, 1974; Sengonca and Leisse, 1984; Webber and Brasier, 1984; Neumann and Minko, 1985; Webber, 1990; Basset *et al.*, 1992; Favaro and Battisti, 1993; Battisti *et al.*, 1994a; Faccoli and Battisti, 1997). In spring, young beetle adults emerging from dead elms fly towards the top of healthy elms for maturation feeding on the crotches young twigs (Gibbs, 1974; Webber and Brassier, 1984). The feeding activity carried by the infected beetles may cause the contamination of the host tissues and the consequent development and diffusion of the fungus within the xylem and vessels. Later beetles move to the trunk of elms attacked on twig crotches the previous year. Here the inner bark provides ideal breeding material on which larvae can develop (Parker et al., 1941). The bark also becomes contaminated with the spores of *O. ulmi* carried by infected beetles when breeding galleries are excavated. The maternal galleries are an ideal micro-environment both for the growth and sporulation of the fungus (Webber and Brassier, 1984). Losses caused by the beetles are not confined to feeding activities alone but also intensifies by disseminating disease pathogens. Their population increases rapidly when there is abundance of decadent tree, wind fall and weakened tree due to water, diseases, nutrients or salt stresses (Wood, 1982).The distribution of bark beetles is largely determined by the distribution and abundance of their host tree species and climate (Lekander et al., 1977). The older, taller elms are preferred for feeding by the bark beetles and therefore much more likely to become diseased compared with younger trees (Sengonca and Leisse, 1984).

The trees infested by the bark beetles may be recognized at a distance by fading foliage of the tree, initially a light green then changing to a light straw colour in a few weeks, and eventually to yellowish-brown. Close inspection may show a fine reddish-

brown boring dust in bark cervices and at the base of the tree (Webber, 1990).

The objective of the present study was to assess the effectiveness of the cultural control in relation to the elm borer, *Scolytus kashmirensis.*

Materials and Methods:

The cultural investigation was carried out from the study areas at Anantnag, Shopian, Baramullah and Ganderbal during the 2009-10.Cultural control was executed by the following methods:

Pruning:

Spring and Autumn pruning were made to investigate its impact on the infestation rate of the shot-hole borer among elm plants.

Sanitation:

It involved the prompt removal and disposal of dead and dying elms to reduce bark beetle breeding sites. The barked elm wood, leaves, twigs were completely disposed off along with their harboring beetles at two sites/locations during the present study in Autumn, 2009. The infestation rate was compared with the control site in the following season.

Removal of brood trees:

It involved the removal of brood trees (unrecoverable-infested trees) followed by their destruction along with harboring grubs. A small proportion of infested trees were sacrificed during the present study in spring and autumn of 2009. Brood trees were removed in two elm nurseries at two sites/locations and the infestation rate was compared with the control plot/site in the next season.

Data analysis:

The observations made during the current study were tabulated and graphically presented. The data was statistically analyzed by different methods. Arithmetic mean± SE (Standard error of mean) and Chi square (X^2) test were used to analyze the data. The means were compared by Student's t-test and the values were considered significant at $P \leq 0.05$.

Results:

Cultural control encompasses all those practices that aim at reducing the pest infestation through the manipulation of regular farm practices. Cultural management practices, viz., seasonal pruning, sanitation, and removal of brood trees were evaluated against *S. kashmirensis*.

Seasonal pruning:

Autumn pruning reduced the borer infestation rate significantly ($P<0.05$) while as spring pruning gave insignificant results ($P>0.05$) (Table 1). Spring pruning reduced the infestation rate of elm shot-hole borer in the next generation by 2.33% as compared to the control plots, whereas autumn pruning reduced it by 63.67% (Figure 1).

Table 1: Effect of Seasonal pruning on infestation rate of *S. kashmirensis*.

Pruning season	No. of sampled trees	% infestation in following spring	% reduction over control	t- value
Spring	50	19.43±1.00	2.33	0.40
Autumn	50	6.73±0.67	63.67	15.00
Control	50	19.66±0.76	0.00	0.00

Pruning is a usual farm practice which involves the removal of infested primary branches. The beetle under study deposited its eggs in the primary branches and the newly hatched grubs made their way into the main stem of the infested trees through the soft pith of these branches. Pruning prevented the newly hatched grubs to colonize in the main stem of elm trees as their earlier instars harbouring the primary branches were destroyed along with the pruned branches.

Sanitation:

Sanitation in two elm plots/nurseries reduced the borer infestation rate by 61.02% in I plot and 63.49% in II plot as compared to control plot/ nursery. (Table 2). Reduction in the borer infestation rate over control was ascribed to the sanitation of elm plots/nurseries (Figure 2).

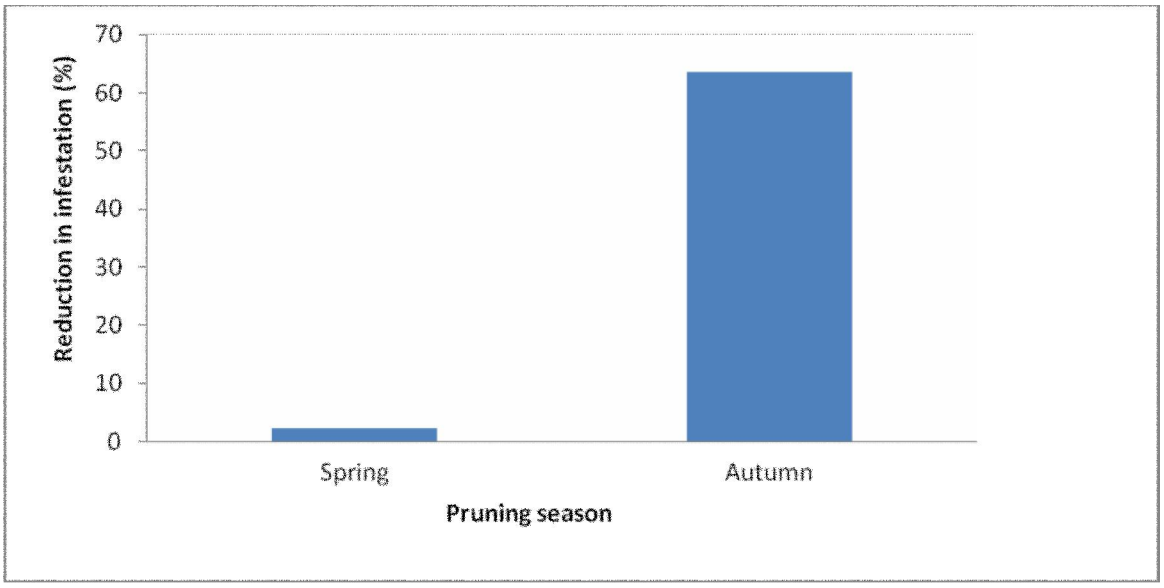

Figure 1: Effect of seasonal pruning on infestation rate of *S. kashmirensis*.

Table 2: Effect of sanitation on elm plots/nurseries.

Treatment in elm plots	No. of trees ascribed to sanitation	Infestation rate (%) in following spring	% reduction over control	t-value
I	100	8.77±0.68	61.02	15.33
II	100	7.55±0.57	63.49	16.00
Control	100	10.43±0.58	0.00	0.00

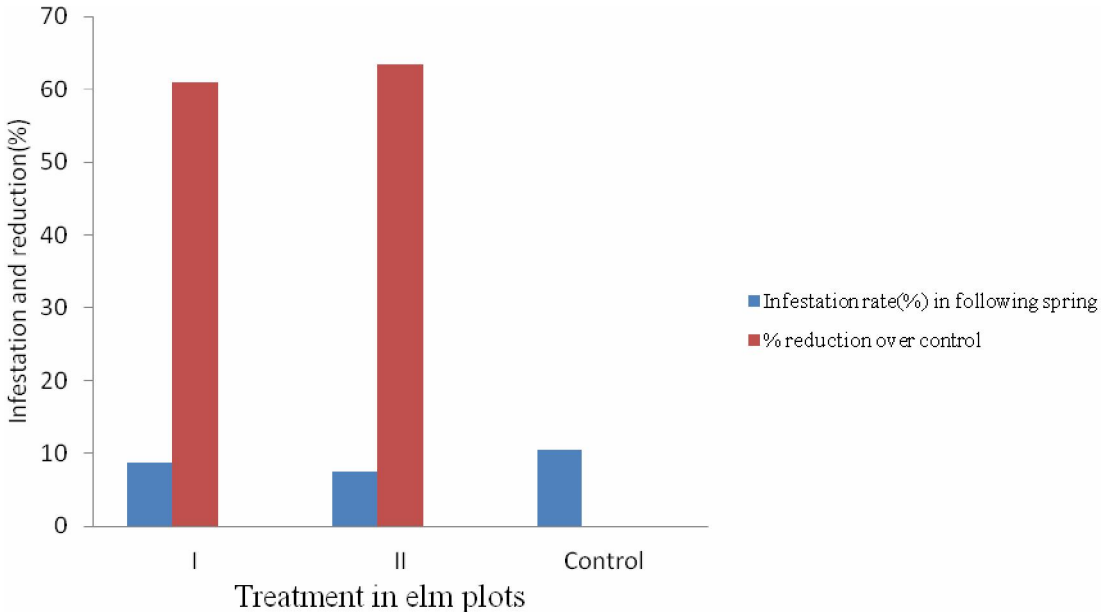

Figure 2: Control of *S. kashmirensis* by sanitation.

Removal of brood trees:

The infestation rate of the borer was reduced in the elm nurseries/ plots significantly (P<0.05) (Table 3) by the removal of brood/ infested trees. During the study period, in the treatment plots the borer infestation rate was reduced by 42.41% while as in control plot infestation rate increased by 5.45%, however, the latter is statistically insignificant (P>0.05).

First treatment involved the removal of 10.33% infested trees and reduced the infestation rate by 17.30%, while as in second treatment 11.55% infested trees were sacrificed which resulted in the reduction of borer attack by 27.31%. A total of 21.88% infested trees were removed and the harboring grubs were killed which in turn resulted in the failure of shot-hole borer populations to regain pretreatment densities which resulted in curtailed mating and subsequent egg laying and finally reduced infestation rate (Figure 3).

Table 3: Efficacy of brood tree removal against *S. kashmirensis*.

Treatment	Pre-treatment infestation (%;mean±SE)	Post-treatment infestation (%;mean±SE)	Reduction over previous generation (%)	t-value
I	20.65±0.62	17.35±0.30	17.30	5.16
II	17.37±0.30	13.29±0.60	27.31	7.43
Control	19.44±0.40	20.50±1.20	-5.45	0.83

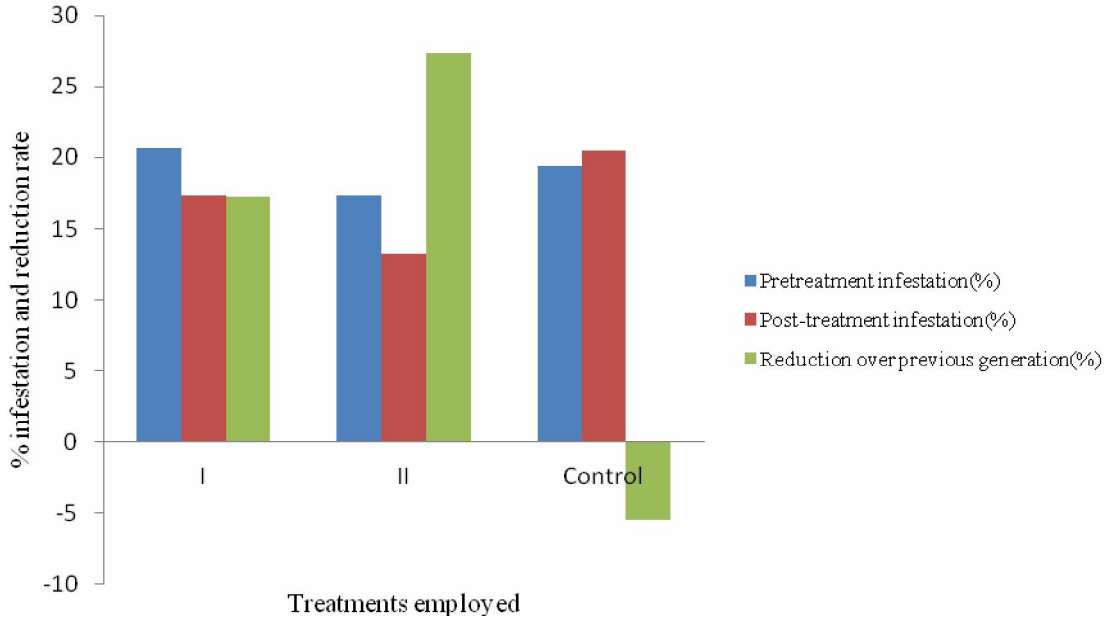

Figure 3: Effect of brood tree removal against *S. kashmirensis*.

Discussion:

Bark beetles are distributed worldwide occupying a wide range of niches on woody and herbaceous plants. The species of genus *Scolytus* (Coleoptera: Scolytidae) attack elm trees (*Ulmus* sp.) in poor physiological condition and are a major cause of tree's decay. Most species of the family are polyphagous causing wide spread mortality among host tree species (Craighead,1950).

Pruning of trees is a cultural operation, an economical tool employed in integrated pest management of perennial plants. The pruning cut for the removal of the branch should be made approximately 10′ behind the point at which healthy wood is first observed (Lanier, 1988). Wounding trees

by pruning will attract the bark beetle vectors of Dutch elm disease (Byers et al., 1980).

The findings of the current study is at par with that of the Lanier (1988) who suggested that ideally, routine pruning should be done in the dormant season or should be restricted to the periods of beetle inactivity, and of Sanborn (1996) who recommended that elm trees should not be pruned from March to September. Spring and Autumn pruning reduced infestation rate of the elm borer by 2.33% and 63.67% respectively.Autumn pruning prevented the elm trees from the borer infestation by destroying the harboring grounds of overwintering larvae along with the pruned branches, thus restricting the infestation in the next season. Spring pruning could not prevent the elm plants from the borer infestation as the twigs sprouted from the spring pruned plants are the preferred oviposition sites for elm shot-hole borer.

Pruning in the management of *S. kashmirensis*, the shot-hole borer under study is appealing for several reasons viz., reduced the borer infestation rate significantly; no environmental hazard encountered; does not interfered in the economics of silviecosystem.

Sanitation is the most important element of management program for existing elms because it removes the elm bark beetles breeding habitat from the system. It consists of the immediate removal of any dead or wounded branches, and the debarking of branches stored for use as lumber and fuel. The present study is at par with the Schreiber and Peacock (1974); van Sickle and Sterner (1976) who suggested that the most effective control measures against the elm bark beetles to date have been based on sanitation programs consisting of prompt removal of recently dead or dying trees, as well as the speedy destruction of all elm material infested by beetles. Lanier (1988) suggested that no borer infestation and thereof Dutch elm disease management program will be successful without good sanitation. Sanitation prevented elm trees from borer infestation as it destroyed the overwintering harboring grounds of the borer. It reduced the borer infestation rate by 61.02% in I elm plot and 63.49% in II elm plot as compared to control plot. Lanier (1988) suggested that sanitation including pruning combined with fungicides gives better disease management than sanitation, pruning or fungicides alone when dealing with a residual infection. Sanitation should be viewed as a community-wide management tactic.

Removal of brood trees as a control measure reported here is based on locating and subsequent removal of heavily infested trees (brood trees) that are unrecoverable which is an attempt to work out the control strategy against the shot-hole borer under study. Brood trees after removal were dissected and the harboring grubs were exposed and killed which in turn resulted in the failure of elm borer to regain pretreatment densities, thus infestation rate automatically reduced. Removal of heavily infested trees reduced elm borer, *S.kashmirensis* attack by 42.41% in two treatments.

Elm trees (*Ulmus* sp.) stressed by unfavorable environmental conditions, disease, defoilation, age, or poor tree care are most susceptible to bark beetle attack (Hagen, 1995). Heavy infestation of Lamiine species cause widespread mortality among host tree species (Yang et al, 1995; Ertain, 2003). Donley (1981) showed that the control of red oak borer, *Enaphalodes rufulus* by removal of infested trees reduced 50% and 90% borer population after treatments in first and second generations respectively.

The present investigation revealed that the shot-hole borer (*Scolytus kashmirensis*) infested elm trees (*Ulmus wallichiana* and *U. villosa*) in Kashmir. The aforementioned borer exploited one or the other tissues of elm plants. The borer, *S. kashmirensis* mine the inner bark (the phloem-cambial region) on twigs, branches or trunks of elm trees and resulted in the stunted growth of infested host tree. The *S. kashmirensis* is of great economic importance as it attacks the living/healthy but weakened elms, lead them to ultimate death and also feed on the dead and dying plant tissues, so plays a significant role in the host plant physiology and/or economy.

Management practices by cultural operations reduced the infestation rate significantly. Seasonal pruning reduced infestation of *S. kashmirensis* significantly; Spring and Autumn pruning reduced it by 2.33% and 63.67% respectively. Sanitation reduced the borer infestation rate by 61.02% and 63.49% in two treated elm plots. Removal of the brood trees reduced *S. kashmirensis* infestation rate by 42.41%. None of the two species of the genus *Ulmus* (*U. wallichiana* and *U. villosa*) offered complete resistance to the attack of the borer under study, however, *U. villosa* though prone to their attack showed slight resistance as compared to the *U. wallichiana* screened in the region which are more or less equally susceptible to the borer.

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Significance of Serum HGF, Bcl-2 and Nitric Oxide in Primary Breast Cancer

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Abstract: The aim of this study was to determine serum concentrations of HGF, Bcl-2 and nitric oxide (NO) in 44 patients with primary breast cancer and 15 healthy individuals as a control group using an ELISA assay for HGF and Bcl-2 while nitric oxide was determined by using colorimetric technique. The measured parameters were correlated with clinicopathological parameters that may affect the outcome of disease. In addition, ROC curve analysis was done to each parameter. The results were as follows, the mean level of HGF was 1198.79 ± 76.32 pg/ml compared with 884.67 ± 66.88 pg/ml for control (p = 0.026). The HGF levels were significantly elevated in the patients with increasing the tumor stage (p = 0.036). In addition, HGF levels were higher in negative estrogen receptor (p = 0.039). The mean level of Bcl-2 in patients was 12.83± 1.97 ng/ml compared with 5.09 ± 0.40 ng/ml for control (p = 0.027). Levels of Bcl-2 were elevated but not statistically significant in patients with GI tumors, negative nodes, ER negative tumors and postmenopausal patients (p = 0.4, 0.8, 0.7 and 0.5, respectively).The patients mean level of the nitric oxide (NO) was 63.07 ± 4.14 µmol/L compared with 43.99 ± 4.21 µmol/L for control (p = 0.014). The levels of NO were elevated but also not statistically significant in patients with tumor size I, GI tumors, ER negative tumors, positive nodes, stage II tumors and postmenopausal patients (p = 0.3, 0.6, 0.3, 0.7, 0.3 and 0.2 respectively). From the ROC curve analysis, it was observed that the area under curve for HGF, Bcl-2 and NO was 0.695, 0.842 and 0.711, respectively. This result indicates the good validity of the above markers especially Bcl-2 parameter to discriminate the positive from the negative samples. In conclusion, this study demonstrates that the serum determination of HGF, Bcl-2 or NO may help in diagnosis of the breast cancer and may aid in disease prognosis. However, larger studies with more patients are required.
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Key words: HGF; Bcl-2; nitric oxide; breast cancer; diagnosis; prognosis.

Introduction

Breast cancer is the second most common cancer in the world, and is the most common cancer in women [1]. In excess of 1.2 million cases are detected every year, affecting 10–12% of women and responsible for approximately 500,000 deaths per year [2]. The ability to detect human malignancy via a simple blood test has long been a major objective in medical screening. The advantages of such an easy to use, relatively non-invasive and operator-independent test are self-evident. In this respect, cancer biomarkers can be DNA, mRNA, proteins, metabolites, or processes such as apoptosis, angiogenesis or proliferation [3].

HGF is a cytokine which induces morphogenesis, proliferation, motility and angiogenesis [4]. In normal mammary development, HGF in collaboration with other growth factors such as neuregulin stimulates tubulogenesis in a tightly controlled paracrine manner [5]. HGF is primarily expressed by mesenchymal/stromal cells, whereas its receptor, Met, is expressed selectively by epithelial cells, thereby creating a paracrine regulatory system [6]. In normal breast tissue, the HGF-and-Met paracrine system has a low basal level of expression [7]. Over-expression of Met [8] and HGF [9] in breast tumors and of HGF in the sera [10] of breast cancer patients has been found to be an independent predictor of recurrence and decreased patient survival. HGF has antiapoptotic effects [11]. Recent studies also suggest that HGF suppresses cell apoptosis by up regulating the expression of Bcl-xl, an antiapoptotic protein [12]. The suppression of apoptosis contributes to carcinogenesis, as well as to a resistance to chemotherapy and radiotherapy [13]. Apoptosis appears to be controlled by several genes. A group of genes with sequences homologous to bcl-2 modulate cell death and can be divided into two functionally antagonistic groups: suppressors, such as Bcl-2, and cell promoters, such as Bax. Homo or heterodimerization are important for the apoptotic regulatory function of the bcl-2-related proteins. The ratio between Bax/Bcl-2 heterodimers appears to be essential in deciding the life or death of a cell. When Bax predominates, apoptosis is accelerated and the antiapoptotic activity of Bcl-2 is antagonized [14, 15].

B-cell lymphoma leukemia-2 (Bcl-2) protein is a member of the bcl-2 family that regulates apoptosis [16] and is expressed in normal glandular epithelium. It's tumourigenic potential has been demonstrated in animal models [17] and is supported by the finding of

over expression of Bcl-2 in a variety of tumors and in lymphomas in which Bcl-2 acts as an oncogene [18]. It is over expressed in 25–50% of breast cancers [19]. High expression of Bcl-2 is considered as a good prognostic factor in cancer since it has been associated with improved survival in patients with breast cancer [20, 21]. High expression of Bcl-2 has been observed in ER-positive breast cancers as well as in progesterone receptor (PR) - positive breast cancers [22, 23].

Nitric oxide (NO) is a free radical acting as a gaseous messenger that affects various biological functions, either at low concentrations as a signal transducer in many physiological processes (e.g., blood flow regulation, smooth muscle relaxation, iron homeostasis, platelet reactivity, neurotransmission) or at high concentrations as a cytotoxic defensive mechanism against pathogens and, perhaps, tumors [24]. Moreover, accumulating evidence suggests that chronically elevated levels of NO are involved in the pathogenesis of some human pathological conditions, such as cancer [25]. NO production is a part of the angiogenic switch in tumor development [26, 27].

NO may promote tumor growth by modulating the production of prostaglandins. NO can activate cyclooxygenase-2 (COX-2) [28, 29] that, by generating prostaglandins, promotes angiogenesis and inhibits apoptosis (e.g., by enhancing Bcl-2 protein synthesis) [30]. The aim of the present work is to determine serum levels of HGF, Bcl-2 and nitric oxide in patients with primary breast cancer and to correlate these parameters with the clinicopathological data of the patients which may affect the outcome of disease. This may help in distinguishing subsets of breast cancer patients and optimizing therapeutic approaches.

Patients and Methods

Forty-four patients with primary invasive breast cancer were included in this study. All the patients met the following criteria: (a) having been diagnosed as having primary invasive breast cancer, (b) having no clinical manifestation of infection, (c) having no other known malignancy. All the 44 patients were women ages 23 to 56 years (median, 36 years). Also, a group of 15 healthy females was used as control. The diagnosis was carried out by biopsy and imaging studies. The data of primary tumor stage, age, estrogen receptor status, progesterone receptor status, tumor size, lymph node status and histological grade were collected. Venous blood samples were collected before the surgery. Sera were obtained by centrifugation and stored at -70 ºC until assayed.

Determination of Serum HGF and Bcl-2 by ELISA

Circulating HGF and Bcl-2 were evaluated by solid-phase Enzyme-linked immunosorbent assay (RayBiotech, Inc and Bender MedSystems GmbH, Europe, respectively) using 96-well microplates in accordance with the manufactures instruction. The color conducted is stopped with stop solution, and the optical density was measured at 450 nm and reference filter 620 nm. A standard curve was constructed by plotting the mean absorbance obtained from each standard against its concentration. The best fit curve through the points of the graph was drawn. From these standard curves, the concentrations of HGF and Bcl-2 for the patients and control under the study were obtained. The concentrations read from the standard curve of Bcl-2 was multiplied by the dilution factor (x 5) due to1:5 dilution of the samples. Detection limit for HGF was less than 8 pg/ml while that of Bcl-2 was less than 0.5 ng/ml.

Nitric oxide Measurement in serum by Colorimetric Method

Quantitative estimation of nitric oxide in serum was carried out colorimetrically according to the method of Montgomery and Dymock [31], using Biodiagnostic nitric oxide kit (Egypt). The principle of the test is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by the colorimetric detection of nitrite as a deep purple azo compound.

Statistical Analysis

Data were expressed as mean ± SEM and were analyzed using Medcal software, version 11 by selecting The Student's t test was used to assess the significance of difference in the levels of HGF, Bcl-2 and NO between the patients group and the control group. One-way ANOVA was performed to differentiate the parameter within the same group of clinical data. The cut-off value was determined for each of the measured serum parameters in that study according to the best discrimination between patients and control regarding optimal values of sensitivity and specificity using ROC curves analysis. AUC of the tests using ROC curve was calculated. $P < 0.05$ was accepted as significant.

Results

The characteristics of the patients are shown in Table 1. The median age was 36 (23–56) years. All patients were with invasive ducal carcinoma, of which 7 (15.9 %) with grade I, 29 (65.9 %) with grade II and 8 (18.2 %) with grade III. Thirty-two patients were Premenapausal (72.7 %) and 12 were postmenopausal (27.3 %). The mean and standard error of mean (SEM) for serum HGF, Bcl-2 and NO levels in patients with breast cancer and control were illustrated in Table 2. Serum HGF concentrations of the breast cancer patients showed significant increase when compared with those of the control (1198.79 pg/ml versus 884.67

pg/ml, respectively, p = 0.026). There was also, significant increase in Bcl-2 serum level in breast cancer patients when compared with those of the healthy control (12.83 and 5.09 ng/ml, respectively, p = 0.027). In addition, Serum NO level revealed significant increase in patients with breast cancer when compared to those of the control (63.07 and 43.99 µmol/L, respectively, p = 0.014).

Table 1. Patient's characteristics.

Parameter	N	%
Age Median	36 (23–56)	
Tumor size		
T1 <2	26	59.1
T2 2–5	11	25
T3 >5	7	15.9
Auxiliary lymph node		
Positive	28	63.64
Negative	16	36.36
Clinical stage		
Stage I	28	63.64
Stage II	16	36.36
Pathological grade		
Grade I	7	15.9
Grade II	29	65.9
Grade III	8	18.2
Estrogen receptor		
Positive	18	40.9
Negative	26	59.1
Progesterone receptor		
Positive	19	43.2
Negative	25	56.8
Menopausal status		
Premenopausal	32	72.73
Postmenopausal	12	27.27

Table 2. The mean serum levels of HGF, Bcl-2 and Nitrate + Nitrite of the patients compared with those of control.

	HGF(pg/ml)	Bcl-2(ng/ml)	Nitrate + Nitrite (µmol/L)
Patients	1198.79 ± 76.32	12.83± 1.97	63.07 ± 4.14
Control	884.67 ± 66.88	5.09 ± 0.40	43.99 ± 4.21
P versus control	0.026*	0.027*	0.014*

Data were expressed as mean ± standard error. (*) significant

Correlations of Serum HGF, Bcl-2 and NO with Clinicopathological Parameters of Patients

Table 3 showed that there was significant elevation of HGF level in sera of the patients with negative estrogen receptor (p = 0.039) compared with that of patients with positive receptors. Also, there was significant elevation of HGF level in sera of the patients with clinical stage II (p = 0.036) compared with that of patients with clinical stage I. On the other hand, Table 3 showed that there was decreasing in the serum Bcl-2 mean level of the patients with increasing the grade, but the difference was not statistically significant (p = 0.4). In addition, there was insignificant increase in the Bcl-2 serum levels in the postmenopausal patients compared with those of premenopausal patients (p = 0.5). Also, table 3. illustrated that there is not significant variation of the nitric oxide levels with the clinicopathological data of the patients.

ROC Curve Analysis

The receiving operating characteristic (ROC) curve was designed for HGF, Bcl-2 and NO (Figures 1, 2 and 3). The cut-off value for HGF, Bcl-2 and NO was >1110, >5.5 and >60, respectively. It was found that the area under curve (AUC) for HGF, Bcl-2 and NO was 0.695, 0.842 and 0.711, respectively. This result indicates the good validity of the above markers especially Bcl-2 parameter to discriminate the positive from the negative samples.

Table 3. Correlations of HGF, Bcl-2 and NO with clincopathological data of patients.

Parameter	HGF(pg/ml)	Bcl-2(ng/ml)	NO (μmol/L)
Tumor size			
T1<2 cm	1147.95 ± 82.48	11.93±2.61	67.34 ± 4.65
T2 2–5 cm	1145.46 ± 171.21	15.36±4.82	61.76 ± 11.59
T3>5 cm	1471.43± 256.12	12.207±2.43	49.28 ± 6.35
Pathological grade			
Grade I	1230.57 ± 146.35	18.50±7.67	72.51 ± 9.48
Grade II	1103.75 ± 92.33	12.33±2.32	62.36 ± 5.40
Grade III	1515.5 ± 194.89	9.66±1.80	57.37 ± 8.50
Estrogen receptor			
Positive	1011.33 ± 110.95	11.94±2.47	57.68±7.18
Negative	1328.57 ± 97.53*	13.44±2.89	66.80±4.92
Progesterone receptor			
Positive	1201.79±126.85	11.989±3.05	54.85± 4.85
Negative	1196.51±95.89	13.470±2.62	69.32± 6.06
Menopausal status			
Premenopausal	1216.65±94.71	12.08±2.09	66.27±5.14
Postmenopausal	1151.17±125.69	14.83±4.71	54.54±6.16
Auxiliary lymph node			
Positive	1111.67±91.36	12.45±2.39	64.33±4.66
Negative	1351.251±131.11	13.50±3.53	60.87±8.13
p-value			
Clinical stage			
Stage I	989.38±97.85	12.40±2.46	59.62±5.12
Stage II	1318.46±100.39*	13.59±3.39	69.11±6.96
p-value			

Data were expressed as mean ± standard error
(*) significant

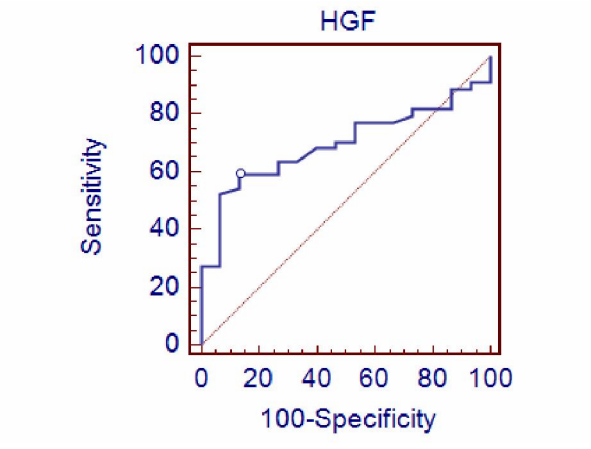

Figure 1. ROC curve of HGF, area under curve equal 0.695, $p = 0.004$.

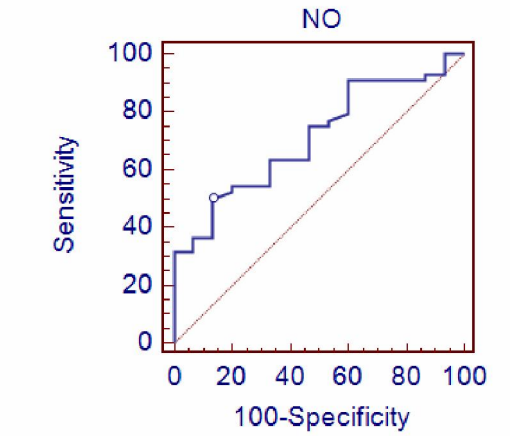

Figure 2. ROC curve of Bcl-2, area under curve equal 0.842, $p = 0.0001$.

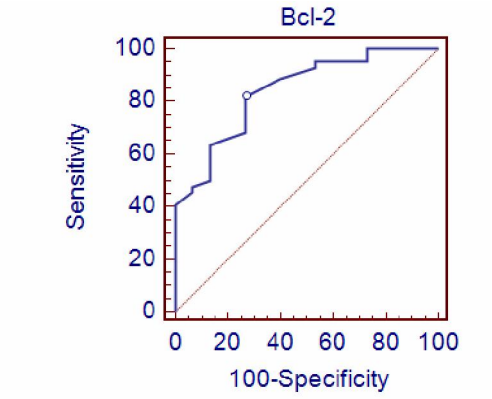

Figure 3. ROC curve of NO, area under curve equal 0.711, $p = 0.0036$.

Discussion

Biomarkers accepted for clinical use in breast cancer, such as CA 15-3, CEA and CA 27-29, have low sensitivity and specificity, and are thus more useful for patients at an advanced stage of breast cancer rather than for early cancer diagnosis [32]. So, there is a needing for new parameters to help in diagnosis and prognosis of primary breast cancer. So, in the present study, serum HGF, Bcl-2 and NO were evaluated and correlated with the clinicopathological parameters. Hepatocyte growth factor was originally identified both as a mitogen for parenchymal liver cells and as a fibroblast-secreted protein responsible for inducing the scattering of polarized epithelial cells (hence the alternative name, scatter factor). HGF and its receptor Met, a tyrosine kinase mediated product of the c-met proto-oncogene, are involved in a number of physiological activities, including cell proliferation, motility and migration, and invasion. In tumors, HGF disrupts adherens junctions and promotes cell dispersal, so stimulating invasive capacity [33].

It was reported that HGF receptor is widely distributed in various epithelial cells including tumor cells but obviously not in mesenchymal cells [34]. On the other hand, HGF production was found in the stromal component but not in the epithelial component of the breast [35]. Because it has been reported that HGF is a modulator of epithelial cell proliferation and motility for a broad spectrum of cell types [36], it is tempting to speculate that HGF originating from breast stromal cells may play a crucial role in facilitating breast cancer cell invasion and metastasis.

HGF is a mitogen for vascular endothelial cells (VECs) [37]. Maejima et al. [37] showed that the proliferative effect was partly dependent on nitric oxide synthesis, which was itself regulated by Src family kinases. Bell et al. [38] found that HGF secreted from adipose cells is involved in local angiogenesis, and specifically in the migration of VECs. Other investigators have demonstrated the induction of VEC protease production by HGF [39], and a consequent stimulation of VEC migration [40] and endothelial tube formation [38].

In the present study, it was observed that HGF serum levels were significantly elevated in the patients compared to those of control (p =0.026). Furthermore, there were significantly higher serum levels of HGF in patients with higher tumor stage (p = 0.036). Thus, the preoperative level of serum HGF may reflect the severity of invasive breast cancer and may be useful to pick up higher risk patients for more aggressive treatment. This result is consistent with Sheen-Chen et al. [41] who indicated that the mean value of serum soluble HGF in patients with invasive breast cancer was higher than that of the control group and the difference was significant, and concluded that patients with more advanced TNM staging were shown to have higher serum soluble HGF and the preoperative serum soluble HGF levels might reflect the severity of invasive breast cancer.

There are studies suggest that HGF suppresses cell apoptosis by up regulating the expression of Bcl-xl, an antiapoptotic protein [12]. Bcl-2 is a cytoplasmic protein involved in apoptosis and oncogenesis; it prolongs the survival of the non-cycling cells and inhibits cycling cells [42]. During the developmental period, bcl-2 is expressed in all tissues, while in adults it is expressed only in proliferating or reserve cells [43]. As far as breast cancer is concerned, bcl-2 protein is generally expressed in 60–80% of invasive breast carcinoma [44, 45]. In breast cancer specimens, bcl-2 expression is associated with well-differentiated tumors, like lower SBR grade, ER positivity and a low proliferation status [46, 47]. Several studies suggested that the low apoptotic response caused by over expression of bcl-2 allows the accumulation of genetic alterations that might be important in metastatic breast cancer potential [48, 49]. Bcl-2 expression has been reported to be associated with better outcomes in metastatic disease as well as in patients with early breast cancer treated with either hormone or chemotherapy [50, 51].

In the present study, Bcl-2 levels were significantly elevated (p = 0.027) in patients with breast cancer compared with those of the healthy control. These results agree with the finding of Kallel-Bayoudh et al. [52] who reported that Bcl-2 expression seems to be a very useful factor that should be in combination with HER2 and ER in breast cancer prognosis.

A strong inverse correlation between Bcl-2 and proliferative activity has been reported to exist in breast cancer, as well as in other tumor types, and the data presented in this study are in line with these findings, as the mean level of bcl-2 in Grade I, II and III was 18.50±7.67, 12.34±2.32 and 9.66±1.81, respectively.

Typically, tumors with low Bcl-2 expression are correlated with high grade histological type, indicating the existence of rapid cell turnover. In fact, similar relationships between apoptosis, proliferation and high tumor grade have been reported for other tumor types [53].

In breast carcinoma patients, a positive correlation between the expression of inducible NOS and metastatic disease has been reported by Martin et al. [54]. Elevated levels of NO production increase tumor vascularity and facilitate tumor metastasis in breast carcinoma patients [55]. NO may promote tumor growth by modulating the production of prostaglandins. NO can activate cyclooxygenase-2 (COX-2) [28, 29] that, by generating prostaglandins, promotes angiogenesis and inhibits apoptosis (e.g., by enhancing Bcl-2 protein synthesis) [30]. Conversely, another study suggested that NO inhibits the proliferation of human breast carcinoma cells, which explains the relationship between NO production and weak tumor aggressiveness [56]. Guntel et al. [57] found elevated level of nitrate+nitrite at operable serum in samples of patients with breast cancer.

In the current study, serum nitrate and nitrite levels showed significant increase in patients (p=0.014) compared with control subjects. These elevated nitrate and nitrite levels in the patients may be a result of increased NOS II activity, which is stimulated by a host defense system against tumor growth. Martin et al. [58] showed that endothelial NO synthetase activity was expressed by human breast tumors. NO synthetase is responsible for the production of NO. Increased NO synthetase activity is necessary for VEGF to stimulate angiogenesis and increase vascular permeability [59]. In addition, no correlation was found between nitrate + nitrite levels, and the prognostic factors of the breast tumor include tumor size, stage and menopausal status.

By ROC curve analysis, the area under (AUC) for HGF, Bcl-2 and NO was 0.695, 0.842 and 0.711, respectively. This indicates the availability of using these parameters in combination with the routine tumor markers such as CA 15.3 to help in diagnosis of breast cancer.

In conclusion, measurement of HGF, bcl-2 and nitric oxide levels might provide useful diagnostic and prognostic information about disease. however, larger studies involving more patients are needed.

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Phenological episodes of *Myriophyllum spicatum* (Haloragaceae); a highly invasive species in Kashmir Himalayan aquatic ecosystems.

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Abstract: Phenological behaviour of *Myriophyllum spicatum*, a highly invasive species in Kashmir Himalayan aquatic ecosystems, was studied in different standing and running water populations for a period of 12 months to monitor the various developmental stages. The plant starts its life cycle with the sprouting of rhizomes and axillary buds in standing water populations, whereas in running waters rhizomes and nodal plantlets contribute to new recruitments. In standing waters flowering phase prolongs when compared to running water populations. In standing water populations a high seed set was observed, whereas in running waters seed formation does not take place. The knowledge of time period and formation of these vegetative and sexual propagules by this invasive species is very important for its effective management and control in these ecosystems.
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Keywords: *Myriophyllum spicatum*; phenology; Kashmir Himalaya; Propagules; management.

Introduction

Many factors affect the reproductive success of flowering plants. Among these factors, the timing, frequency and duration of the flowering period collectively referred to as phenology is obviously of great importance (Rathcke and Lacey, 1985). The phenology of a species not only encompasses when, how often, and how long reproduction takes place but also determines the degree of reproduction synchrony with other plant species . Synchrony among species might be advantageous if the presence of one species facilitates increase in pollinator visitation and thus fruit/seed set in another species (Rathcke and Lacey, 1985). Phenology in general and reproductive phenology in particular is a critical and important trait of a plant because it determines the growth, developmental pattern and number of potential mates in a population thus providing a mechanism for reproductive isolation or speciation over time (Rathcke, 1983; Bronstein *et al.*, 1990). Researchers always and continuously try to identify environmental factors that correlate with phenological events such as initiation of flowering, the synchronization of flowering, the length of the flowering phase and variation in flower abundance (Opler *et al.*, 1980; Borchert, 1983; Inouye *et al.*, 2002). Environmental factors that initiate the onset of a particular phenophase include photoperiod, temperature and precipitation (Rathcke and Lacey, 1985). The same environmental factors can delimit a particular phenophase including flowering season in some specific ecoedaphic conditions or environments (Borchert, 1980; Inouye and McGuire, 1991).

The *Myriophyllum*, commonly known as watermilfoil, is among the species rich (68 spp.) genus of the aquatic "Core eudicots" (APGII, 2003). It shows a cosmopolitan distribution (except Antarctica), with a centre of diversity in Australia (42 spp., 34 endemic), North America (14 spp., 7 endemic) and Asia (16 spp., 8 endemic) also harbor a significant continental diversity and share seven common species . (Moody and Les, 2010). *Myriophyllum* is well-known for its invasive species. The aggressive *M. spicatum* L. (Eurasian watermilfoil) has now established on most continents and listed as noxious weeds in United States. Hybridization also has been shown to play a role in North American invasions with two hybrid lineages recognized which are *M. spicatum* x *M. sibiricum* and *M. heterophyllum* x *M. laxum* (Moody and Les, 2002*)*.

From the Indian subcontinent, five species of *Myriophyllum* have been reported (Hooker, 1879). In the Kashmir Himalaya, earlier the genus has been reported to comprise of two species: *M. spicatum* and *M. verticillatum* (Kaul and Zutshi, 1965), while as later on three species have been reported viz. *M. spicatum, M. verticillatum* and *M. tuberculatum* (Kak, 1990).

M. spicatum is native to Europe, Asia and North America (Couch and Nelson, 1985). It was introduced into North America between 1880's and 1940's and now occurs in both Canada and the United States (Reed, 1977, Couch and Nelson, 1985, Aiken *et al.*, 1979). From the initial point of introduction in the North America, *M. spicatum* has spread to 44 states and at least three Canadian provinces (Creed, 1998) and is now considered a major nuisance species throughout the Northeast, Northern Midwest and Pacific Northwest of

the United States (Couch and Nelsons, 1985; White *et al.*, 1993). *M. spicatum* has spread to 46 states and three Canadian provinces of North America (Jacono and Richerson, 2003; Kim, 2005). *M.spicatum* is categorized among the five most noxious wetland plants and it is the most widely managed aquatic need in the United States (Bartodziej and Ludlow, 1998). The knowledge about phenology of invasive plants is very important for their effective management and can be utilized to identify week points in their life cycle and long term management.The present study was undertaken with the same objective.

Materials and Methods

Healthy individuals of the species were selected from different standing and running water populations, tagged and examined throughout the growing season to study the life history pattern and mode of reproduction operative in the species in relation to habitat condition of the study sites. The tagged individuals were monitored to record data on various reproductive phenophases such as initiation of budding,vegetative growth, peduncle growth and anthesis, duration of flowering and seed formation. The axillary bud formation was examined regularly and their number was recorded to evaluate the importance of these propagules in the reproduction and fitness of the species.

Results:

The species is a submerged, perennial herb with pinnately divided leaves inhabiting both standing and running water habitats. The phenological behaviour of the species was studied in both standing and running water populations. The phenology starts with the sprouting of rhizomes and axillary buds in the first week of March and continues upto first week of April in standing water populations, whereas sprouting of rhizomes and formation of nodal plantlets commences in the second week of March and continues upto second week of April in running water populations. The planlets grow vegetatively from second week of April upto first week of June in standing waters, while in running waters the process is completed between second week of April and second week of July. The mature plantlets enter into the sexual phase during first week of June and flowering continues upto last week of September in standing waters. However, in running waters this phase starts during fourth week of June and lasts upto third week of September. In standing waters fruiting sets from second week of September and ends in third week of October. In running waters however fruits are not formed. The senescence of the above sediment parts starts in the second week of October and continues till first week of December in standing water and in running waters the process starts during the fourth week of October and continues upto the last week of December (Table 1 and Figure 1).

Table 1. Phenological behavior of *Myriophyllum spicatum* in standing and running water populations in the Kashmir Himalaya.

Phenophase	Habitat	Duration	Number of days
Sprouting of vegetative propagules(rhizomes,axillary buds, nodal planlets)	SW RW	1 * (3) ** - 1 (4) 2 (3) - 2 (4)	27 32
Vegetative growth	SW RW	2 (4) - 1 (6) 2 (4) - 2 (7)	62 88
Flowering phase	SW RW	1(6) - 4 (9) 4(6) - 3(9)	110 81
Fruiting phase	SW RW	2 (9) - 3(10) -	39 -
Senescence	SW RW	2 (10) - 1(12) 4 (10) - 4(12)	54 60

SW = Standing water
RW = Running water
* = Week
( ) ** = Month

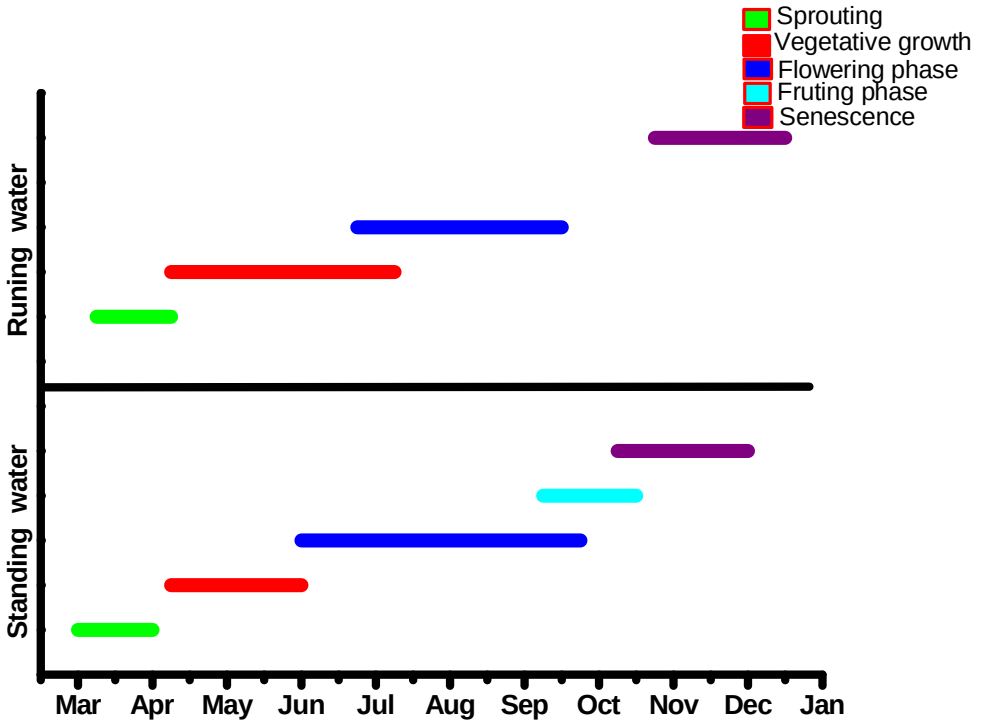

Figure 1: Phenological behaviour of Myriophyllum spicatum in different standing and running water populations.

Discussion

Myriophyllum spicatum overwinters by means of rhizomes and winter buds. These structures start sprouting during the first week of March and continue upto second week of April when environmental conditions such as temperature and light are available, because these factors are important for the initiation of a particular phenophase (Rathcke and Lacey, 1985). The vegetative growth commences during April and continues till June; however in running water populations the vegetative growth phase is longer than in standing water populations. The flowering commences from June to September and continues for 1-4 months in standing water populations and for 1-3 months in running water populations. The longer vegetative phase, delayed and shorter flowering phase in running waters may be due to the lower allocation of resources to sexual reproduction and permanent exposure of plants to mechanical stress (Niklas, 1998; Henery and Thomas, 2002; and Hodges *et al.*, 2004). The fruiting phase completes from September to October in standing water populations where as in running water populations fruits are not formed. The senescence starts from second week of October and continues till December. These phenological events are in agreement with the work of Patten (1956) and Spencer and Lekic (1974).*M. spicatum* produces flowers, seeds and axillary buds during June-October. Therefore removal of ramets before June can prove an effective method for control of this aggressive species. This is supported by the work of Caffery and Monahan (2006) who reported that in *Myriophyllum verticillatum*, removal of turions yielded desirable results in control of this species as compared to annual treatment with dichlobexil, followed by mechanical cutting. The present study revealed that knowledge about various life history traits, such as different types of sexual and vegetative propagules, their time of formation and germination is very important for long term management of this aggressive species in the Kashmir Himalayan aquatic ecosystems.

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3/17/2011

Antibiotic Susceptibility Profiles of Enteric Bacterial Isolates from Dumpsite Utisols and Water Sources in a Rural Community in Cross River State, Southern Nigeria.

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Abstract: A survey was conducted to establish the effects of bacterial contamination from dumpsite effluents on utisol and water and the antimicrobial susceptibility profiles of the bacterial isolates. A total of 504 each of soil and water samples from different locations were sampled between the months of May and November, 2009. *Proteus* sp(70.24%), *Pseudomonas* sp(59.13%), *Bacillus* sp(58.33%), *Escherichia coli* (58.33%), *Campylobacter* sp(45.63%), *Klebsiella* sp(35.12%), *Shigella* sp(30.96%), *Salmonella* sp(27.98%), *Aeromonas* sp (27.98%) and *Vibrio cholerae* (10.91%) were isolated from polluted utisols, while *Bacillus* sp (86.51%), *Pseudomonas* sp (71.23%), *Escherichia coli* (60.71%), *Aeromonas* sp (52.58%), *Salmonella* sp (47.02%), *Klebsiella* (26.19%) and *Vibrio cholerae* (13.10%) were isolated from various water sources. The prevalence of the bacterial species in the two environmental sources differed significantly ($P<0.05$). All isolates were resistant to Gentamicin, Chloramphenicol and Amikacin, while low resistance values were recorded in Erythromycin (25%) and Nalidixic acid (37.50%). Adequate treatment of dumpsite effluents and the use of Erythromycin and Nalidixic acid as therapeutic measures are recommended to reduce possible health hazards.
[Ikpeme Emmanuel, Nfongeh Joseph, Eja Matthew Egbebor, Etim Lawrence and Enyi-Idoh Kingsley. **Antibiotic Susceptibility Profiles of Enteric Bacterial Isolates from Dumpsite Utisols and Water Sources in a Rural Community in Cross River State, Southern Nigeria.** Nature and Science 2011;9(5):46-50]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Dumpsite effluents**,** enteric bacteria, antibiotic susceptibility, Southern Nigeria.

1. Introduction

The various uses of water for drinking, bathing, washing and cooking are well known. Water meant for human consumption should be free from pollution, and should be safe and acceptable. Indeed the microbial quality of potable water should not exceed limits specified in the water quality guideline (APHA, 1998). However, the microbial quality of water in several rural Nigerian communities has been reported to be poor, unsafe and not acceptable for human consumption (Obi *et al*, 2004). Enteric bacterial pathogens have been shown to thrive for long periods in water in spite of a large number of antagonistic populations (Hoge *et al*, 1989). These pathogens are variously incriminated in cases of diarrhea which in turn accounts for a substantial degree of morbidity and mortality in different age groups worldwide (Black, 1993 and Obi *et al*, 1997). Water sources in Akansoko are recipients of heavy microbial load through anthropogenic activities. Akansoko is a community located on the outskirts of Calabar and situated strategically on the banks of one of the tributaries of the lower Cross River. Its immediate neighborhood serves as one of the numerous dumpsites for municipal wastes.

Isolation of pathogens from sources connotes a serious public health risk to consumers. This risk is further exacerbated by the widely reported cases of resistance of enteric bacterial pathogens to several antibiotics (Hoge *et al*., 1998; Ash *et al*., 2002). For example, in 1984, 82% of *Campylobacter* strains from Lagos, Nigeria, were sensitive to erythromycin and 10years later only 70.8% were sensitive (Coker and Adafaso, 1994). In Thailand, ciprofloxacin resistance among *Campylobacter* species increased from 0% before 1991 t0 84% in 1995 (Hoge *et al*., 1998). In the United States, several rivers were reported to be reservoirs of antibiotic resistant bacteria (Ash *et al.*, 2002). Even though antibiotic resistance is common, antibiotics are still used in the management of diarrhea. Antibiotics shorten the duration of diarrhea, decrease stool output, and may mitigate complications (Black, 1993.)

This study was therefore aimed at evaluating the antibiogram of enteric bacterial isolates from water sources and dumpsites effluents in Akansoko community using commonly used antibiotics scheduled for diarrhea cases.

2. Materials and Methods

2.1 Study Area.

The study area is Akansoko village, located approx. 15 km Southeast of Calabar, the Cross River State capital, Nigeria. It is situated between latitudes $4^{o}30^{/}$ and $5^{o}00^{/}$N and longitude $5^{o}15^{1}$ and $8^{o}45^{1/}$E. Major sources of water supply are dug-out wells, streams and slow flowing portions of great Kwa River

estuary, which forms part of the lower Cross River estuarine system (Eja *et al*., 2003).

2.2. Sample Collection

Sampling was done thrice weekly from May to November 2007.

Water samples were collected from two sources, two stream sources and an estuary located within the study area. Duplicated samples were collected in sterile one liter Nalgene containers and transported in ice box at 4^{o}C to the microbiology laboratory within 6h for analysis.

Effluent contaminated soils from dumpsites were collected from 6 stations within the study community. Each duplicate sample was collected in sterile polyethene bags and transported in an ice-cold container to the microbiology laboratory, Cross River University of Technology for analysis within 8h.

2.3 Sample Analysis

The organisms were isolated using standard methods (APHA, 1998)

For the isolation of *Campylobacter*, Skirrows and Butchers' medium (LAB-M) was used. Blood Agar plates were incubated at 42^{o}C under microaerophilic conditions by placing in candle jar for 72h. Colonies were considered to be *Campylobacter* if they were S-shaped, Gram negative, motile and oxidase positive .

For the isolation of *Salmonella* and *Shigella* 1ml water sample and 10^{-1} soil dilution were inoculated in 9ml selenite-F-broth and incubated for 18-24h at 37^{o}C for enrichment. The enriched samples was plated on *Salmonella-Shigella* Agar (Oxoid) and incubated for 48h at 37^{o}C. Small colourless colonies were subcultured on nutrient agar slants and identified using methods described by Cowan and Steel (1985).

For the identification of *Vibrio cholerae*, 1.0ml of water sample and 10^{-1} soil dilution were enriched in alkaline peptone water (pH 8.6) and incubated at 37^{o}C for 8hrs. Several loopfuls of the peptone water culture (taken from the surface) were streaked on Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar (Difco) and incubated at 37^{o}C for 24h. Yellow colonies were subcultured on nutrient agar slants to produce pure cultures. Microscopic and biochemical tests were used to identify *Vibrio cholerae* as described by Cowan and Steel (1985).

Other enterobacteria were identified by culturing samples on MacConkey agar (Oxoid) and characteristic colonies subcultured on nutrient agar slants for further biochemical tests.

Non-enterobacterial species were identified by culturing on nutrient agar and subsequent biochemical tests carried out as described by Cowan and Steel(1985).

2.4. Antibiotic Susceptibility Testing

Pure isolates were cultured for antibiotic susceptibility assessment using the disc diffusion method (Obi *et al.,* 2004). Antibiotic-impregnated discs were placed on Mueller Hinton Agar (Difco) and incubated at 37^{o}C for 24h. Zones of inhibition were measured and compared with standard values. Antibiotics in the panel included Tetracycline(30µg),Gentamycin(120µg),Erythromycin(10µg), Ampicillin(10µg), Chloramphenicol(30µg), Nalidixic acid(30µg) and Amikacin(30µg) with concentrations as recommended by the National Committee for Clinical Laboratory Standards(NCCLS, 1996).

3. Results

Bacterial isolates obtained from soils at various dumpsites in the study community are shown in the Table 1.0. *Proteus* sp, *Bacillus* sp, *Pseudomonas* sp, and *Escherichia coli* were isolated from more than 50% of the total samples. The prevalence of the various bacterial species differed significantly ($P<0.05$) throughout the sampling period. All species were isolated in the various sampling stations.

Table 2.0 shows the occurrence of bacterial isolates from water sources in the community. *Bacillus* sp, *Pseudomonas* sp, *Escherichia coli* and *Aeromonas* sp recorded prevalence values higher than 50% in all samples analyzed. The prevalence of all species were also observed to differ significantly ($P<0.05$). Enteric pathogens such as *Vibrio cholerae*, *Salmonella* sp, *Escherichia coli* and *Shigella* sp had higher prevalence values in samples from streams and estuary.

Antibiogram results of the various isolates as presented in Table 3.0 show multidrug resistance by all isolates. Resistance of isolates (100%) was observed on Gentamicin, Chloramphenicol and Amikacin. Similarly, 87.5% and 75% of isolates were resistant to tetracycline and Ampicilin, respectively. However, Erythromycin and Nalidixic acid displayed better performing index with 25% and 37.5% isolates resistance, respectively.

Table 1.0: Occurrence of bacterial isolates from dumpsites soils at various sampling stations

Bacterial Isolates	Sampling station 1 N=84(%)	Sampling station 2 N=84(%)	Sampling station 3 N=84(%)	Sampling station 4 N=84(%)	Sampling station 5 N=84(%)	Sampling station 6 N=84(%)	Total Frequency N=504(%)
Bacillus sp	63(75.00)	65(77.38)	82(97.62)	21(25.00)	43(51.19)	20(23.81)	294(58.33)
V.cholerae	10(11.90)	17(20.24)	6(7.14)	12(14.29)	2(2.38)	8(9.52)	55(10.91)
Aeromonas sp	38(45.24)	53(63.10)	14(16.67)	10(11.90)	7(8.33)	11(13.10)	133(26.39)
Salmonella sp	41(48.81)	30(35.71)	34(40.48)	10(11.90)	17(20.24)	9(10.71)	141(27.98)
Shigella sp	49(82.14)	34(40.48)	36(42.86)	15(17.86)	10(11.90)	12(14.29)	156(30.95)
E. coli	69(82.14)	73(86.90)	58(69.05)	13(15.48)	61(72.62)	20(23.81)	294(58.33)
Campylobacter sp	59(70.24)	62(73.81)	31(36.90)	17(20.24)	28(33.33)	33(39.29)	230(45.63)
Pseudomonas sp	47(55.95)	52(61.90)	75(89.29)	61(72.62)	32(38.10)	29(34.52)	298(59.13)
Klebsiella sp	27(32.14)	34(40.48)	51(60.71)	40(47.62)	11(13.10)	14(16.67)	177(35.12)
Proteus sp	82(97.62)	66(78.57)	77(91.67)	48(57.14)	53(63.10)	28(33.33)	354(70.24)

Total $P<0.05$

N=Total No of samples analyzed

Table 2.0: Occurrence of bacterial isolates from water sources at various sampling locations

Bacterial Isolates	Well 1 N=84(%)	Well N=84(%)	Well 3 N=84(%)	Stream 1 N=84(%)	Stream 2 N=84(%)	Estuary N=84(%)	Total Frequency N=504(%)
Bacillus sp	71(84.52)	69(82.14)	65(77.38)	75(89.29)	67(79.76)	79(94.05)	436(86.51)
V. cholerae	3(3.57)	11(13.10)	5(5.95)	18(21.43)	13(15.48)	16(19.05)	66(13.10)
Aeromonas sp	38(45.24)	62(73.81)	13(15.48)	17 (20.24)	54(62.29)	81(96.43)	265(52.58)
Salmonella sp	67(79.76)	24(28.57)	41(48.81)	35(41.67)	21(25.00)	49(58.33)	237(47.02)
Shigella sp	9(10.71)	24(28.57)	12(14.29)	20(23.81)	28(33.33)	16(19.05)	109(21.63)
E. coli	44(52.38)	52(61.90)	22(26.19)	61(72.62)	69(82.14)	58(69.05)	306(60.71)
Campylobacter sp	12(14.29)	19(22.62)	4(4.76)	9(10.71)	17(20.24)	23(27.38)	84(16.67)
Pseudomonas sp	56(66.67)	63(75.00)	41(48.81)	60(71.43)	65(77.38)	74(88.10)	359(71.23)
Klebsiella sp	11(13.10)	27(32.14)	14(16.67)	38(45.24)	12(14.29)	30(35.71)	132(26.19)
Proteus sp	7(8.33)	2(25.00)	4(4.76)	31(36.90)	29(34.52)	(18(21.43)	91(18.06)

Total $P < 0.05$

N = Total No of samples analyzed

Table 3.0: Antibiotic susceptibility profile of major pathogenic bacterial isolates from dumpsites and water sources

	Antibiotics susceptibility (Zone of inhibition in mm)						
Isolates	TET	GEN	ERT	AMP	CLP	NAL	AMK
V. cholerae	R(16)	R(18)	R(16)	S(20)	R(16)	S(20)	R(17)
Aeromonas sp.	R(14)	R(10)	R(12)	R(8)	R(11)	R(16)	R(11)
Salmonella sp.	R(18)	R(16)	S(28)	R(11)	R(10)	S(26)	R(16)
Shigella sp	R(16)	R(10)	S(21)	R(10)	R(13)	S(20)	R(18)
E. coli	R(16)	R(9)	S(26)	R(10)	R(12)	S(29)	R(14)
Campylobacter sp.	R(17)	R(18)	S(27)	R(19)	R(10)	S(26)	R(19)
Pseudomonas sp.	R(16)	R(11)	S(25)	R(9)	R(10)	R(12)	R(13)
Klebsiella sp.	S(27)	R(12)	S(20)	S(26)	R(16)	R(11)	R(9)
Total Resistance (%)	7(87.50)	8(100.00)	2(25.00)	6(75.00)	8(100.00)	3(37.50)	8(100.00)

R = Resistant, S = Susceptible, TET= Tetracycline, GEN = Gentamicin, ERT= Erythromycin, AMP = Ampicillin, CLP= Chiliramphenicol, NAL= Nalidixic acid, AMK =Amikacin

4.1 Discussion

Many anthropogenic activities have been known to be major causes of pollution of soil and water sources. The community under study relies on water sources which are devoid of treatment for their domestic needs. Most of these sources are situated at proximal distances to various dumpsites.

Bacterial species isolated from soils at dumpsites showed high prevalence of *Proteus* sp (70.21%), *Pseudomonas* sp (59.13%), *Bacillus* sp (58.33%) and *Escherichia coli* (58.37%). Other major enteric pathogens such as *Salmonella* sp (47.02%), *Shigella* sp (21.63%) and *Vibrio cholerae* (13.10%) were also isolated. The presence of these pathogens may be due to domestic wastes which usually form the main component of wastes at the dumpsites. Udo and Nfongeh (2005) working in Adim community, Cross River State, Nigeria isolated *Vibrio cholerae* (14.7%), *Salmonella paratyphi* (12.5%), *Salmonella typhimurium* (8.8%) and *Shigella sonnei* (6.3%) as major enteropathogens associated with diarrhea cases. The isolation of these pathogens at dumpsites directly confirms the presence of faecal wastes at various dumpsite since it was common practice to dump human excreta in the sites which could also be used as latrines at night.

Similarly, *Bacillus* sp (86.51%), *Pseudomonas* sp (71.23%), *Escherichia coli* (60.71%), *Aeromonas* sp (52.58%) and *Salmonella* sp (47.02%) were the major bacterial species isolated from water sources proximal to dumpsites. These species are directly related to those isolated from soils at dumpsites. This similarity suggests possible percolation of pathogens in dumpsite effluents through the soil into water sources. The residents used the sampled water sources as their main source of water supply for domestic needs.

Antibiograms of the major enteric pathogens isolated reveal resistance to preferred drugs. All isolates were resistant to Gentamicin, Chloramphenical and Amikacin while Erthromycin (25%) and Nalidixic acid (37.50%) had low resistance percentages for the isolates. The fact that several bacterial species are known to be resistant to a wide array of antibiotics was confirmed by Aseffa *et al.,*(1997). The marked resistance of strains of *Salmonella* and *Shigella* to Ampicillin and Chloramphenicol as shown in the present study agrees with the findings of Ash *et al.*, (2002) working on rivers in the United States and probably accounts for the major outbreaks of salmonellosis and shigellosis worldwide. Similarities in antibiograms among isolates from both environmental sources indicate a possible infiltration of pathogens from dumpsite effluents to water sources. Persistent multiple drug resistance of most isolates to appropriate drugs of choice are of great public health concern and calls for periodic monitoring of antibiograms to detect possible changing patterns.

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Characteristics of rural women in developing countries

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Abstract: Women's productive activities has affective role to increase revenue, rural family welfare, and its consequents is: foods status improvement, health, preventing irregular migration, literacy enhancement and development of rural family social status. Despite clearness of affective women's role at production, economy of village and country, they don't enjoy proper social base and they were deprived of educational and welfare programs especially at rural and nomadic area. Thus women and their roles should be considered particularly in order that they would find that first they are important and efficient; second they have educational needs and many technical gaps; third they shouldn't forget efforts for enabling themselves. As girls and women's discussion and solving their historical lag and restoring their social right are important and necessary, it is sensitive and accurate equally, because dominant patriarchal cultures at rural societies, put women at lower status. So that at some societies, women's duties are just upbringing and reproduction and maybe they are considered as workforce, and they are deprived of decision making and opining at family and society environment.
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Introduction:

While, access opportunities to different sources and needed inputs at agriculture activities for them should be prepared. thus , women at village have activity by help farming and other rural affairs such as ranching, nurturing poultry , gardening and … and recently by participating at related programs to rural development including organizing cooperative associations related to women , establishing handicraft factory at village and even marketplace for it, organizing occupation plan for inhibited girls and women, related activities to filling rural women's leisure, participating at rural affaires which previously managed by men and etc . Now, women alongside men attend at all rural affaires and even at some case it has be seen that programming related to provide different services, were done by women at villages. All we can say that women in current villages enjoy better freedom and they do rural affairs by managing not as their duties and obligation like in the past. Now, women are not seen as passive receiver of help to improve their welfare, but as active social propagators who can change women and men's life condition (Farghdan, 2001).

Aside from the economic role of women that clearly has been made in the past decades, the vital role of women in social and cultural dimensions of development process in rural areas has remained hidden from the polls. they train the next generation of farmers and teach them the next generation nessessary knowledge. A Chinese proverb says, "If training a man, just training a man but if you teach a woman you teach a family." Women are local knowledge and local educators themselves, in preparing and providing food, health treatments and cultural values are the next generation.

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 part and possibility to institutionalized appropriate infrastructure, we would have suitable attitude toward development process. Agriculture part has critical responsibility, as one of the productive part of country for supplying needed food security, that it can assist this part 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.

Means participation of women in all stages of development, evaluate needs, identify problems, planning, management, implementation and evaluation is. Equity participation in a patriarchal society was not easily achieved, such matter requires the participation of women, especially rural women in projects is the way that they are concerned. Rural population of Iran always different roles in the production and distribution have

been responsible. Agricultural sector, supplier of about one third of employment, food needs of more than Chharpnjm country, half of exports, do not need the agricultural products industry and one-fifth of GDP countries.

In all communities, rural women as an important factor in achieving rural development goals were discussed and in fact, half of rural human resources development are needed, however, the rural population of Iran, the ruling class (the owners of capital) and rural people, between urban and rural, between literate and illiterate, between men and women, there is a deep cleft.

Women, especially in villages of fewer possibilities in terms of investment, credit and enjoy the power. Miran role of rural women more than men, influenced by various factors, conditions and economic, social, cultural and ecological is.

Rural women, either directly (production of crops, livestock, handicrafts and rural) or in terms of helping the agricultural sector (as labor) considerable potential in the community are considered. About 5/6 million women in the production Iran's agricultural sector involved. Activities related to planting, and harvesting, processing and preparation of animal feed, preservation and care of livestock and poultry and some related activities including marketing and sales field role and participation of rural women to sue.

One of the problems that governments and governments today should know the world should talk TVs? Especially in rural undeveloped countries. In these countries for lack of proper planning policies and improving quality of life of people in these areas, rural migration to cities has increased significantly is Urbanization and the incidence and growth problems and mental disorders, social, cultural and economic sectors especially in agriculture and animal husbandry provides guidelines and rural development requires a deep study and research in this field is that the governments can Planning and economic policy, social and cultural to help.

Creation of local organizations and regional organizations with a presence with the participation of women and rural residents to resolve problems such an important and influential cases that regional planning should be considered. Drfraynd system development and change advanced agricultural economy, the value of a woman who previously worked as unpaid family labor force was converted to money and he is placed at the disposal. Except for the agricultural sector is the main areas of rural women work in the other two parts of the economy, namely industry and services as well as the presence of rural women is very important in discussions of social and political participation of women, the most important issue in planning, decision making, implementation of decisions and evaluation results are.

Conclusion and discussion:

Aghaee in on research as “rural women’s role at economy of agriculture and their success at agriculture development programs “ further assessing their status at different countries and also emphasis on their participation at production activity of family , has expressed factors that led to ignorance of their role at economy of agriculture .

Lahsaee Zade at research as “assessing Iranian rural women’s role at economy arena: first assessed their position at occupation structure then has compared it with rural men’s occupation base. He expressed that rural women have equal importance compared to men.

Safiri, in his doctoral dissertation as “assessing quantitative and qualitative women’s occupation ant its relation to economic development” has considered some of their problems of occupation due to obstacles which refers to structure of countries. And some contain social-economic and cultural obstacles.

Changizi Ashtiani in one research as “counting women’s share at production of country” addressed that studying historical process of social and economic development of countries represent that at development process , lagged countries are those which proper and favorite balance between men and women’s participation wasn’t created , and also there isn’t any fair and equal opportunities to flourish creativity .

Rasool Pararabi , in his thesis as “assessing women at economic activities in rural area of Ramsar “ has shown that more than 96% of rural women , at least had participated at economic activity that was supplement for family income . But they don’t participate at basic decision making of family, in spite of their affective role and vast attendance at economy of family, and also they enjoy owning production factors, less.

Development Realization is impossible without women’s participation at different social-economic aspect. Therefore, to understand unknown, researchers should strive and take basic step in this regard. Some programs should be provided at national level as long term projects at the field of education and Cultural Revolution in order to create needed knowledge in society and in women to identify their rights, education and extending modern techniques, creating infrastructure facilities and also rural development.

Since, village is suitable place for agriculture and additional related activities, so it can be said that women’s role at village and possibility to institutionalize proper infrastructure, we able to have suitable perspective toward development process.

Agriculture part as one of the most important productive parts of country have critical responsibility in preparing needed food security that can help this part to access its major goal according to efficiency of

workforce up to proper level . In this regard, women play critical role. Nevertheless, they couldn't represent their abilities at this field, due to various limitations which women face. Among this especial attention to this group of society and preparing them supporting, educational and extensional services for them can help to remove their vast future problems , according to major role of this forgot group at agriculture activities and finally lead to increase and improve their efficiency about agriculture and consequently lead to increase welfare and comfort of rural society .

In order to be able to remove obstacles and problems of women's activity at villages , we should reinforce stamina if women's work by one exact and codified programming in order to be able to progress at one correct direction.

Villager access to education at different level , possibility to enjoy suitable occupation opportunities and also industrial , technical and healthcare equipment has caused that cities go out from concentration and attraction of inside and outside capitals , and so possibility of fair distribution of resources and facilities between city and village be provided , and government instead of bear heavy cost of urban population , spend these costs for rural development and support rural women whom get more damage while face lack of facility and compared to men enjoy less migration rate and also have to adopt existing conditions and use available facilities . In today world, it is impossible to achieve development goals without applying abilities of half of people of society (i.e. women).

Women at most countries, have low access to economic resources at the field of economic activity. They should reinforce them at this field by supplying economic facilities. Another part that changed women's attendance at economic affairs is agriculture activities. Opportunities which they gain at this part can have important impact on economic function and related social relations

Same discussions were presented about identifying women's role on environment changes (especially in preserving natural sources) that related to women's life and job. Women's access to agriculture credits, because increasing and improving their efficiency at agriculture. Women's membership at cooperatives, also help them to receive facilities in order to supply needed inputs of agriculture, sale productions and make some production with aim of increasing efficiency. Most of researches found that women's education is related to their agriculture efficiency. Indeed, years which women used educational programs, related to their productions meaningfully. So, by identifying their needs, demands and interests and also by determining their issues, resources and preferences, we should prepare proper extensional and educational programs for them.

Also literacy programs and generally their basic education should be considered specifically with aim of better women's enjoyment of extensional and educational programs. And also access opportunities to different resources and needed inputs at agriculture activity should be provide for them. Development programs for rural women mostly have certain importance that should be considered at extension activity.

Empowering women is one of principal discussions of development process for many countries of today world. existing factors contain women's education , their ownership sample , their occupation opportunities and function of labor market but if we go beyond this rather classic variables , these factors also contain occupational relations nature , how to behave family and generally society with economic women's activity and economic and social conditions which encourage or prevent change at these moods .

Last conclusion is that men and women, play role at agriculture programs and rural development but each has different needs and knowledge base on kind of their activities, since total people activities were done to supply their needs and so governments should consider regional programming in their policy making and programming. This issue dose not achieved unless by identify climate, population, cultural, economic and politic constituents of each region and also kind of relation of these constituents with constituents outside village and region.

These kinds of study and recognition have provided causes of better programming and adopted with needs of region, and prevent loss of investment. If education, health, occupation, cure and … facilities be provided in village and improving rural life level be considered, so migration would be regulated. At the other hand, protecting agriculture and livestock products and local industry, and attracting well condition markets for it, by governments, can be affective for villager's interest about rural life. Finally, positive attitude of development programmers, would help significantly to improve condition of one benefited rural family, and would act as a factor to diminish gap between urban family and rural family.

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Interactions between insulin like growth factor 1, thyroid hormones and blood energy metabolites in cattle with postpartum inactive ovaries

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Abstract: The relationship among insulin-like growth factor-1, thyroid hormones, energy metabolites and ovarian activity was investigated in cattle with postpartum inactive ovaries. The study was conducted on two groups of cows. The first group consisted of 10 cows with postpartum inactive ovaries (non-cyclic cows) based on rectal and ultrasonographic examination. The second group consisted of 8 cows in estrus (cyclic cows). The evaluated parameters included serum concentrations of variables of energy metabolites such as glucose (GLU), total lipids (TL), triglycerides (TG) and total cholesterol (TCH). Serum concentrations of total proteins (TP) were also measured. The hormones evaluated in this study included metabolic hormones such as insulin like growth factor-1 (IGF-1), thyroxine (T4), and triiodothyronine (T3) in addition to hormonal indicators of ovarian activity as progesterone (PRO) and estradiol (E2). The results revealed that serum levels of E2 was significantly lower (P<0.05) in the non-cyclic cows compared with the cyclic group. Serum GLU concentrations showed a significant decrease (P<0.05) while TL and TCH were significantly increased (P<0.05). Metabolic hormones profile demonstrated a significant decrease (P<0.05) in IGF-1, T4 and T3 in cows with inactive ovaries compared to the cyclic cows. Correlations between the monitored variables indicated that there was a significant positive correlation (P<0.05) between GLU and E2 and a significant negative relationship between TL, TCH and E2 (P<0.05). We reported a significant positive correlation (P<0.05) between IGF-1 and E2, GLU, T4 and T3. T4 was positively correlated (P<0.05) with E2, GLU, IGF-1 and T3, while a significant negative relationship between T4 and both TL and TCH was recorded. There was also a significant positive correlation between T3 and E2, GLU, IGF-I and T4. Significant positive correlation between TL and TCH was found while serum glucose showed no strong correlations with other energy-related metabolites. These results suggest that incidence of low reproductive performance in the postpartum lactating cows is associated with a decrease of some metabolic hormones such as IGF-1, T4 and T3 and the alterations seen in these hormones could be tightly related with changing in energy metabolites suggesting that energy influences ovarian activity in postpartum lactating cows possibly through changes in secretory patterns of these metabolic hormones.

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1. Introduction

Inactive ovary, also called true anestrus is a condition in which the ovaries are quiescent without signs of cyclicity or cycle related ovarian structures (Zulu et al., 2000). The condition is most frequently observed in high yielding dairy cows, first calf heifers or in postpartum lactating cows. From an economic point of view, poor reproductive efficiency caused by ovarian inactivity is the most important obstacle for increasing an animal productivity that can result in considerable economic losses in Egypt and all over the world (El-Khadrawy et al., 2008).

Many factors can predispose to and exacerbate the problem such as nutritional intake, high production, energy deficiency, parasites, adverse climatic conditions, management stress and diseases. These factors compounded with lactation can further extend the postpartum anestrus period (Ahmed, 2007).

Nutrition has long been known to have a profound influence on reproductive performance of female cattle, but measures of postpartum ovarian activity have been more closely related to energy balance (EB) (Beam and Butler, 1999; Gong, 2002; Armstrong et al., 2003; Butler et al., 2006,). Lack of energy has been proved to adversely affect the size and ovulatory fate of the dominant follicle (Lucy, 1992; Kruip et al., 1996; Diskin et al., 2003) but the underlying mechanism remains poorly understood.

While, many investigations focused on the modulatory effect of energy intake on hypothalamic-pituitary axis, recent studies have tested the hypothesis that energy balance influences ovarian follicle development in cattle possibly through changes in some metabolic hormones that act as nutritional signals exert a direct effect at the ovarian level (Gong, 2002; Zulu et al., 2002; Spicer and Aad,

2007; Rhoads, 2007) and whose their production and release are dynamically regulated by energy balance (Spicer et al., 1991).

Insulin-like growth factor (IGF-I) was documented to be one of the most important potential hormonal mediators of ovarian function and has been reported to play a critical endocrine role controlling nutrient metabolism in cattle (Spicer et al., 1990; Giudice, 1992). The hormone has been reported to act in synergy with FSH and LH in stimulating bovine granulosa and luteal cell steroidogenesis (Ahmad et al., 1996; Stewart et al., 1996). Also other factors such as thyroid hormones have been evaluated as potential regulators in ovarian steroidogenesis (Spicer et al., 2001). Very little data about the relationship between these metabolic hormones and postpartum ovarian activity in cows under our environmental conditions are available. Therefore, the aim of the current investigation was first to identify the patterns of secretion of IGF-l and free thyroid hormones in cows with postpartum inactive ovaries. A second objective was to evaluate associations of serum concentrations of these metabolic hormones with some blood energy metabolites and indicators of ovarian activity to throw light on the role of these hormones on the ovarian activity through establishing the effect of energy on secretory profiles of these hormones.

2. Material and Methods

Cattle:

A total of 18 lactating cows were used in this study. The cows were divided into two groups; the first group (non-cyclic cows) consisted of 10 cows aged from 5-8 years with inactive ovaries that were not observer in postpartum oestrus for over than 4 months. Inactive ovaries were diagnosed by two consecutive rectal examinations which revealed bilateral small ovaries which were flat and smooth. The diagnosis was confirmed by ultrasonographic examination. Ultrasonography was done by linear array scanner which produces a real time B-mode image (scanner 480-Vet-scan, Pie medical Co.). Scanner was equilibrated a five MHz transducer designed for intrarectal insertion in cow. The second group (cyclic cows) was consisted of 8 cows in estrus and was considered as control. The cows were apparently healthy, in good body condition and had no history of other reproductive disorders. No treatments with hormones were administered during the last 2 months.

Blood samples:

Blood samples were collected from both groups and serum harvested from blood samples was kept at -20 ^{0}C until assayed for the following parameters:

Hormonal indicators of ovarian activity:

Serum progesterone (PRO) and estradiol (E2) levels were determined by Enzyme-linked Immunosorbent assay (ELISA) micro-well technique using kits supplied by Hellabio biokits company (USA) and following the manufacturer's instructions.

Blood energy metabolites:

The serum values of variables of energy metabolism including glucose (GLU), total lipids (TL), triglycerides (TG) and total cholesterol (TCH), in addition to total protein (TP) were assessed by spectrophotometric method using commercial diagnostic kits of Spinreact S.A.Co (Spain).

Metabolic hormones:

Serum concentrations of IGF-1, thyroxin (T4) and triiodothyronine (T3) were measured by radioimmunoassay (RIA) using commercial kits supplied by Synbiotics Corporation, 11011 via Frontera, San Diego, according to the manufacturer's instructions.

Statistical analysis:

All results were expressed as mean ±standard error (SE). Statistical differences between the two groups were compared using Student's t-test at 0.05 level of probability. Correlation between two specific monitored variables was determined with the Pearson's simple correlation method. A difference was considered significant at $P < 0.05$.

3. Results

Hormonal indicators of ovarian activity:

The results shown in (Table 1) reveals that serum concentrations of estradiol were significantly decreased in the non-cyclic cows ($P<0.05$) compared to the cyclic group. No significant differences in serum levels of progesterone between the two groups were observed.

Blood energy metabolites & total proteins:

Cows with inactive ovaries demonstrated a significant decrease in the mean values of blood glucose levels ($P<0.05$) in comparison to the control cyclic group (Table 2). The mean values of serum total lipids and total cholesterol were significantly higher ($P<0.05$) in the non-cyclic cows. The average values of triglycerides and total protein did not show significant differences between the two groups.

Table 1. Serum concentrations of PRO and E2 in cows with postpartum inactive ovaries compared to the control cyclic group (Values are means ± SE).

Hormone	Cyclic	Non-cyclic
PRO (ng/ml)	0.47±0.01	0.44±0.01
E2 (pg/ml)	14.56±0.23	9.02±0.16*

Significant differences in the values between the two groups were indicated by * P < 0.05.

Table 2. Serum values of some energy metabolites and total protein in non-cyclic cows compared to the control cyclic group. (Values are means ± SE).

Variable	Cyclic	Non-cyclic
GLU (mg/dl)	110.00±5.20	73.40±1.71*
TL (mg/dl)	404.43±1.94	414.00±1.73*
TG (mg/dl)	80.46±0.31	79.70±0.35
TCH (mg/dl)	162.13±1.15	174.64±3.51*
TP (g/dl)	7.66±0.03	7.06±0.07

Significant differences in the values between the two groups were indicated by * P < 0.05.

Metabolic hormones:

When compared to the cyclic group, non-cyclic cows showed a significant decrease (*P*<0.05) in the mean values of IGF-1, T4 and T3 (Table 3).

Table 3: Serum concentrations of IGF, T4 and T3 in cows with inactive ovaries compared to the control cyclic group (Values are means ± SE).

Hormone	Cyclic	Non-cyclic
IGF-1 (ng/ml)	53.16±1.41	38.46±1.58*
T4 (µg/ml)	5.12±0.08	3.23±012*
T3 (ng/dl)	108.17±0.57	87.86±2.38*

Significant differences in the values between the two groups were indicated by * P < 0.05.

Correlations between the monitored variables:

By assessment of correlations of changes in means between the monitored hormones and variables of energy metabolism, we recorded a significant positive correlation (*P*<0.05) between GLU and E2 and a significant negative relationship between TL, TCH and E2 (*P*<0.05) (Table 4). A significant positive correlation (*P*<0.05) was found between IGF-1 and E2, GLU, T4 and T3. T4 was positively correlated (*P*<0.05) with E2, GLU, IGF-1and T3, while a significant negative relationship between T4 and both TL and TCH was reported (Table 4). There was also a significant positive correlation between T3 and E2, GLU, IGF-1, and T4. By evaluation of correlation between variables of energy metabolism we found significant positive correlation between TL and TCH while serum glucose showed no strong correlations with other energy-related metabolites (Table 4).

4. Discussions

Cattle play an important role in the agricultural economy in many countries in the world. It is well known that the reproductive disturbances in cows are the main cause of their infertility, or even sterility, and reduced their productive efficiency (El-Khadrawy et al., 2008). Inactive ovary is a major and important reproductive hindrance in which, the ovarian follicles fail to reach mature size and ovulate (Zulu et al., 2000). The reason of this condition may be insufficient release or production of gonadotropins to induce follicular development or it may reflect the failure of ovaries to respond to gonadotropins (Ahmed, 2007).

In the present study cows with inactive ovaries were lactating and were not observed in postpartum oestrus for over than 4 months. Laboratorially inactive ovaries were indicated by the significant decrease in the serum concentrations of E2 and the low levels of serum PRO (0.44±0.01ng/dl) (Ahmed et al., 2010). Serum concentrations of PRO in both cyclic and non-cyclic cows were almost similar. In this study, the cyclic cows were in estrus phase (follicular phase) which is usually characterized by its low levels of PRO (Stabenfeldt et al., 1969; Díaz, 1986; Jazayeri et al., 2010).

Resumption of postpartum ovarian function is controlled by numerous systemic and intraovarian factors but previous reports have found that the ability of postpartum lactating cows to resume estrus is dependent upon their EB (Kendrick, 1997).

In this regard, the results of the present study revealed a significant decrease in serum concentrations of glucose in cows with inactive ovaries which may indicate lack of energy resulting from NEB due to increasing demands of the mammary glands for glucose for milk synthesis (Seifi et al., 2007; Gabriel Kováč et al., 2009). Glucose is the principal source of energy for the life processes of the mammalian cell. The mammary gland is a major glucose utilizing tissue, principally for biosynthesis of lactose, the predominant molecular species in milk (Kaneko et al., 1997). The only one precursor of lactose is plasma glucose (Kaneko et al., 1997). The two main sources of plasma glucose are absorption from the gut and gluconeogenesis (Kaneko et al., 1997). In ruminants, little glucose is absorbed from the gut, so the overwhelming bulk of it is synthesized through the process of gluconeogenesis that occurs mainly in liver by utilizing other substrates such as ruminal volatile fatty acids that result from fermentation of dietary glucose in the rumen (Kaneko et al., 1997).

Table 4. The correlation between the selected hormones and variables of energy metabolism in the non-cyclic group (Pearson's correlation test).

Parameter	IGF-1	T4	T3	PRO	E2	GLU	TL	TG	TCH
IGF-1	1	0.950*	0.968*	0.608	0.943*	0.985*	-0.737	0.628	-0.620
T4	0.950*	1	0.947*	0.778	0.987*	0.961*	-0.827*	0.557	-0.814*
T3	0.968*	0.947*	1	0.590	0.963*	0.927*	-0.811	0.770	-0.644
PRO	0.608	0.778	0.590	1	0.705	0.699	-0.460	0.142	-0.653
E2	0.943*	0.987*	0.963*	0.705	1	0.933*	-0.885*	0.608	-0.812*
GLU	0.985*	0.961*	0.927*	0.699	0.933*	1	-0.704	0.506	-0.659
TL	-0.737	-0.827	-0.811	-0.460	-0.885*	-0.704	1	-0.561	0.905*
TG	0.628	0.557	0.770	0.142	0.608	0.506	-0.561	1	-0.216
TCH	-0.620	-0.814*	-0.644	-0.653	-0.812*	-0.659	0.905*	-0.216	1

* Correlation is significant at the 0.05 level.

In lactating cows, if there is a mismatch between mammary drain of glucose for lactose synthesis and gluconeogenesis via inadequate energy intake, the cow will experience NEB and hypoglycemia will result (Kaneko et al., 1997).

On the other hand, the present results demonstrated significant higher values of total cholesterol in the non-cyclic cows. Increased lipolysis due to hypoglycemia will result in increased serum levels of low density lipoproteins (LDLs). The rate of cholesterol synthesis is increased with the associated increase in plasma LDLs concentrations and thus hypercholesterolemia results (Meyer and Harvey, 1998; Anna et al., 2004).

Hypothyroidism seen in the non-cyclic cows may be another strong reason for this hypercholesterolemia. Thyroid hormones stimulates LDLs receptors and promotes uptake of cholesterol, therefore lack of thyroid hormones results in decreased LDLs receptors and decreased cholesterol uptake (Anna et al., 2004) .This approach is further supported by the significant negative relationship seen between T4 and TCH (Table 4). Total lipids significantly increased in the non-cyclic cows probably due to hypercholesterolemia.

In the present work, we found significant positive correlations between the mean values of glucose and estradiol suggesting a role for glucose in the regulation and resumption of estrous cycling after parturition. Glucose appeared to play a role in the nutritional regulation of GnRH release and in turn pulsatile LH secretion. (Diskin et al., 2003). The same was mentioned by (Kruip et al., 1996) who suggested that lack of energy leads to a lower glucose concentration, inducing lipolysis and resulting in a lower LH release. Low glucose levels also could reduce follicular responsiveness to LH and ultimately shut down follicular estradiol production (Diskin et al., 2003). Decreased dietary energy intake in cyclic heifers was associated with decreased concentrations of progesterone in follicular fluid of small and medium follicles, and decreased size of large follicles (Spicer et al., 1991) while increasing EB was proportional to the number of large follicles in postpartum dairy cows with significant differences in follicular development related to body condition (Lucy et al., 1991). Poor expression of estrus has been reported to be associated with reduced plasma levels of L H and estradiol and inconsistent growth and development of ovarian follicles caused by NEB (Grimard et al., 1995).

The underlying mechanism of the effect of EB on reproductive activity remains poorly understood**.** However, several reports have implicated involvement of various metabolic hormones such as IGF-1in relation to the energy balance in the dynamic growth of ovarian follicles. These studies suggested that the changes in ovarian activity due to energy

shortage are a result of direct actions of these metabolic hormones on the ovary (Beam and Butler, 1999; Gong, 2002; Armstrong et al., 2003) but such reports are very little in Egypt.

IGF-1 has been postulated as a potent activator of ovarian follicular growth. Specifically, IGF-1 in conjunction with gonadotropins have been reported as an important stimulators of mitosis and ovarian steroid production by granulosa and theca cells, which are required for normal oocyte development and hormonal feedback signalling to the hypothalamus and pituitary (Spicer and Aad, 2007; Tosca et al., 2008; Grado-Ahuir et al., 2009; Kolesarova1 et al., 2009).

Although many of the previous studies tended to monitor the levels of IGF-1 in the follicular fluid, there is strong evidence that direct nutritional effects on ovarian function appear to operate through hepatic rather than follicular regulation of IGF-1, and on systemic concentrations of IGF-I (Diskin et al., 2003).

Based on these reports, we evaluated the serum secretory pattern of IGF-1 in relation to the variables of energy metabolites and elements of reproductive activity. The data demonstrated that serum concentrations of IGF-1 were significantly lower in cows with inactive ovaries (Table3). Similar findings were reported by Zulu et al., (2002) who found that IGF-1 levels were higher and rose sharply in cows that cycled normally than in cows with inactive ovaries.

Moreover, significant positive relationship was found between IGF-1 and serum concentrations of E2 supporting the identification of IGF-1 as an important metabolic modulator of postpartum ovarian activity in cows.

In cattle, increases in the population of small ovarian follicles were associated with increases in circulating concentrations of IGF-1 (Gong, 2002). Stimulatory effects of IGF-1 on estradiol production by mammalian granulosa cells were also well documented. Some studies reported that follicular fluid IGF-1 levels increased as follicular fluid estradiol and follicle size increased (Echternkamp et al., 1994). These effects were due in part to its ability to enhance the action of gonadotropins on ovarian follicular steroidogenesis by increasing theca-interstitial cell LH binding affinity and/or binding capacity (Cara et al., 1990).

Since many studies have suggested that the influence of IGF-1 on ovarian activity is related to EB, statistical correlations between IGF-1, estradiol and some variables of energy metabolites were performed (Table 4). The results indicated strong positive relationships between changes in blood glucose, peripheral IGF-1 and estradiol. Thus, it appears that IGF-1 is likely acts as a mediator of energy induced alterations in ovarian function in the postpartum period in cows. Rhoads et al., (2008) stated that in liver, growth hormone receptor and IGF-1 production are dynamically regulated by lactation and energy balance. Reduced IGF-1 secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing the expression of estrus (Spicer et al., 1990).

At the molecular level, dietary energy intake has been proved to affect the expression of mRNA encoding components of the ovarian IGF system and these changes can directly increase the bioavailability of intrafollicular IGF-1. This, in turn, can increase the sensitivity or response of follicles to FSH and is one mechanism through which nutrition can directly affect follicle recruitment (Armstrong et al., 2003).

In regard to the thyroid hormones, the present results revealed that significant lower values of T4 and T3 were observed in cows with inactive ovaries with a strong positive relationship was detected between the two hormones and E2.

An association between thyroid hormones and reproductive efficiency was plausible. Thyroid hormone receptors and /or their mRNA have been detected in porcine (Maruo et al., 1992) and human Zhang et al., 1997). Others demonstrated an augmentation of progesterone secretion and, to a lesser extent, estradiol secretion into the human granulosa cells medium by the addition of thyroid hormone to the medium of human granulosa cells in vitro (Wakim et al., 1995).

In buffalo cows hypothyroidism was associated with cessation of behavioral signs of estrus as well as low plasma progesterone levels (Ahmed and Ezzo, 1998).

In cattle studies demonstrated that follicular fluid contains the free fractions of thyroid hormones suggesting that thyroid hormones are required for bovine ovarian follicular function (Blaszczyk et al., 2006).

The exact mechanism by which the thyroid hormones regulate steroidogenesis is not well known. It was supported that thyroid hormones may have direct stimulatory effects on ovarian function via synergistic action with follicular stimulating hormone (FSH) to induce the differentiation of granulosa cells (Spicer et al., 2001). Although T3 and T4 had little or no effect on aromatase activity, they could provide important estrogen precursors to granulosa cells and thus indirectly increase estradiol production which is the primary hormone stimulating estrous behavior (Spicer et al., 2001). Interestingly, Blaszczyk et al (2006) found a significant negative correlation between levels of T4 and T3 and cholesterol in the follicular fluid of bovine follicles suggesting that the

modulatory influence of thyroid hormones on steroidogenesis in bovine follicles may consist in free thyroid hormones participating in intra-follicular metabolism of cholesterol which is the primary substrate of steroid synthesis. This hypothesis is further confirmed in this study by the significant negative relationship found between T4 and TCH (Table 4). Additionally, a significant positive relationship was reported between T4 and T3 and GLU. Hypoglycemia has been reported to be associated with a decrease in hypothalamic thyrotropin-releasing hormone and pituitary thyroid stimulating hormone (Leung et al., 1975). Also an increase in serum T3 was observed following glucose ingestion (Koh et al., 1994) or during a short term glucose infusion (Langer and Gschwendtova, 1999). These findings suggest that thyroid hormones are important modulators of bovine ovarian function partly via alterations that involve energy metabolism.

5. Conclusions

The results presented above can provide new supportive evidence about the importance of energy balance in the resumption of postpartum reproductive efficiency in the lactating cows. Our results also demonstrate that the incidence of low reproductive performance in the postpartum lactating cows is associated with a decrease of some metabolic hormones such as IGF-1, T4 and T3 which are equally important metabolic modulators of postpartum ovarian activity. The results suggest, however, a necessity to carry out further studies in order to profile the secretory patterns of these hormones in peripheral blood together with the pattern of their concentrations in the follicular fluid in the same animal. Additionally, the positive relationships between changes in some energy metabolites, peripheral IGF-1, thyroid hormones and blood indicators of ovarian activity support the identification that the effect of alterations in energy balance on postpartum ovarian function could be mediated through the effect of these alterations on secretory patterns of these metabolic hormones. Finally these data may improve our understanding of some factors associated with the ovarian activity in the postpartum lactating cows and how these factors are interacted which may lead to better methods for reproductive management.

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Nuclear Research Reactors Accidents Diagnosis Using Genetic Algorithm/Artificial Neural Networks

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Abstract: The Nuclear Research Reactors plants are expected to be operated with high levels of reliability, availability and safety. In order to achieve and maintain system stability and assure satisfactory and safe operation, there is increasing demand for automated systems to detect and diagnose such failures. In recent years, both Genetic algorithms and neural networks, which are inspired by computation in biological systems, are emerged as established techniques for optimization and learning. Genetic algorithms have been used in conjunction with neural networks in three major ways: First, genetic algorithms have been used to construct neural network topologies. Second, they have been used to set the weights in fixed architectures. Third, they have been used to select training data and to interpret the output behavior of neural networks. This paper is concerned with the construction of Artificial Neural Networks (ANNs) using Genetic algorithms (GAs) for the nuclear accidents diagnosis. MATLAB ANNs toolbox and GAs toolbox are employed to optimize an ANN for this purpose. When we apply the results obtained from genetic algorithms on the back-propagation algorithm, the results are similar but the design of ANNs using GAs is useful in terms of automating and optimizing the design and finding weights and biases for the suggested construction. The results obtained show the efficiency of using genetic algorithm, which can construct the high performance neural network structure for the nuclear reactor's input data. The best structure obtained is two layers ANN with correspondence values of weights and biases that are required to construct such network. [Abdelfattah A. Ahmed; Nwal Ahmed Alfishawy; Mohamed A. Albrdini, and Imbaby I. Mahmoud. Nuclear Research Reactors Accidents Diagnosis Using Genetic Algorithm/Artificial Neural Networks. Nature and Science 2011; 9(5):64-74]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Genetic algorithms (GA), Artificial Neural Networks (ANN), Nuclear Reactors, Accidents Diagnosis, MATLAB.

1. INTRODUCTION

As the systems become more complex and they need to operate with minimum malfunctioning or breakdown time, reliable automated fault detection and diagnosis system are increasing rapidly. The early detection of plant's failure could prevent system malfunction or serious damage, which could also lead to disaster. Therefore, an intelligent fault detection and diagnosis system to deal with inaccurate information has also been greatly required [1-5].

Genetic Algorithms and Neural networks are two techniques for optimization and learning, each with its own strengths and weaknesses. The two have generally evolved along separate paths. However, recently there have been attempts to combine the two technologies. The genetic algorithms implemented in the toolbox let to solve optimization problems with nonlinear, linear, and bound constraints. The genetic algorithm improves the chances of finding a global solution, due to its random nature. [5-10].

In this paper, diagnoses of nuclear reactor accidents are investigated. A computer program is developed using MATLAB environment for this purpose. Genetic algorithm routine is implemented and employed to construct an artificial neural network, to diagnose the nuclear reactor accidents. The program is capable of plotting the various relations between the neural network output and the required target. The program stops calculation when the mean square error or sum square error reaches a desired minimum or met one of the default stopping criteria. The proposed NN constructed by GA outperforms backpropagation alone, which is the standard training algorithm, on our reactor accidents data case. This performance gain comes from tailoring the genetic algorithm to the domain of training neural networks (reactor accidents data). The results of this program are demonstrated by a series of screens shots and output plots. Comparing with results published in [1], a significant improvement is obtained. This paper is organized as follows: Section 2 presents the problem formulation. Section 3 describes the proposed system. Section 4 shows the results and discussions of the proposed system performance. Section 5 is devoted to conclusion.

2. PROBLEM FORMULATION

The system is designed to construct an ANN using Genetic algorithms (GAs) for accidents diagnosis of the nuclear reactor's input data. MATLAB toolboxes

are used to implement the GA which produces the optimized values of weights and biases that are required to construct such network. The data used in the application were collected by the aid of reactor operation crew and Safety Analysis Report (SAR) of the reactor, in addition to the Atomic Energy experts. The data sets are for the eight accidental cases (Classes) listed below; plus the normal operation case as shown in Figure (1). So the total cases, which we have, are nine. The result is that each accident is represented as a 15 by 1 grid of Boolean values [1, 2].

```
[0 0 0 0 1 1 1 1 1 1 1 1 1 1 1]
[0 0 0 0 0 1 1 1 1 1 1 1 1 1 1]
[1 1 0 1 1 0 0 1 1 1 1 1 1 1 1]
[1 1 1 1 1 1 1 0 1 1 0 1 1 1 1]
[1 1 1 1 1 1 1 0 0 1 0 0 1 1 1]
[0 0 0 0 1 1 1 0 0 0 1 0 1 1 1]
[1 1 1 1 1 1 1 1 1 1 1 0 0 0 0]
[1 1 0 0 1 1 1 1 1 1 1 0 0 0 0]
[1 1 1 1 1 1 1 1 1 1 1 1 1 1 1]
```

Figure (1): Sample of reactor accidents data patterns.

In optimization problems a set of parameters is selected that will give the best solution to a particular problem. To present the initial values of these parameters to a GA, it must be encoded into a string so that crossover and mutation can be applied. Binary encodings are the most common, due to the fact that Holland [9] used them in his early pioneering work. In choosing an encoding scheme the nature of the problem will play a major role. So, in the reactor accidents diagnosis, the binary encoding becomes practical as the accidents patterns are in binary form. Any base can be used, as it is just a different method of encoding the same information, but the lower the base the longer the string will be [6-9].

3. PROPOSED SYSTEM

In this section, we will explain the proposed system modules. It is consisted of an m file program for GA computation and a Graphical User Interface (GUI) for easy communication with the program and experimentation with alternatives. Matlab environment is used to implement the system.

3.1 Program Description

The graphical user interface (GUI) is depicted in figure (2), which is used to guide the user for the optimization process.

Calculation is started by clicking *'Calculate'* button, where the calculation program begins with calculating the different parameters (the weights and biases, the transfer functions and the performance functions) for the two and the three layers of the ANN.

3.2 The Implemented GA.

A block diagram of the implemented GA is shown in Figure (3). The genetic algorithm begins by creating a random initial population. If the minimal point for the fitness function is known approximately where it lies, the initial range should set so that the point lies near the middle of that range. However, the genetic algorithm can find the minimum even with a less than optimal choice for initial range.

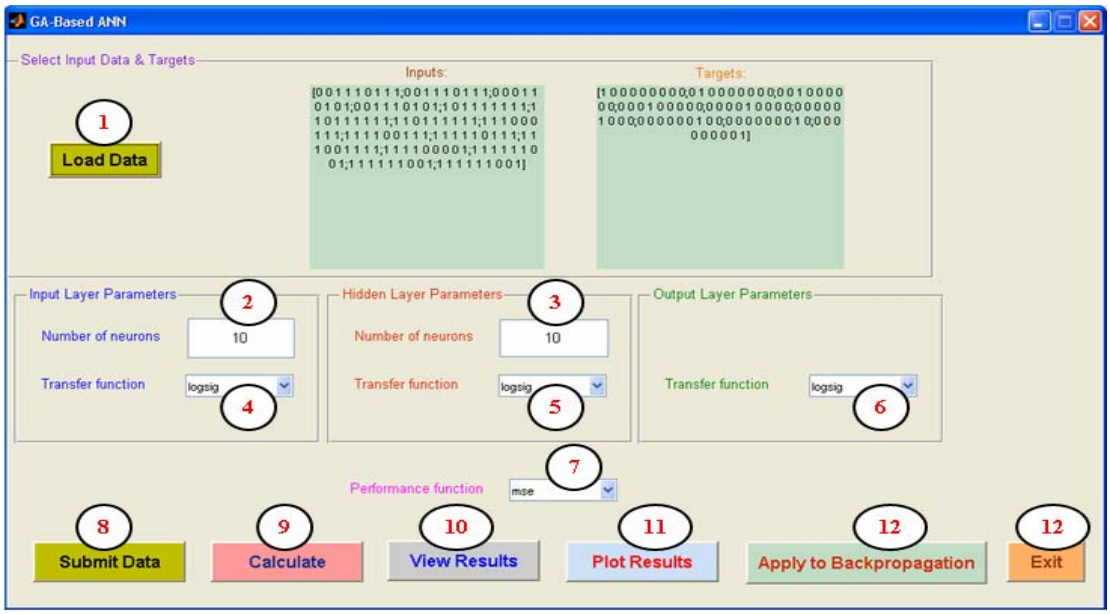


Figure (2): The program graphical use interface (GUI)

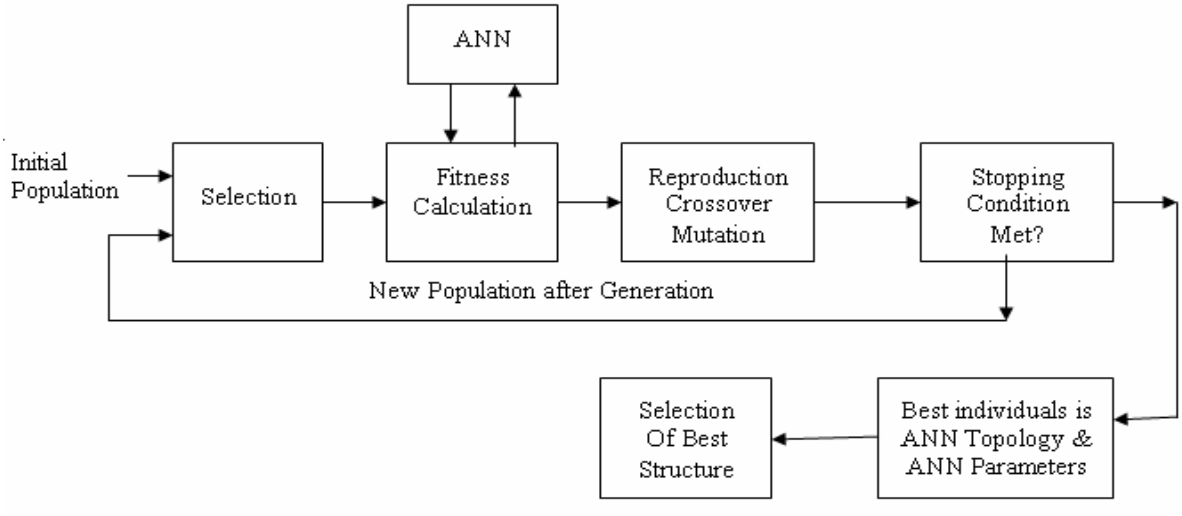


Figure (3): Artificial Neural Network and Genetic Algorithm

A genetic algorithm is used to find a good topology and parameters for a neural network. Using neural network as the *fitness function*, GA determines the fitness level of each *topology and parameters*. Using fitness values, the genetic algorithm would then evolve a new population for the network to try. After several generations, a population of several "good" structures with parameters evolves and fittest topology and parameters are used as the best construction of the neural network.

The genetic algorithm uses the individuals in the current generation to create the offspring that make up the next generation. Then, the algorithm creates a sequence of new populations. To create the new population, the algorithm scores each member of the current population by computing its fitness value. The Fitness Value of the individual Vi of GA is calculated as follows:

- The chromosome V_i string and the corresponding settings of ANN with reference the choices from the program GUI.
- Designed ANN model as per the string and the network choices. Predicted output y_j is given as $y_j = f(x_j, W, b)$, where x_j is input vector, W is weight matrix and b is bias matrix.
- Performance is checked by calculating fitness values and is expressed in terms of Mean-Squared Error (*MSE*) or Sum-Squared Error (according to the choice of the performance function from GUI) as:

$$SSE = \sum_{i=1}^{N_p} \sum_{k=1}^{K} (t_{i,k} - y_{i,k})^2 \quad \text{--(1)}$$

$$MSE = \frac{1}{N_p K} \sum_{i=1}^{N_p} \sum_{k=1}^{K} (t_{i,k} - y_{i,k})^2 \quad \text{--(2)}$$

Where N_p and K denote the number of patterns and output nodes used in the training respectively, i denotes the index of the input pattern (vector), k denotes the index of the output node, $t_{i,k}$ and $y_{i,k}$ express the desired output (target) and actual output values of the k^{th} output node at i^{th} input pattern, respectively. The calculation of the output is according to figure (4) for two layers network using equation (3) and figure (5) for three layers network using equation (4)

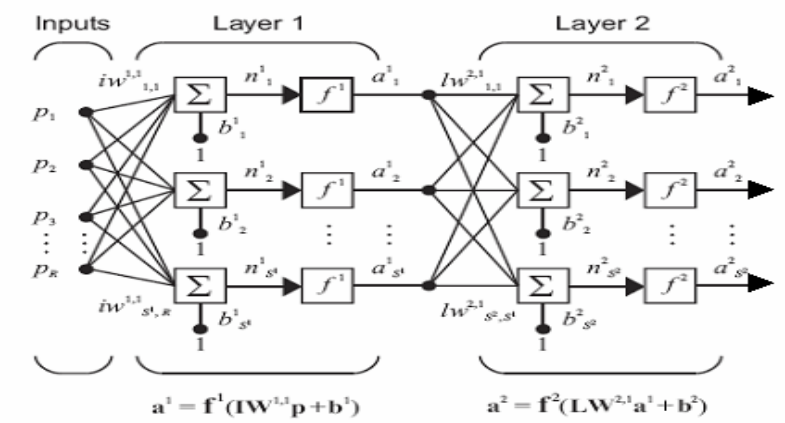


Figure (4): The output of two layers Artificial Neural Network structure

$$a^2 = f^2\,(LW^{2,1}\,(f^1\,(LW^{1,1}p + b^1) + b^2) = y_j\,, \qquad (3)$$

where j is neurons in the output layer,

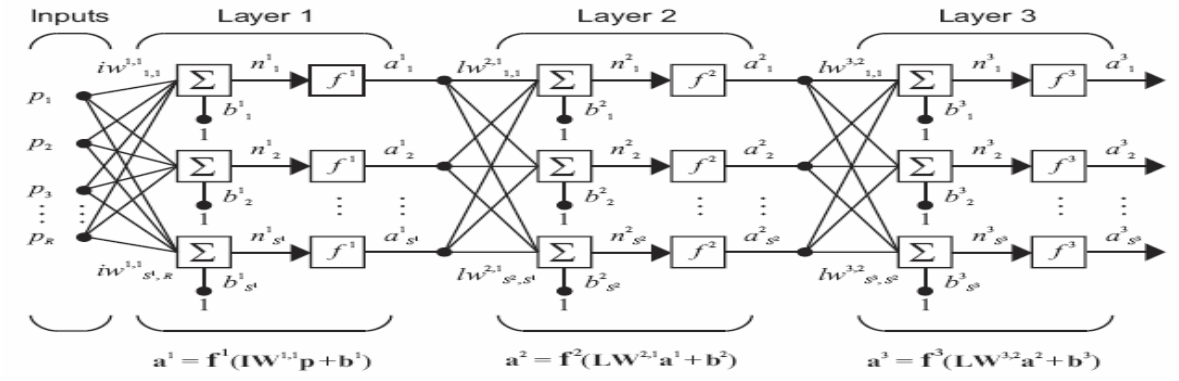


Figure (5): The output of three layers Artificial Neural Network structure

$$a^3 = f^3\,(LW^{32}\,f^2(\,LW^{2,1}\,(\,f^1\,(\,LW^{1,1}p + b^1\,) + b^2) + b3) = y_j\,, \text{--(4)}$$

where j is neurons in the output layer,

The setting for GA is shown in Table (1). Based on speed consideration some values set for population size and evaluation generation a little small.

3.2.1 Algorithmic Setting

Table (1): Parameters setting for GA.

Setting Type	Value
Encoding Scheme	Binary Encoding
Population Size	100
Selection	Stochastic Uniform
Crossover	Uniform
Mutation	Random
Elitism	Yes
Stall Time Limit	20
Display	iteration

a) The **selection** function chooses parents for the next generation based on their scaled values from the fitness scaling function. An individual can be selected more than once as a parent. The default selection option, *stochastic uniform*, lays out a line in which each parent corresponds to a

section of the line of length proportional to its scaled value. The algorithm moves along the line in steps of equal size. At each step, the algorithm allocates a parent from the section it lands on.

b) Some of the individuals in the current population with the best fitness values are chosen as *elite*. The default setting is 10% of the current population size. These *elite* individuals are passed to the next population. Setting Elite count to a high value causes the fittest individuals to dominate the population, which can make the search less effective.

c) Besides elite children, the algorithm:

- Creates *crossover* children by selecting vector entries, or genes, from a pair of individuals in the current generation and combines them to form a child. The default setting is 80% of the current population size, after excluding the elite children. With some probability *P* (typically between 0.5 and 0.8), it is decided how parent contribute to the gene values in the offspring chromosome. For example:
  Parent1 : 11001010
  Parent2 : 00110111
  If *P* is 0.5 :
  Offspring 1 : Parent1 contributes ***odd*** positions, Parent2 contributes ***even*** positions
  Offspring 2 : Parent1 contributes ***even*** positions, Parent2 contributes ***odd*** positions
  This is means approximately half of the genes in the next offspring will come from Parent1 and the other half will come from Parent2. Example:
  - Contribution of Parent1 : 1 0 1 1
  - Contribution of Parent2 : 0 0 1 1
  ----
  * New Offspring1 : 10001111
  * By the same way New Offspring2 : 01100010
- Creates *Mutation* children by applying random changes to a single individual by altering one or more genes in the chromosome from its initial state. The default setting is 20% of the current population size, after excluding the elite children. The mutation process is illustrated as the following:
  7 6 5 **4** 3 2 1 0 bit position
  Before Mutation : 1 0 1 **1** 0 1 1 1
  After Mutation : 1 0 1 **0** 0 1 1 1
  Random mutation exchanges a random selected gene with a random value within the range of the genes minimum and maximum value.
- Crossover rate generally should be high, and mutation rate should be very low

d) Replaces the current population with the children to form the next generation.

e) The stopping criteria, as Generations, Time limit, Fitness limit, Stall generations (The algorithm stops when the weighted average change in the fitness function value over Stall generations is less than Function tolerance), Stall time limit (The algorithm stops if there is no improvement in the objective function during an interval of time in seconds equal to Stall time limit.), Function Tolerance (The algorithm runs until the weighted average change in the fitness function value over Stall generations is less than Function tolerance.) and Nonlinear constraint tolerance (The Nonlinear constraint tolerance is not used as stopping criterion. It is used to determine the feasibility with respect to nonlinear constraints.), stops the algorithm as soon as any one of these conditions is met. You can specify the values of these criteria in the Stopping criteria pane in the Optimization Tool or by the function 'gaoptimset' from the command line.

4. RESULTS AND DISCUSSION

4.1. Results of Constructed ANN by GA

The output response, after finishing the calculation, show a 9-by-9 matrix with diagonal values larger than 0.9, as in the figure (6-a). Then, when these diagonal values rounded, it approximately equal 1, as in the figure (6-b), and that is exactly the required target. This means that the constructed neural network is the best for the reactor accidents data. While the best structure is obtained for the ANN, the correspondence values of weights and biases, that are required to construct such network, are also calculated by the program. Figures from (7-1) through (7-4) display the calculated output provided by the proposed system. Figure (7-1) show layer1 weight matrix 10x15, that is required to construct layer one. Figure (7-2) show layer1 bias matrix 10x9, that is required to construct layer one. Figure (7-3) show layer2 weight matrix 9x10 that is required to construct layer two. Figure (7-4) show layer2 bias matrix 9x9, which is required to construct layer two.

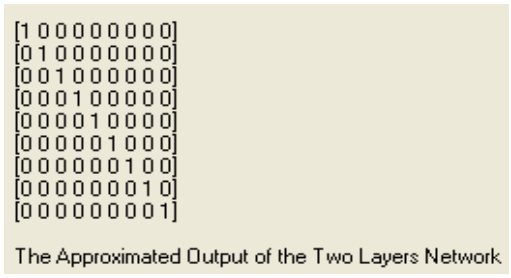


Figure (6-a):

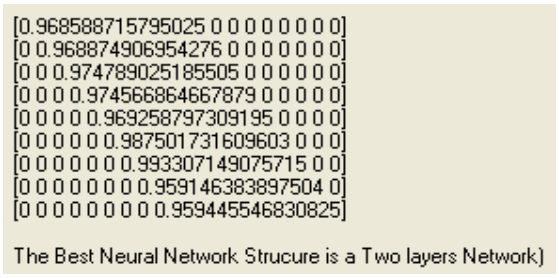


Figure (6-b):

1.294174	0.403967	0.022914	0.244113	0.616213	0	0	0.55837	0.852117	0.220866	0.100933	0	1.338927	0.308076	0.159786
0.718047	0.114096	0.052566	0.104471	0.093289	0.10646	0.10646	0.596243	0.143794	0.168883	0.095424	0.116833	0.101025	0.015186	0.190239
0.119424	0.121144	1.467614	0.196043	1.05597	0.666791	0.107896	0.546831	0.134914	0.109544	0.701517	0.139553	0.339345	0.095098	0.921141
0.002896	0.237737	0.134907	0.821212	0.007631	0.354783	0.006918	0.004494	0.030706	0.004918	0.808678	0.607187	0.003464	0.003464	0.634602
0.232903	0.24205	0.24126	0.771206	0.396973	0.410102	0.410102	0.877658	0.303567	0.405242	0.312016	0.506578	0.460419	0.460419	0.490105
0.896784	1.11265	1.020514	6.09E-06	0.411697	8.71E-05	1.1482	0.342579	1.000721	0.020681	0.175393	0.061857	0.228265	4.93E-05	0.245899
0	0	0.105134	0	0	0	0.120325	0.13839	0.445451	0.43366	0.809465	0.809116	0	0.81693	0.813953
0.41986	0	0	1.18536	0.001899	2.22027	0	0.562003	0.133793	0.496002	0.15001	0.004008	0.505742	0	0
0	0	0	0	0	0.000324	0.072711	0	0	0	0.081883	0	0	0	0.203292
0.842985	0.001318	0.287045	0.741289	0.547496	0	1.04216	0.696164	0.749939	0.657586	0.002687	0	0.001152	0.738568	0.428855

Figure (7-1): Layer1 weight matrix (IW11) 10x15

4.69E-06	0.821539	0.22262	0.000174	0	0.113669	0.341775	0	0
0.016709	0.186577	0.023529	0.019666	0.297388	1.23353	0.02192	1.204812	0
1.136521	0.019397	0.043312	0.322597	0.593459	0.043481	0.258947	0.615361	0.520055
0.000974	0.07822	1.316873	0.488916	1.6907	0.34788	0.534693	0.003247	0.978904
0.855429	0.785634	0.315264	0.12171	1.303084	0.297257	1.475311	2.56E-06	0.23909
0.688354	1.090398	0.8214	0.67968	0.516206	8.85E-05	1.302364	0.391155	1.968722
0.461741	2.115235	0.826562	0.964289	0	0.668071	0	0.765233	0.84226
0.034372	0.366686	0.27244	0.764833	0.344254	0.336583	0.74381	0.527595	1.80E-06
0.903312	0.000124	0.367796	0.05299	0.785721	0.030262	0.018436	0.849421	0.81607
0.766573	0.000381	7.37E-09	0.536192	0.892741	0.804116	0.000496	2.02E-06	0.198955

Figure (7-2): Layer1 bias matrix (bi1) 10x9

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
0.819534	0.261904	0	0.662799	0.539179	0	1.520552	2.64707	0.779306	0.738034
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	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Figure (7-3): Layer2 weight matrix (LW11) 9x10

2.843221	0	0	0	0	0	0	0	0
0	5	0	0	0	0	0	0	0
0	0	2.922706	0	0	0	0	0	0
0	0	0.349029	6.40E-05	0	0	0.191728	0.441004	0.908459
0	0	0	0	3.37511	0	0	0	0
0	0	0	0	0	4.089659	0	0	0
0	0	0	0	0	0	2.945033	0	0
0	0	0	0	0	0	0	2.871964	0
0	0	0	0	0	0	0	0	2.877407

Figure (7-4): Layer2 bias matrix (bi2) 9x9

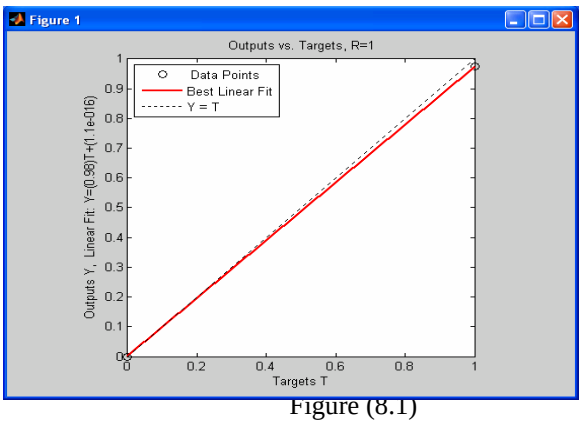
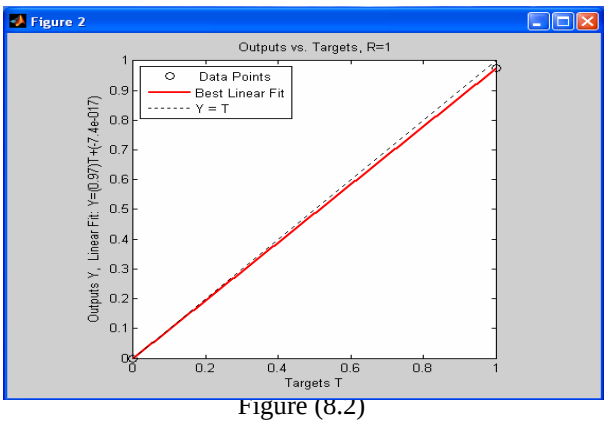


Figure (8.1)



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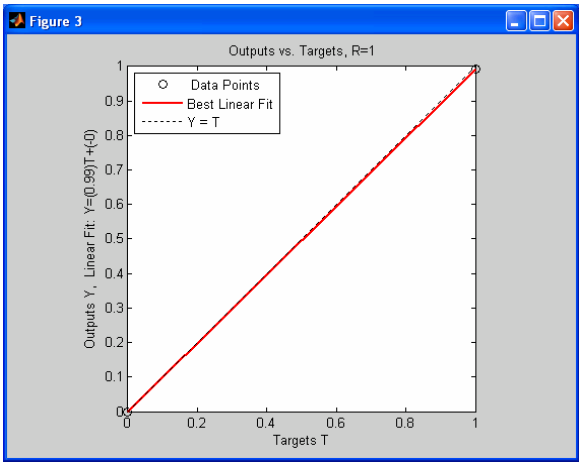

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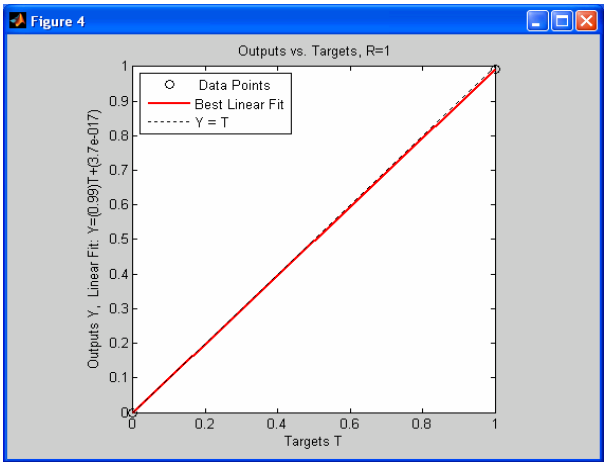

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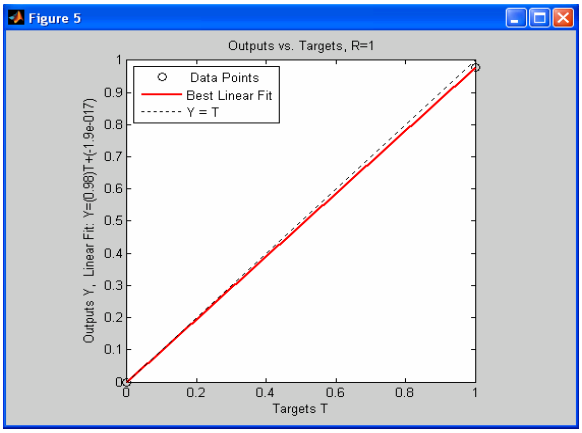

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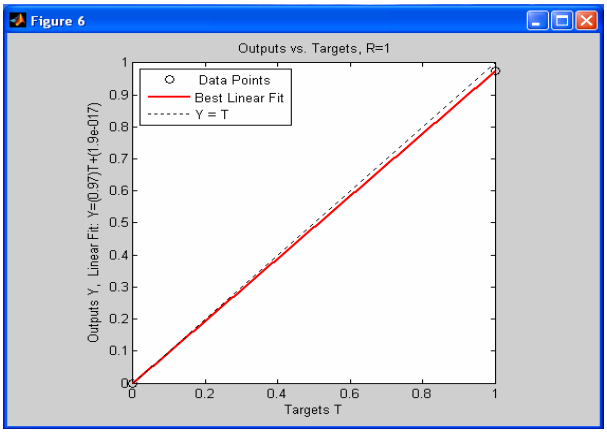

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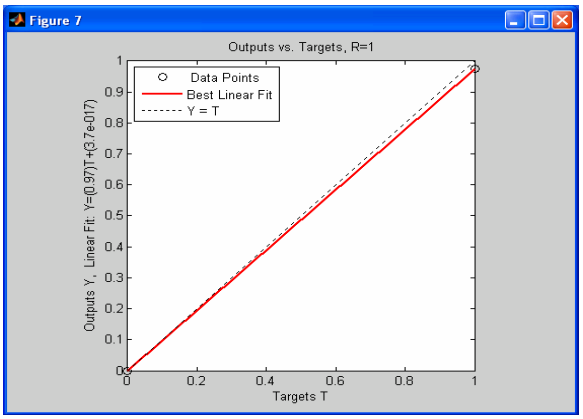

Figure (8.7)

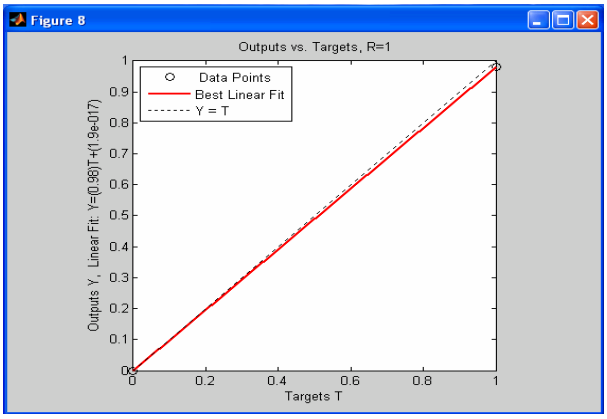

Figure (8.8)

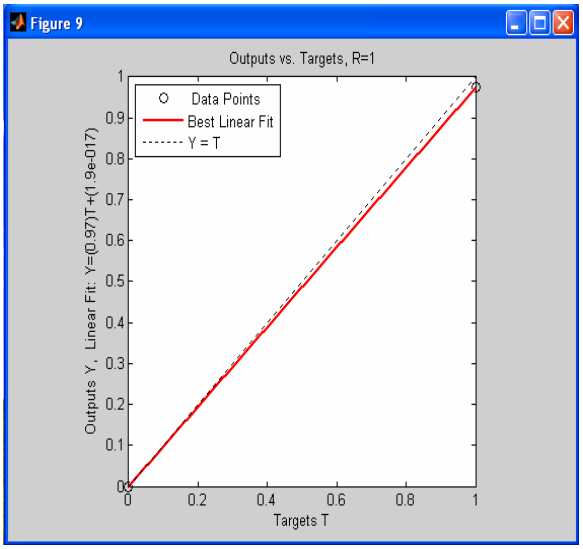

Figure (8.9)

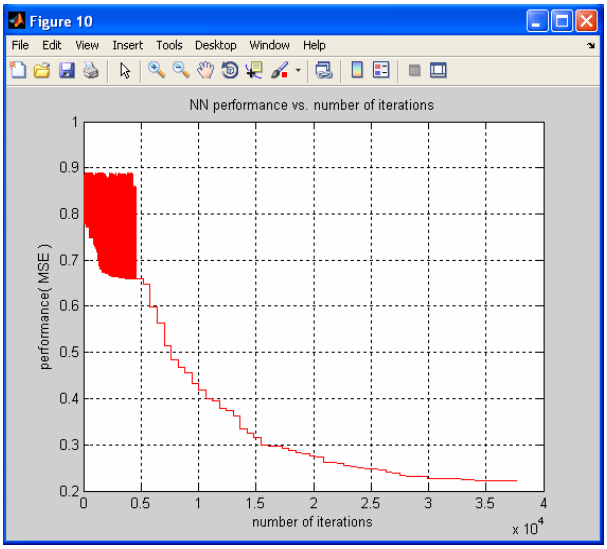

Figure (9): ANN performance vs. Number of iterations

The performance of a constructed network can be measured to some extent by investigating the network response in more detail. A regression analysis between the network response and the corresponding targets is proposed.

The network output and the corresponding targets are passed to regression analysis function, and it returns three parameters. The *first one* is the slope (m), the second is the y-intercept (b) of the best linear regression relating targets to network outputs and the *third* variable is the correlation coefficient (R-value) between the outputs and targets. If there were a perfect fit (outputs exactly equal to targets), the slope would be 1, and the y-intercept would be 0. In our program, we can see that the numbers are very close. It is a measure of how well the variation in the output is explained by the targets. If this number is equal to 1, then there is perfect correlation between targets and outputs. In clear from figure (4-a) and figure (4-b), the number is very close to 1, which indicates a good fit.

The following figures from (8.1) through (8.9) illustrate the graphical output provided by regression analysis function. The constructed neural network outputs are plotted versus the targets as open circles. The best linear fit is indicated by a dashed line. The perfect fit (output equal to targets) is indicated by the solid line. In these figures, it is difficult to distinguish the best linear fit line from the perfect fit line because the fit is so good. Is also clear that the outputs seem to track the targets reasonably well, and the R-values are equal 1.0, this means that there is perfect correlation between targets and outputs. Accordingly, this is another demonstration for the suitability of the suggested construction of neural network using genetic algorithm.

Figure (9) displays another performance measure to the constructed neural network using genetic algorithm. The result shown in this figure is reasonable, where in the first iterations from 0 to $0.5x10^4$, there is a big variation in performance (MSE) error and the error is still below the value 0.9, and it doesn't appear that any significant variation has occurred after iterations point $0.5x10^4$.

4.2 Comparing Results to Backpropagation Algorithm ANN

In this section, the parameters obtained from the construction of the neural network optimized by genetic algorithm; such as number of layers, number of neurons in each layer, activation function in each layer, performance function and the training function are compared to traditional backpropagation algorithm. Also, the initial conditions

were taken into account such as the mean of sum-squared error goal, momentum constant and number of epochs, to investigate the effect of these parameters on the network performance. Figure (10) and figure (11) show the plot of performance versus number of epochs.

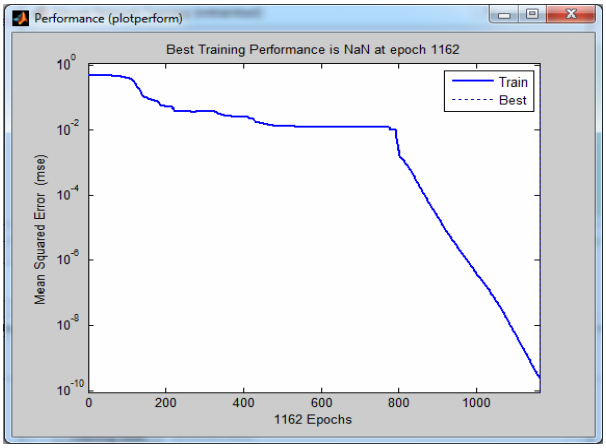

Figure (10): performance vs. number of epochs (GA parameters applied to backpropagation

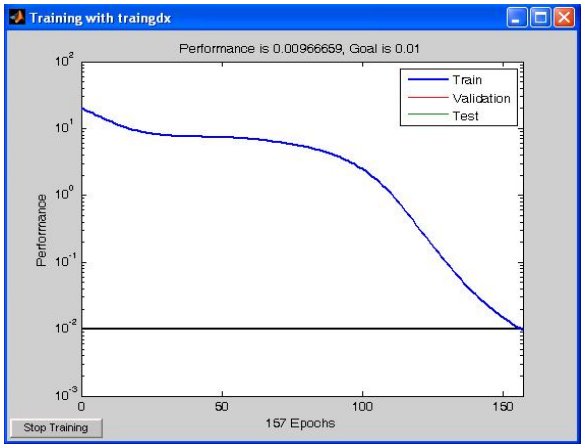

Figure (11): performance vs. number of epochs (Running backpropagation alone)

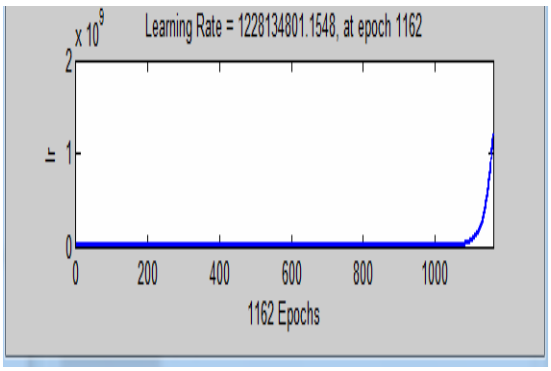

Figure (12): learning rate vs. number of epochs (GA parameters applied to backpropagation)

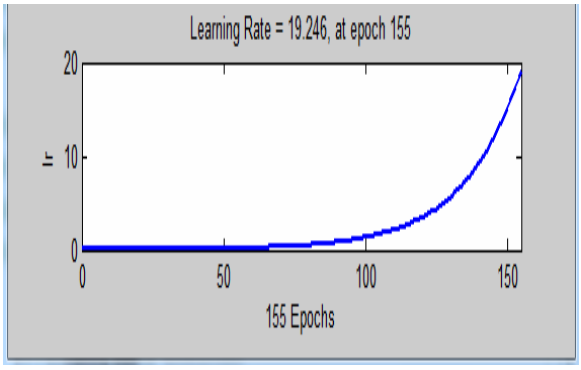

Figure (13): learning rate vs. number of epochs (Running backpropagation)

Table (2): Comparison between results when applying GA parameters to backpropagation and running backpropagation alone

Statement	**GA parameters applied to backpropagation results issued at epoch *1162***	**running backpropagation alone results issued at epoch *155***
Performance	$2.42\ e^{-10}$	$959.00e^{-5}$
Gradient	$9.90\ e^{-11}$	$5.37\ e^{-5}$
Learning rate	1228134801.1548	19.246

From the result shown in figure (9), the best performance (MSE) is 0.22 and has occurred after iterations point $3.5x10^4$. This means long time is needed for the GA to produce results, but it is acceptable with the current advances in computer technologies. From figure (12), figure (13) and Table (2), the difference between the values of the mean square error (performance), that results from applying GA parameters to backpropagation and running backpropagation alone, (using Matlab[11]), is highly large (approximately double of value). Also, for the gradient, the difference is highly large (approximately double of value), but this comes in the expense of large learning rate. This means much faster convergence when using backpropagation algorithm, ($959.00e^{-5}$ at epoch 155), but it is the best when using GA, ($2.42\ e^{-10}$ at epoch 1162).

The valuable benefits from GA are automating and optimizing the design; and finding weights and biases for a suggested construction of Artificial Neural Networks as explained in section (4.1).

5. CONCLUSION

In this paper a method was proposed for constructing an optimized neural network by employing genetic algorithm. In this method we implemented a genetic algorithm which can construct the high performance neural network structure for a given input nuclear reactors measured data, and the corresponding target accident. This method can be applied to any problem, where a data of inputs and outputs has the same form. Moreover, in this paper we present encoded nuclear reactors data (into a string), so, the genetic algorithm can be applied. In the reactor accidents diagnosis, the binary encoding becomes practical as the accidents patterns are in binary form. A comparison is conducted between a GA constructed ANN, GA initialized ANN and traditional backpropagation ANN. By applying the results obtained from genetic algorithms as initialization values on the backpropagation algorithm, the results showed much faster training, better convergence (smaller mean square error). This work demonstrated the method of automating and optimizing the design and finding weights and biases for a suggested construction of Artificial Neural Networks by Generic Algorithm in the domain of nuclear reactors.

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3/12/2011

Yield Productivity and Energy-Saving Advantages at Applying Slow-Release Nitrogen Fertilizer in Upper Egypt

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Abstract: Two experiments have been conducted in New Valley and Assiut agriculture research stations "*Typic Torripsamments, hyperthermic and Typic Torriorthents*, coarse loamy, hyperthermic" to evaluate the fertilization of two cropping sequences of oil crops (sunflower, safflower and peanut & sesame and canola) with ureaform (UF) as a slow release nitrogen fertilizer (SRNF) comparing with ammonium nitrate (AN) as a conventional one.
- First sequence: the applied rates of UF-fertilizer have been 45, 67.5, 90, and 112.5 kg N.fed^{-1} added as side banding only at planting the first crop ,Sunflower, followed by safflower planting in the same previous plots and then peanut to determine the residual effect of UF-fertilizer. Ammonium nitrate (AN) has been applied in one rate of 45, 45 and 20 kg N.fed^{-1} (recommended rate) for each crop of the sequence and in the same order. It has been taken as a scale to estimate the performance of UF, in addition to no-fertilized one (Control).
- Second sequence: UF-fertilizer has been applied in the same rates mentioned in first sequence against the recommended rate of ammonium nitrate (45 kg N.fed^{-1} for each crop) with or without application of clay sediments and control treatment. Yield and its components, Nitrogen & energy consumption ability, net return and investment factor have been recorded. The results show that:
- Firstly, the UF-fertilizer has almost had strong positive effect on yield and its components for both two cropping sequences.
-Secondly, calculations of nitrogen-consumption ability have demonstrated that the UF-fertilizer has had much more efficiency at donating its nitrogen than that of AN one where their values at first cropping sequence have been (on average) 70 and 110 kg N. ton^{-1} dry matter (yield) for UF and AN respectively, as well as 92.66 and 158.72 at second cropping sequence.
-Thirdly, calculations of Energy-consumption ability have illustrated that the saved energy with application of UF-fertilizer to produce one ton dry matter (yield) has been (on average) 36.54% for first cropping sequence and 41.6% for second cropping sequence calculated of those of AN-one. In other words, the saved energy with using UF has been (on average) 83.75 and 105.69, Liter of diesel fuel.ton^{-1} dry matter for first and second cropping sequence respectively which equivalent to 3132.25 and 3952.81 M. Joule or 0.53 and 0.67 barrel of diesel fuel or L.E. 92.13 and L.E.116.26. This would undoubtedly reduce CO_2 emissions, the first accused in global worming case.
- Fourthly, all treatments have been almost implemented reasonable profitability (IF >3) either at first or second cropping sequences. The economic application of UF has been fulfilled when it had been applied in high rate and then it is enough to fertilize two crops. It is also observed that the added clay has positively affected net return; however it has not given profitability. In spite of marked superiority of UF-net return value to those of AN, their IF values have been approximated.
-Fifthly, the cost of consumed energy related to nitrogen fertilization has been reduced to about 1/2 by using UF fertilize

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Key words: ureaform; slow release nitrogen fertilizer (SRNF); clay sediments; oil crops; nitrogen-consumption ability; energy-consumption ability

1-Introduction

One of the most important axes of agriculture modernization in Egypt is that related to the application of innovations and new technologies to promote plant productivity and environmental protection. In fertilization field, the use of slow release nitrogen fertilizers (SRNFs) is the most important one of them for environmental reasons representing the reduction of nitrogenous losses from conventional nitrogen fertilizer in forms of seeped nitrate through soil profile into water system, ammonium volatilization and nitrous oxide emission (Horgan *et al.*, 2002 and Fernandez *et al.*, 2004). Moreover, their application increased the crop yield, Nitrogen use efficiency and net economic return in spite of their higher costs (Abbady *et al.*, 2006, Abbady *et al.*, 2008 and Abd El-Aal *et al.*, 2008) as well as conserved soil fertility (Abbady *et al.*, 1999). The response of such fertilizers was more effectively illustrated when they applied on new reclaimed soils where the coarse texture, high infiltration rate, nutrients poverty and its low retention are presented. The used SRNF, here, is the ureaform (UF) which

proved its success under Egyptian condition in several works, for example, El-Mallah *et a*l.,1998, Hegazy *et al*.,1998 and Awaad *et al*., 2003. Ureaform fertilizers are combinations of various methylene-urea polymers such as methylene-diurea, dimethylene-triurea, trimethylene tetraurea and so on (Abbady, *et al.,* 1992)

2-Materials and Methods

Two field experiments have dealt with two cropping sequences of oil crops and two forms of nitrogen fertilizer. First experiment has been conducted at Agricultural Research Station of New Valley governorate "*Typic Torripsamments, hyperthermic"**. The cropping sequence has been sunflower (*Helianthus annuus*) Giza, 102 variety, safflower (*Carthamus tinctorius*. L) Giza, 1, and peanut (*Arachis hypogaea L*.) Giza, 6. Second experiment has been conducted at Agricultural Research Station of Assiut "*Typic Torriorthents*, coarse loamy, hyperthermic"*. The cropping sequence has been sesame (*Sesamum indicum*) Shandawel, 3, and canola (*Braaica napus*) Serw, 4. Physical and chemical properties of both two soils have been presented in table 1. The soil analyses have been performed according to Jackson, 1958.The used nitrogen fertilizers have been ureaform (UF) as a slow release nitrogen fertilizer (SRNF) with 40% nitrogen and 60% activity index, prepared by Abbady *et al*., (1992) and ammonium nitrate (AN, 33%N) as a conventional one which used in official recommended rate representing the standard level to compare the different effects of UF-fertilizer.

In first experiment, UF-fertilizer has been applied in 4 rates; 45, 67.5, 90 and 112.5 kg N.fed^{-1} and added just only before planting first crop (sunflower) in one dose. AN-rate has been applied in one rate split into two doses for each crop; 45, 45 and 20 kg N.fed^{-1} for sunflower, safflower and peanut at the same order, Moreover non-fertilized treatment (control). The experiment has been carried out on a complete randomized design with four replications consisting of 6 treatments.

In second experiment, a spilt plot design has been used. The main plots (a) have been for clay sediments (Table 2) achieved in two treatments 0.0 and 3 ton fed^{-1}. Subplot treatment (b) have come as 45, 67.5, 90 and 112.5 kg N.fed^{-1} of UF-fertilizer used as mentioned in first experiment in addition to AN-one in rate of 45 kg N.fed^{-1} split into two doses for each crop (sesame and canola). Control treatment has been also included. Four replicates for every treatment have been achieved. Then the experiment has consisted of 12 treatments.

For more confirmation, both experiments have been started with first crop followed by second then third which have been planted in the same plots of preceding crop. Recommended rates of calcium super phosphate and potassium sulphate have been applied. Statistical analysis has been carried out according to the procedures outlined by Snedecor and Cochran, 1980.

The yield and some yield components of each crop have been recorded. Nitrogen & energy consumption ability calculations and economical analysis have been performed to evaluate the application of UF-fertilizer as a model for SRNFs against conventional one; they have been calculated using the models: 1, 2, 3, and 4 respectively.

- Nitrogen-consumption ability = N-kg.fed^{-1} / yield ton.fed^{-1}……. (1)
- Energy-consumption ability = Liter of diesel fuel.fed^{-1} / yield, ton.fed^{-1} ……. (2)
- Liter of diesel fuel.fed^{-1} = Quantity of diesel fuel (Liter) required to manufacture 1 kg nitrogen for fertilizer multiplied by N-rate.fed^{-1}

One liter of diesel fuel as an energy = 37.4 M Joule

M Joule =10^6 Joule

- Net return = gross return - total cost…….. (3)
- Investment factor = gross return / total cost…… (4)

where: Gross return = yield x sale price.

Table 1. Some physical and chemical properties of representative soil samples for the experimental sites

Soil properties	Experiment sites	
	Assiut Exp. Station	New Valley Exp. Station
Sand (%)	66.3	81.5
Silt (%)	28.0	4.00
Clay (%)	5.7	9.84
Texture class.	Sandy loam	Loamy sand
pH (1:1 soil-water suspension).	8.46	7.6
Ec dS/m (1:1 soil-water extract)	0.66	0.58
Soluble cations me 100 g^{-1} soil		
Ca^{+2}	0.31	0.25
Mg^{+2}	0.26	0.22
Na^{+1}	0.09	0.07
K^{+1}	0.01	0.04
Soluble anions me100 g^{-1} soil		
CO_3+ HCO_3	0.31	0.2 2
Cl	0.30	0.30
SO_4	0.07	0.06
Total $CaCO_3$ (%)	17.63	3.3
Total N%	0.041	0.079
$NaHCO_3$ extractable P (mg/kg)	4.35	7.90
NH_4OAC extractable K (mg/kg)	120.9	218.8
DTPA extractable micronutrients (mg/kg)		
Fe.	2.15	4.33
Mn.	1.02	2.00
Zn.	0.34	0.51

Table 2. Some chemical properties of the used clay amendments

Property	pH (1:2.5)	EC (1:2.5) dSm^{-1}	Total Macronutrient %			Available Macronutrient $Mg.Kg^{-1}$			CEC Mme .100g^{-1}
			N	P	K	N	P	K	
Value	7.89	7.13	0.02	0.03	0.12	53	12	45	20.50

3-Results and Discussion

Yield, yield components, nitrogen consumption ability (N-CA), energy consumption ability (E-CA) as well as net economic return (NE) and investment factor (IF) of two cropping sequences; sunflower-safflower-peanut and sesame-canola have been studied to determine their affection by application of ureaform (UF) as a slow-release nitrogen fertilizer comparing with conventional one; ammonium nitrate (AN).

3-1- Agronomic appraisal

3-1-1-First cropping sequence

Data listed in Table 3 show that there have generally been significant differences among the yield and its components values affected by different N–treatments. At first crop (sunflower), plant yield, seed index and yield of UF-treatments (on average) have surpassed those of AN-treatment. It is also observed that the yield and its components have gradually increased with increasing UF-N rates. However, the yield of AN-treatment (45 kg $N.fed^{-1}$) has not significantly differed from its corresponding of UF-treatment. At second crop, the results of this crop have not more varied than those of previous crop, however the yield of AN-treatment has approximated with that of UF_2–treatment which has proven that the residual part from UF-fertilizer has certainly managed to nourish another crop. At the third crop, the picture has entirely differed where the yield and its components of UF treatments have been inferior to that of AN-treatment except at UF_4 (its N-rate = 2.5 times of AN-rate). This result has emphasized upon existence of the UF-fertilizer in soil continuously releasing its nitrogen to meet the plant demands and no nitrogen loss has occurred whatever its rate was.

Table 3 Yield, some yield components of first cropping sequence (sunflower, safflower and peanut) and % relative increase calculated of AN-treatment yield as affected by different treatments.

Treatments	Sunflower (first crop)					Safflower (second crop)					Peanut (third crop)				
	N rate Kg.fed^{-1}	Plant yield (g)	Seed Index* (g)	Yield ton.fed^{-1}	Relative Increase %	N rate Kg.fed^{-1}	Plant Yield (g)	Seed Index* (g)	Yield ton.fed^{-1}	Relative Increase %	N rate Kg.fed^{-1}	Seed Index* (g)	Shelling**	Yield Ton. Fed^{-1}	Relative Increase %
Control	0.0	12.0	2.10	0.284	-54.78	0.00	27.7	3.15	0.841	-25.90	00.0	49.2	30.8	0.878	-46.08
AN	45.0	26.6	5.10	0.628	-	45.0	53.3	5.73	1.135	-	20.0	96.1	60.3	1.628	-
UF1	45.0	31.3	6.07	0.580	-07.29	0.00	33.6	5.58	1.081	-4.77	00.0	76.4	53.5	1.253	-23.04
UF2	67.5	36.3	6.45	0.670	06.69	0.00	39.5	7.63	1.128	-0.62	00.0	88.0	52.1	1.305	-19.84
UF3	90.0	47.2	6.80	0.950	51.27	0.00	48.8	8.09	1.346	18.59	00.0	91.2	58.4	1.433	-11.98
UF4	112.5	58.2	7.23	1.026	63.38	0.00	91.0	9.40	1.710	50.66	00.0	91.2	59.7	1.568	-03.69
Means of UF treat		43.25	6.64	0.807	28.51	00.0	53.23	7.68	1.315.8	15.97	00.0	86.7	55.93	1.389	-14.64
L.S.D 0.05		3.8	0.51	0.105			5.0	0.89	0. 20			11.1	3.2	0.56	

* Seed index = weight of 100-seed (g) for each crop

**Shelling % = Seed weight (g) / Pod weight (g)

Table 4 Sum of 1st & 2nd crop yield (summation $_1$) and 1st, 2nd and 3rd crop yield (summation $_2$) (ton.fed^{-1}), % yield relative increase of UF-treatments calculated of AN-treatment yield, yield increased (ton.fed^{-1}), NCA (kg N. ton^{-1} yield), total consumed energy (diesel fuel L. fed^{-1}), ECA (L. ton^{-1} yield).

Treatments	Summation of first and second crop yield data (summation 1)							Summation of first, second and third crop yield data (summation 2)						
	N rate Kg.fed^{-1}	Yield ton.fed^{-1}	Relative increase %	Yield increased ton.fed^{-1}	N-CA kg N. ton^{-1} yield	Consumed Energy diesel fuel L. fed^{-1}	ECA L. fuel. ton^{-1} yield	N rate Kg.fed^{-1}	Yield ton.fed^{-1}	Relative Increase %	Yield increase ton.fed^{-1}	N-CA kgN ton^{-1} yield	Consumed Energy diesel fuel L. fed^{-1}	E-CA L. fuel. ton^{-1} yield
Control	00.0	1.125	-36.19	-	-	-	-	00.00	2.003	-40.93	-	-		
AN	90.0	1.763	-	0.638	141	144	226	110.0	3.391	-	1.387	79	176	127
UF1	45.0	1.661	-05.79	0.536	84	72	134	45.00	2.914	-14.07	0.911	49	72	79
UF2	67.5	1.798	01.98	0.673	100	108	160	67.50	3.103	-8.49	1.100	61	108	98
UF3	90.0	2.296	30.25	1.171	77	144	123	90.00	3.729	9.97	1.726	52	144	83
UF4	112.5	2.736	55.23	1.611	70	180	112	112.5	4.304	26.92	2.301	49	180	78
Means of UF treat.		2.123	20.40	0.998	83	126	132		3.512	3.59	1.510	53	126	85

N-CA = N Kgm.fed^{-1}/ yield, ton.fed^{-1}

E-CA = Consumed diesel fuel liter.fed^{-1} / yield, ton.fed^{-1}

Consumed energy, diesel fuel liter.fed^{-1} = nitrogen rate. Kg.fed^{-1} x 1.6

where 1.6 is the international average of consumed diesel fuel quantity (Liter) to produce one kg nitrogen for fertilizer.

Relative increase values of UF- treatments yield calculated as percentage of AN- treatment yield (Table 3 and Figure 1) have been amounted (on average) 28.42 for first crop, 15.97 for second and -14.64 for third. Such tendency would have illustrated that no need to plant third crop unless the applied UF was in high rate.

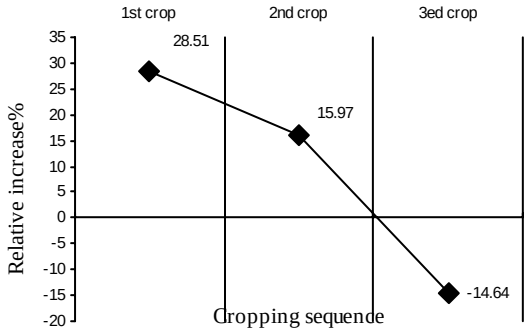

Figure 1 Pattern of % relative increase of UF-treatments yield (on average) calculated of AN-treatment yield for first cropping sequence

Data given in Table 4 represent comprehensive view for obtained results of first & second crop yield $(summation)_1$ and the same results in addition to third crop results $(summation)_2$. Examination of such two summations has shown that:

Firstly, in spite of magnitude of both yield and yield increased of UF-treatments (on average) at $summation_2$ comparing to their corresponding at $summation_1$, their relative increase value (on average) has been very low (3.59%) which has again confirmed that no need to cultivate third crop at application of UF-fertilizer because its residual quantity at this interval (third season), seemingly is not enough to give recompensed yield. These results have been in agreement with those of Abbady *et al*., 2006.

Secondly, the fertilizing role of N-fertilizer could be evaluated by application of nitrogen- consumption ability (N-CA) supposal. It is calculated as in model (1).

N-CA (kg $N.ton^{-1}$) = N-rate $kg.fed^{-1}$/yield $ton.fed^{-1}$(1)

It represents the nitrogen quantity consumed from fertilizer nitrogen to produce one ton of yield. The data in Table 4 also illustrated that N-CA from UF-fertilizer has been less than that from AN-one in both two summations, i.e. the UF-fertilizer has had more efficiency at donating its nitrogen. It is also observed that N-CA values at $summation_2$ have been less than that at $summation_1$, due to severe depletion of UF-nitrogen performed by the three crops.

Thirdly, this part has been concerned the energy consumption; according to the reports of Goering, 1989 and Bhat *et al*., 1994 as pointed out in the introduction , it can be inclusively concluded that manufacturing one kilogram of nitrogen for fertilizer requires the energy equivalent to from 1.36 to 1.82 liter of diesel fuel. Taking the average value 1.6 to calculate consumed energy ($Liter.fed^{-1}$) as recorded in materials & methods, energy consumption ability (E-CA) could be imitatively calculated to N-CA using the model (2).

E-CA ($L.ton,^{-1}$ yield) = consumed energy, Liter of diesel $fuel.fed^{-1}$/ ton $yield.fed.^{-1}$……..… (2)

It is noticed that E-CA (Liter of diesel fuel. ton^{-1}) at using UF-fertilizer (on average) has been much less than that at using AN-one, also their values have been gradually decreased with increasing the N-rate of UF either at $summation_1$ or at $summation_2$, this due to increasing obtained yield quantity at those rates. Moreover, E-CA at $summation_2$ for UF-fertilizer (on average) has been much less than it is (itself) at $summation_1$.Considering the E-CA of AN-fertilizer (on average) equals 100, then this of UF-one equals 61.11, i.e. the saved energy by using UF has been 38.89% as shown in Fig. 2. In other analysis; the saved-energy by using UF-fertilizer (on average) has been amounted 90.0 and 50.0 L. diesel $fuel.ton^{-1}$ dry matter (sunflower, safflower, peanut) representing 3366 and 1870 M Joule* at $summation_1$ and $summation_2$ respectively and those have represented the energy content of 0.57 and 0.32 barrel** or L.E. 99.00 and L.E.55.0

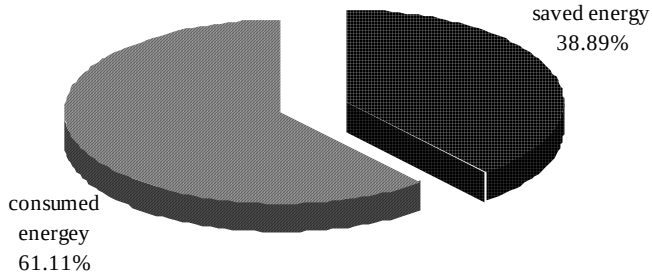

Figure 2 Energetic position at using UF-fertilizer for first cropping sequence

* One Joule is the work done, or energy expended, by a force of one Newton moving one Meter along the direction of the force.

** American barrel = 158.984 Liters

3.1.2. Second cropping sequence

Data presented in Table 5 show that regardless of type and rate of nitrogen fertilizer at first crop, the clay addition has had insignificant effect on seed index and significant one on both plant yield and crop yield. The added clay has increased the yield.fed^{-1} with 25.9%. At second crop, the clay addition has given significant effect on each of plant yield, seed index and yield.fed^{-1}; the yield increase has been 10.37%. This is expected because the added clay would improve soil physical and chemical properties specially water holding capacity and cation exchange capacity in addition to its nutrients content. These results are in a harmony with those obtained by Al-Omran *et al.,* 2002 and Suganya and Sivasamy, 2006.

Examination of summed up data of the two crops has demonstrated that the added clay has increased the yield by 11.81%, however it has not had effect on averages of yield increased and nitrogen & energy consumption.

About the effect of different treatments, the data show almost significant differences between AN-treatment and UF-ones as well as amongst UF-treatments themselves; at first crop in case of no adding clay, the plant yield, seed index and crop yield values of AN-treatment have nearly equaled to those of UF_1&UF_2 and been inferior to UF_3&UF_4 while in case of adding clay, such estimates of AN-treatment have been inferior to those of UF-treatments. Also, % yield relative increases of UF-treatments calculated of AN-treatment yield (as a standard level) have ranged from -8.79 to 30.78 at no-adding clay and from 14.93 to 32.46 at adding clay. At second crop, the strong superiority for UF-treatments effect (residual part) to AN-treatment (current fertilization) has been evidently shown; the values of plant yield , seed index and crop yield for former have been greater than those of latter. Such effect has dominated either in case of adding clay or not. % yield relative increases have ranged from 5.81 to 22.09 at no- adding clay and from 8.82 to 38.6 at adding clay. As it is appeared, the UF-fertilizer has been more donations for its nitrogen at second crop than that at first one although the second crop has been nourished on the residual part of UF previous-added at first crop planting, this action has emphasized on two facts: 1-The residual part in soil from UF has been sufficient to grow another crop, this result was much obtained before, El-mallah *et al.*, 1998; Awaad *et al.*, 2003 and Abbady *et al.*, 2006

2-It seems that the adaptations between UF fertilizer and soil microorganisms that are responsible about its breaking-down during first crop growing period have a bit lagged especially, that the applied soil is obviously not enough fertile (Table 1.)

Speculation of the summed up effects of UF and AN-treatments (Table 5) has illustrated that UF treatments have fulfilled high preponderancy in the matter of the studied-estimates comparing with those of AN-treatment; their total yield and its % relative increases have been gradually increased with the N-rate increase. It is worthy to clarify that 90 kg N in form of conventional nitrogen fertilizer (AN) have given yield as much as 45 or 67.5 kg N in form of slow release nitrogen fertilizer (UF).

In regard to N-CA, it is noticed that the values belonging to UF-treatments have been got much lower than that of AN-treatment. Also, using UF has been reduced the consumed - nitrogen (on average) with 31.28% in case of no-adding clay and 49.83% in case of adding clay. This may be due to its improving effect on soil characteristics which would reflect upon plant productivity. Here, it must be pointed out that saving 40.63% (on average) of used nitrogen may promote the application of SRNFs.

The trend of E-CA values of UF-treatments has taken the same as the N-CA trend ; they have been increased with increasing N-rate, however, they have been never exceeded that of AN-treatment, yet the discountable quantity as a percent of that of AN-treatment reached 31.44% in case of no-adding clay and 49.83% in case of adding clay. This has been logically expected because saving nitrogen means saving energy (Figure 3a). For more illustration, to obtain 1 ton of plant product (sesame and canola), the consumed is 254 liter of diesel fuel (on average) at applying conventional fertilizer and 148.27 liter when the SRNF has been applied. The difference is 105.73 liter; this represents 41.63% saved energy (Figure 3b) if UF-fertilizer has been in use and equivalent to 3952.81 M Joule or energy content of 0.67 barrel diesel fuel or L.E.113.30.

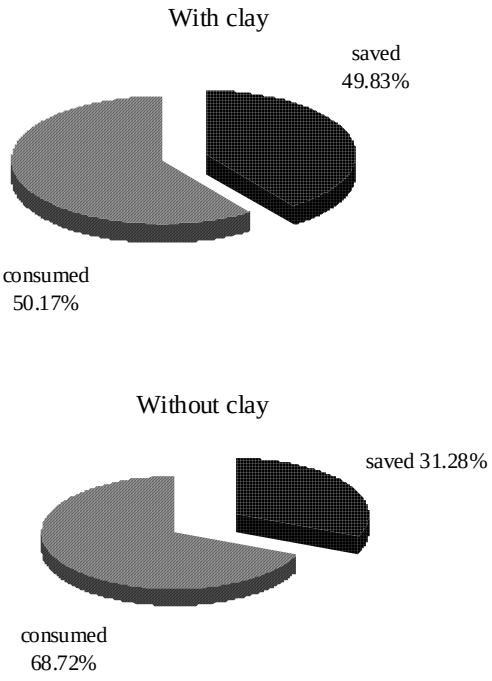

Figure 3a. Energetic position at using UF fertilizer as affected by added clay for second cropping sequence

Table 5 Yield, some yield components of second cropping sequence (Sesame and Canola), their summation, % relative increase calculated of AN-treatment yield, N-CA and E-CA as affected by different treatments.

Treatments		Sesame					Canola					Summation					
Clay (A)	Ureaform (B)	N rate Kg.fed^{-1}	Plant yield g	Seed index g	Yield ton. fed^{-1}	Relative Increase %	N rate Kg. Fed^{-1}	Plant yield g	Seed index g	Yield ton. Fed^{-1}	Relative increase %	Yield ton. fed^{-1}	Relative increase %	Yield increase ton. fed^{-1}	N-CA kg N. ton^{-1}	Consumed energy L. fed^{-1}	E-CA L.fuel ton^{-1}
Without Clay	Control	0.00	8.15	1.51	0.154	-66.15	0.00	6.55	1.33	0.521	-39.42	0.675	37.0	-	-	-	-
	AN	45.0	16.9	2.33	0.455	-	45.0	10.38	1.79	0.860	-	1.315		0.640	140.63	144	225.0
	UF1	45.0	18.5	1.90	0.415	-8.79	00.0	10.79	1.85	0.910	5.81	1.325	0.76	0.650	69.23	72.0	110.77
	UF2	67.5	16.5	3.20	0.420	-7.69	00.0	11.03	1.89	0.940	9.30	1.360	3.42	0.685	97.83	108.0	156.52
	UF3	90.0	23.6	2.60	0.560	23.08	00.0	11.40	1.94	0.985	14.54	1.545	17.44	0.870	103.45	144.0	165.52
	UF4	112.5	25.1	2.90	0.595	30.78	00.0	11.93	2.02	1.050	22.09	1.645	25.10	0.970	115.98	180.0	185.57
	Means of treatments		18.13	2.41	0.433	-7.19		10.35	1.8	0.878	3.08	1.311	18.7	0.763	105.42	129.6	168.68
	Means of UF treatments		20.93	2.65	0.498	9.35		11.29	1.93	0.971	12.94	1.469	11.69	0.794	96.62	126.0	154.60
With Clay	Control	0.00	11.05	1.81	0.241	-102.91	00.0	7.84	1.65	0.621	-29.90	0.862	-37.37	-	-	-	-
	AN	45.0	17.3	3.10	0.489	-	45.0	10.48	1.80	0.873	-	1.362	-	0.509	176.82	144	282.91
	UF1	45.0	24.3	2.50	0.562	14.93	00.0	11.11	1.90	0.950	8.82	1.512	11.01	0.659	68.29	72.0	109.27
	UF2	67.5	26.3	2.50	0.612	25.15	00.0	11.70	1.99	1.021	16.95	1.633	19.90	0.780	86.54	108.0	138.46
	UF3	90.0	27.3	2.50	0.641	25.56	00.0	12.75	2.15	1.150	31.73	1.791	31.50	0.938	95.95	144.0	153.52
	UF4	112.5	29.1	2.70	0.724	32.46	00.0	13.25	2.22	1.210	38.60	1.934	30.83	1.081	104.07	180.0	166.51
Means of treatments			22.56	2.52	0.545	4.81		11.19	1.95	0.971		1.516	18.1	0.784	106.33	129.6	170.13
Means of UF treatments			26.75	2.55	0.635	24.53		12.2	2.07	1.083	24.03	1.718	23.31	0.856	88.72	126.0	141.94
LSD A	0.05		n.s	n.s	n.s			0.54	0.08	0.066							
LSD B	0.05		2.2	n.s	0.003			0.45	0.07	0.054							
LSD AB	0.05		3.1	n.s	0.005			0.63	0.10	0.077							

*Seed index = weight of 1000-seed (g)

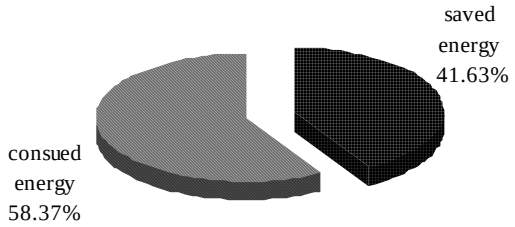

Figure 3b. General energetic position at using UF-fertilizer for second cropping sequence

3.2. Economic appraisal:

The choice to use either UF-fertilizer or AN-one should not be based on nitrogenous content, because it's both the same nitrogen. UF-fertilizer has the benefit of being less likely to leach into ground or surface water (environmentalist's viewpoint). The farmer's viewpoint, however, is entirely different, the loss or gain is his concern. Here, the economic appraisal for UF-fertilizer against AN-one through their use to fertilize the two cropping sequences (sunflower-safflower-peanut & sesame-canola) has been achieved. Perhaps, if the results have satisfactorily come, there should be little resistance to adopting the SRNFs application.

Expenses of inputs have represented only in the purchase and application of nitrogen fertilizers, because the cost of all other agricultural processes have not been included. It would be pointed out that the UF-fertilizer has not had credible price and its cost has been the costs of the chemicals used in preparing it (in laboratory).Then the inputs have been as follow:

1-L.E.4500 to prepare one ton of UF (L.E.3000 for formalin and L.E.1500 for urea)
2- L.E.1300 for one ton ammonium nitrate.
3- L.E. 35 for one ton clay sediments.
4- L.E.25.00 for laborer per day.
5- L.E. 1.10 for liter of diesel fuel.

The outputs have represented selling price of different oil crops under study and which have come as follow:

1- L.E. 3000 for one ton sunflower.
2- L.E. 3000 for one ton safflower.
3- L.E. 5300 for one ton peanut.
4- L.E. 7000 for one ton sesame.
5- L.E. 1400 for one ton canola.

3.2.1. First cropping sequence:

Data recorded in table 6 and table 7 demonstrates that: Firstly, regardless of kind or rate of applied nitrogen fertilizer, all treatments at position$_1$ (sum of 1st+2nd crop economical data) and position$_2$ (sum of 1st +2nd +3rd crop ones) have generally been profitable because of their IFs (IF$_1$+IF$_2$) have been more than 3 (FAO, 2000). Secondly, rational approximation for the net return (NR) values of UF-treatments to those of AN-treatment has been occurred. They have been proportionally amounted 0.82, 1.4 and 0.9 of those of AN-treatment after each of first, second (sum of 1st +2nd) and third crop (sum 1st +2nd +3rd) in the same order. This has been expected due to the high cost of UF. Thirdly, economic application of UF has been seemingly prospered when it had been applied in high rates (Table 7) where their IF$_2$ and IF$_3$ values have been approached to those of AN-fertilizer (as a standard treatment). This could be explained on the basis of severe nitrogen depletion of applied UF at planting first crop and consumed at all 3 crops growing period long. To discuss the only UF data (Table7 and Figure 4), it would be illustrated that NR$_1$ &IF$_1$ calculated for first crop data, NR$_2$ & IF$_2$ calculated for sum of first and second crop data and NR$_3$ & IF$_3$ calculated for sum of first, second and third crop data.

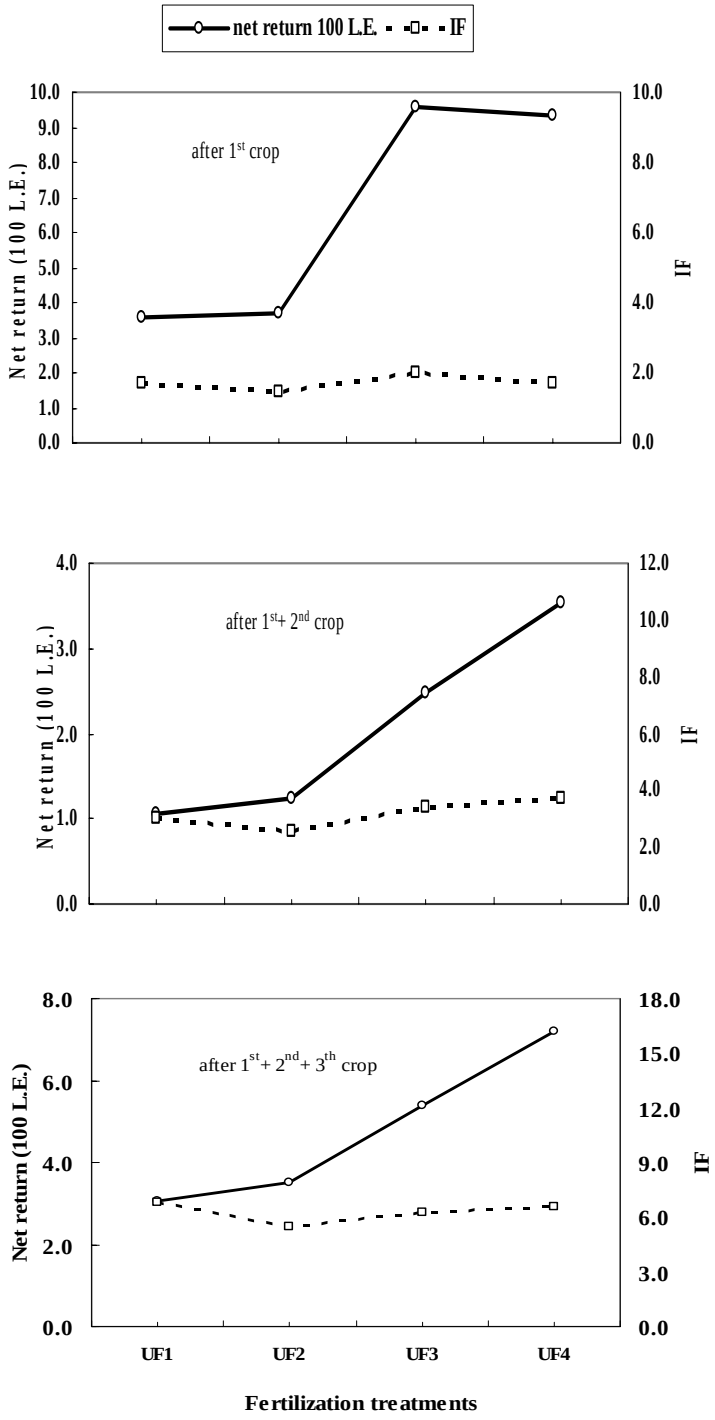

Figure 4.UF treatments in relation to net return and investment factor of first cropping sequence

Table 6 Total cost, gross and net return produced from N-fertilization for first cropping sequence.

Treatments	Sunflower (first crop)								Safflower (second crop)							Peanut (third crop)						
	App. fert. Kg. fed^{-1}	Cost fert. L.E. fed^{-1}	Lab-or L.E. fed^{-1}	Total Cost L.E. fed^{-1}	Yield increase ton. fed^{-1}	Gross return L.E. fed^{-1}	Net return $_1$ L.E. fed^{-1}	IF_1	App. fert. Kg. fed^{-1}	Cost fert. L.E. fed^{-1}	Lab-or L.E. fed^{-1}	Total Cost L.E. fed^{-1}	Yield increase ton. fed^{-1}	Gross return L.E. fed^{-1}	Net return L.E. fed^{-1}	Appl. fert. Kg. fed^{-1}	Cost fert. L.E. fed^{-1}	Lab-or L.E. fed^{-1}	Total Cost L.E. fed^{-1}	Yield increase ton. fed^{-1}	Gross return L.E. fed^{-1}	Net return L.E. fed^{-1}
AN	143.3	186.3	50	236.3	0.344	1031.1	794.8	3.36	143.3	186.3	50	236.3	0.293	879.9	643.6	60	78	50	128	0.750	3975.0	3847.0
UF1	112.5	506.3	25	531.3	0.296	887.1	355.3	1.67	0.0	0.0	0.0	0.0	0.239	717.6	717.6	0.0	0.0	0.0	0.0	0.375	1987.5	1987.5
UF2	168.8	759.4	25	784.4	0.386	1157.1	372.7	1.48	0.0	0.0	0.0	0.0	0.286	858.6	858.6	0.0	0.0	0.0	0.0	0.428	2268.4	2268.4
UF3	225.0	1012.5	25	1037.5	0.666	1997.1	959.6	1.92	0.0	0.0	0.0	0.0	0.504	1513.2	1513.2	0.0	0.0	0.0	0.0	0.555	2941.5	2941.5
UF4	281.3	1265.6	25	1290.6	0.742	2225.1	934.5	1.72	0.0	0.0	0.0	0.0	0.869	2605.5	2605.5	0.0	0.0	0.0	0.0	0.690	3657.0	3657.0
UF treatments means				866.0	0.523	1566.6	655.61	1.9					0.473	1423.7	1423.75					0.512	2713.6	2713.6

Table 7 Economic position of N-fertilization for 1st and 2nd crop yield (position) $_1$and for 1st, 2nd and 3rd crop yield (position)$_2$ in first cropping sequence.

Treatments	Economic position for 1st and 2nd crop (position)$_1$				Economic position for 1st, 2nd and 3rd crop (position)$_2$			
	Total cost L.E. fed^{-1}	Gross Return L.E. fed^{-1}	Net return $_2$ L.E. fed^{-1}	IF_2	Total cost L.E. fed^{-1}	Gross return L.E. fed^{-1}	Net return $_3$ L.E. fed^{-1}	IF_3
AN	472.6	1911.0	1438.4	4.04	600.6	5886.0	5285.4	9.80
UF1	531.3	1604.7	1073.4	3.02	531.3	3592.2	3060.9	6.76
UF2	784.4	2015.7	1231.3	2.57	784.4	4284.1	3499.7	5.46
UF3	1037.5	3510.3	2472.8	3.38	1037.5	6451.8	5414.3	6.22
UF4	1290.6	4830.6	3540.0	3.74	1290.6	8487.6	7197.0	6.58
UF treatments means	910.9	2990.3	2079.3	3.18	910.9	5703.9	4792.9	6.25

It is, thereon, observed that NR_1, NR_2 and NR_3 have been increased with increasing applied UF-rate, while IF_1 have been somewhat lowered and nearly not changed (varied from 1.48 to 1.92), IF_2 values have been increased with increasing N-rate of UF, whoever, they still relatively low and IF_3 has given values bit greater than that of IF_2. From table 7 and figure 5, it is noticed accelerated increasing for net return values and delayed one for IF values (on average) from beginning first crop to third one ($position_2$) passing by second one ($position_1$) have been occurred . Hence, it may be decided that the economic rate of UF is UF_3 (90 kg $N.fed^{-1}$) and planting only two crops under the condition similar to that of this experiment is quite sufficient.

3.2.2. Second cropping sequence:

Generally, it seems that the poverty of soil fertility (Table 1) has negatively affected the plant productivity which would essentially reflect upon the NR and IF values for both two crops which have been somewhat reduced. It is however found according to data given in table 8 that all treatments either slow or conventional nitrogen fertilizer have been implemented some profitability where their IF-values have frequently been more than 3. About clay application, it is observed that the adding clay has given NR greater than no adding clay. Whoever, its added costs has obscured the appearance of profitability where IF (on average) of the former has nearly equated to that of latter.

Evidently, NR produced from UF - application (on average), at second crop has been superior to those produced from AN-one, either with adding or no-adding clay which may due as mentioned before to the UF - decomposition in second season of experiment has been more activating , subsequently more efficient nitrogen release.

As for the case of amongst UF-treatments themselves, data in table 8 and figure 6 illustrate that summed up impact (1^{st} crop $+2^{nd}$ crop) of UF-treatments on NR and IF values have been affected by adding clay. Markedly, adding clay has given NR higher than that of no-adding. At the same time IF values have not been changed and approximated in both two cases. It is also illustrated that the UF1 rate (45Kg N fed^{-1}) has been the most profitability either in case of adding clay or not.

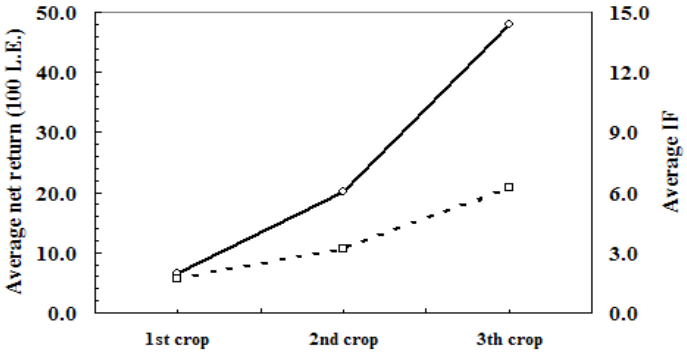

Figure 5 Accumulation of the economic data for first cropping sequence

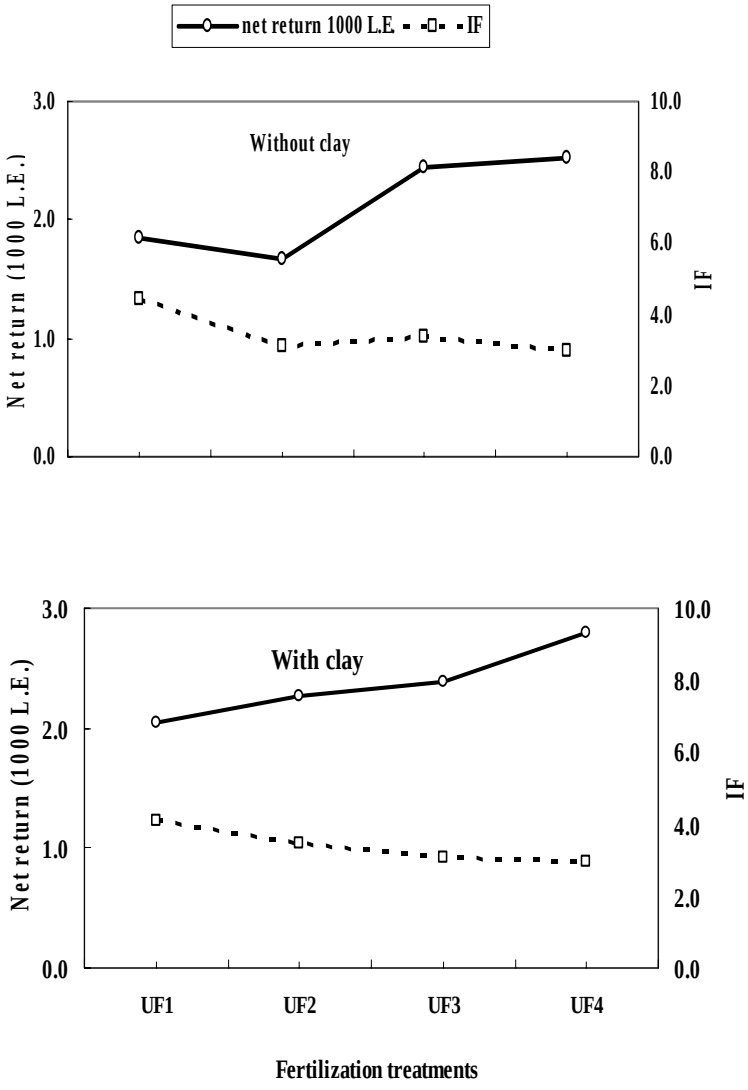

Figure 6 UF-treatments in relation to NR and IF of second cropping sequence

Table 8 Economic position of N-fertilization for 1st and 2nd crop in second cropping sequence

Treatments		Sesame (first)										Canola (second)									First economic position for 1st and 2nd crop			
		App. fert. Kg. fed^{-1}	Cost fert L.E. fed^{-1}	Fert. lab. L.E. fed^{-1}	App . clay ton. fed^{-1}	Cost of Clay App. and trans. LE. fed^{-1}	Total Cost L.E. fed^{-1}	Yield income ton. fed^{-1}	Gross return L.E. fed^{-1}	Net return. fed^{-1}	IF	App. fert. Kg. fed^{-1}	Cost fert L.E. fed^{-1}	Fert. lab. L.E. fed^{-1}	App. clay L.E. fed^{-1}	Cost of Clay App. fed^{-1}	Total cost L.E. fed^{-1}	Yield income ton. fed^{-1}	Gross return L.E. fed^{-1}	Net return. fed^{-1}	Cost L.E. fed^{-1}	Gross Return L.E. fed^{-1}	Net Return L.E. fed^{-1}	IF
Without clay	AN	143.3	186.3	50	0	0	236.3	0.301	2107.0	1870.7	8.92	143.3	186.3	50	0	0	236.3	0.339	474.6	238.3	472.6	2581.6	2109.0	5.46
	UF1	112.5	506.3	25	0	0	531.3	0.261	1827.0	1295.8	3.44	0.0	0.0	0	0	0	0.0	0.389	544.6	544.6	531.3	2371.6	1840.4	4.46
	UF2	168.8	759.4	25	0	0	784.4	0.266	1869.0	1077.6	2.38	0.0	0.0	0	0	0	0.0	0.419	586.6	586.6	784.4	2455.6	1664.2	3.13
	UF3	225.0	1012.5	25	0	0	1037.5	0.406	2842.0	1804.5	2.74	0.0	0.0	0	0	0	0.0	0.464	644.6	649.6	1037.5	3486.6	2454.1	3.36
	UF4	281.3	1265.6	25	0	0	1290.6	0.410	3087.0	1290.6	2.39	0.0	0.0	0	0	0	0.0	0.529	740.6	740.6	1290.6	3827.6	2031.2	2.97
UF treatments means							910.9	0.336	2406.3	1367.1	2.74							0.450	629.1	630.4	911.0	3035.4	1997.5	3.48
With clay	AN	143.3	186.3	50.0	3.0	130.0	366.3	0.248	1736.0	1369.7	4.74	143.3	186.3	50.0	0.0	0.0	236.3	0.261	365.4	129.1	602.6	2101.4	1498.8	3.49
	UF1	112.5	506.3	25.0	3.0	130.0	661.3	0.321	2247.0	1585.8	3.40	0.0	0.0	0.0	0.0	0.0	0.0	0.330	462.0	462.0	661.3	2709.0	2047.8	4.10
	UF2	168.8	769.4	25.0	3.0	130.0	924.4	0.371	2597.0	1682.6	2.81	0.0	0.0	0.0	0.0	0.0	0.0	0.409	572.6	572.6	924.4	3169.6	2255.2	3.43
	UF3	225.0	1012.5	25.0	3.0	130.0	1167.5	0.400	2800.0	1632.5	2.40	0.0	0.0	0.0	0.0	0.0	0.0	0.538	753.2	753.2	1167.5	3553.2	2385.7	3.04
	UF4	281.3	1265.6	25.0	3.0	130.0	1420.6	0.483	3381.0	1960.4	2.38	0.0	0.0	0.0	0.0	0.0	0.0	0.598	837.2	837.2	1420.6	4218.2	2797.6	2.97
UF treatments means							1043.4	0.394	2756.3	1715.3	2.75							0.469	656.3	656.3	1043.5	3412.5	2371.6	3.38

3.3. Energetic economical situation:

Again, it must be mentioned that the discussed energy is the energy which has been spent at nitrogen fertilizer manufacturing. Data given in Table 9 show general picture about the cost of consumed energy to produce one ton of yield of first or second cropping sequences: Firstly, the cost at first copping sequence has been less than that at second one. This due to that the obtained yield of first has been more than that of second. Secondly, the cost in case of adding clay has been less than that in case of no adding it.

Thirdly, at using UF fertilizer, the cost (on average) has been much less than that of AN treatment; the reduction has amounted 61% and 62% of that of AN treatment for $summation_1$ and $summation_2$ respectively, for first cropping sequence while at second one, it has amounted 69% and 50% for case of no adding clay and adding it respectively due to high nitrogen use efficiency of UF fertilizer.

Table 9.Cost of consumed energy to obtain a ton of dry matter (Yield) of first and second cropping sequences as affected by different treatments.

Treatments	*Cost of energy L.E. ton^{-1}			
	First cropping sequence		Second cropping sequence	
	$(Summation)_1$	$(Summation)_2$	Without adding clay	With adding clay
AN	253	143	247.50	311.20
UF_1	154	88	121.85	120.20
UF_2	176	99	172.17	152.31
UF_3	132	88	182.07	168.87
UF_4	121	88	204.13	183.16
Means of UF -treatments	154	88	170.06	156.13

*Cost of energy, L.E. ton^{-1} = E-CA, L. Diesel fuel $.ton^{-1}$ X L.E. 1.10 (current price)

In conclusion, in spite of the superior performance of UF-fertilizer in the matter of yield production to that of AN-one, their both profitabilities have come proximate. To produce 1 ton of oil crops using UF-fertilizer, it has been consumed (on average) about 78.45 kg N produced with 4725.49 M Joule or 126.35 liter of diesel fuel while at using ammonium nitrate, the consumables have been about 132.00 kg N produced with 7941.14 M Joule or 212.33 liter of diesel fuel. The application of UF has saved (on average) about 53.55 kg $N.ton^{-1}$(yield) and 3215.65 M $Joule.ton^{-1}$ or 85.98 liter of diesel fuel $.ton^{-1}$ which produce at its combustion 229.57 kg of CO_2 emissions (85.98x*2.67). Thus, the application of SRNFs has not only contributed to maintaining the environment (air, water and soil) but it has also helped to enhancing the efficiency of energy application which is the main concern of environmental policies targeting to reduce the CO_2 emissions causing the global worming.

*Carbon coefficient of a diesel fuel liter = 2.67Kg CO_2 **(**U.S. Environmental Protection Agency**,** 2005)

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Response of Snap Bean *(Phaseolus vulgaris* L) Plants to Nitrogen Fertilizer and Foliar Application with Methionine and Tryptophan

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Abstract: Two field experiments were carried out in two successive seasons of 2008 and2009 at the Agricultural Experimental Station of the National Research Centre, EL-Nubaria, Elbehira Governorate, Egypt, to study the effect of different combinations of three levels of nitrogen fertilizer (100%, 65% and 35% of the recommended dose) with two levels of foliar spray methionine and tryptophan (100 and 200mgL^{-1}) on growth, pod yield, quality and some chemical constituents of snap bean plants. Results showed that fertigated snap bean plants with the highest nitrogen dose increased the vegetative growth, yield and quality. tryptophan (100mgL^{-1}) improved vegetative growth, yield and quality. Foliar application of tryptophan at both concentrations increased free amino acids content and phenolics content in the leaves. In addition, both concentrations of methionine increased free amino acid, protein percentage and nitrogen percentage in pod. It can be concluded that nitrogen fertilizer can be reduced to 65% with sprayed tryptophan amino acid (100mgL^{-1}) to obtain the highest vegetative growth, yield and quality of snap bean plants.
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1. Introduction

Snap bean or 'French bean' (also referred to as green beans or string beans) is a strain of common bean, (*Phaseolus vulgaris* L), which is grown as a cash crop at large scale and smallholder farmers. So, snap bean is an important vegetable crop for local consumption and export.

Mineral fertilizers application is essential for plant growth, development and productivity of snap bean plants. With fertilizers, farmers can produce more food and cash crops of better quality, especially in the low soil fertility which has been over-exploited. Nitrogen is one of the 17 chemical elements required for plant growth and reproduction. On the other hand, frequent or excessive amounts of nitrogen fertilizer would led to un-favorite effect on the growth and yield of snap bean plants and will lead to increase the losses of nitrogen fertilizer. So, the adequate amounts of nitrogen fertilization led to improve growth, yield and quality of pods.

Nitrogen is of vital importance for plant growth due to being a part of amino acid, protein, enzymes and chlorophyll molecule (Devlin and Witham, 1986). Many investigators reported that increasing NPK levels application improved the plant growth, yield and green pod quality of snap bean (Singer *et al*., 2000, Saxena *et al*., 2003, Hafez *et al*., 2004, Abdel-Mawgoud *et al*., 2005, Souza *et al*., 2008, and El-Bassiony *et al*., 2010). All vegetative growth parameters were gradually and significantly increased by increasing the level of nitrogen fertilizer application (Asmaa *et al*., 2010). Insufficient available N leads to reduced growth, reduced light interception, limited yield and early crop senescence. On the other hand, excessive available N can result in reduced and delayed yield and reduced dry matter content (Kleinkopf *et al*., 1981).

El-Tohamy *et al* (2009) reported the possibility of using slow release N fertilizer such as Ensyabine to maximize growth, yield and quality of bean plants grown under new-reclaimed sandy soils. This approach also provides an efficient way of applying nitrogen to such soils to increase the efficiency of N application and to minimize leaching as well as to prevent environmental pollution by the excess of nitrogen in the soil.

Bioregulator substances were shown to enhance the biosynthesis of certain chemical constituents in plants. In this respect the amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000). Maxwell and Kieber (2004) indicated the link of methionine to the biosynthesis of growth regulating substances, e.g. cytokinins, auxins and brassinosteroids in plants. Whereas the link of tryptophan to the biosynthesis of auxins, the phytoalexin camalexin, phenyl propanoids and other related natural products in plants was recently reported (Tao *et al*. 2008). Studies have proved that amino acids can directly or indirectly influences the physiological activities of plant growth and development. Many studies reported that the foliar

application of amino acids caused an enhancement in plant growth, fruit yield and its components (El-Shabasi *et al*., 2005) on cucumber, and (Awad *et al.*, 2007) on garlic.

Thus, this study aimed to investigate the interactive effect of different nitrogen fertilizer levels in combination with foliar spray of methionine and tryptophan on the growth, productivity, quality and some chemical constituents of snap bean plant.

2. Materials and Methods:

A field experiment was carried out at the Agricultural Experimental Station of National Research Centre, Nubaria, Elbehira Governorate, Egypt, during two successive summer seasons of 2009 and 2010 to study the effect of the interaction between 3-rates of nitrogen fertilizers application (100%, 65% and 35% of the recommended dose i.e. 100 unit N/feddan 300Kg ammonium nitrate) and two concentrations of methionine and tryptophan (100 and 200mgL^{-1}) in addition to the control on the growth, yield and pods quality of snap bean (*Phaseolus vulgaris* L.cv. Paulesta). Seeds of snap bean were sown on the first week of April in the two seasons. Seeds were sown on two sides of the ridge10 cm apart, ridge was 80 cm width and 4 m length and the plot area was 12.8 m^2. Nitrogen fertilizer was applied through fertigation three times per week. At 30 and 45 days after sowing methionine and tryptophan were sprayed on snap bean plants.

The experimental soil was sandy and the physical and chemical analyses are presented in Table (1). The normal agricultural practices required for snap bean production were applied as recommended.

Table (1): Physical and chemical properties of the experimental soil.

Physical properties		Chemical analysis	
Sand	90.08	Ca (Mg/L)	7.02
Clay	9.26	Mg (Mg/L)	0.527
Silt	0.66	Na (Mg/L)	0.982
Texture	Sandy	K (Mg/L)	0.31
F.C. %	16.57	HCO_3 (Mg/L)	1.3
W. P. %	5.25	Cl (Mg/L)	0.566
E. C. (ds/m)	1.7		
PH	8.2		

Data recorded:

Vegetative growth: A sample of 5 plants from each plot was taken randomly at 45 days after sowing and the following characters were recorded: plant length, leaves number, branches number, fresh and dry weight of snap bean per plant.

Pods yield:

At harvest stage, the mature pods of bean for each experimental plot were collected along the harvesting season and the total pods yield was recorded as ton/fed (feddan=4200m^2).

Pods quality:

Random sample of 50 pods from each plot was taken and average pod weight and pod length were recorded.

Chemical constituents:

Photosynthetic pigments (chlorophyll a, b, carotenoids and total photosynthetic pigments) content were estimated in snap bean leaves according to Wettstein (1957). Protein percentage was determined according to A.O.A.C. (1990). Total Free amino acids were determined using the ninhydrin colorimetric method defined by Plummer (1978). Total phenolic compounds were estimated according to Snell and Snell (1952).

The treatments were arranged in a split plot design with four replicates where, nitrogen fertilizers rates were arranged in main plots, while methionine and tryptophan foliar application treatments were in addition the control distributed in the sub plots. The obtained data were statistically analyzed according to the method described by Gomez and Gomez (1984).

3. Results and Discussion:

Effect of nitrogen and amino acids on vegetative growth of snap bean plants:-

1- Effect of nitrogen level:-

Data in Table (2) cleared that the application of nitrogen fertilizer at recommended dose (100 %) increasing significantly the vegetative growth criteria (i.e. plant length, leaves number/plant, number of branches/plants and fresh and dry weight of leaves/plant) in the two seasons. While the lowest values of the vegetative growth criteria was obtained with the application of nitrogen at 35% of recommended dose. These results were true in the two seasons. This result may be due to nitrogen is vital importance for plant growth due to being a part of amino acid, protein, enzymes and chlorophyll molecule (Devlin and Witham, 1986).

2- Effect of amino acids:-

Spraying snap bean plants with amino acid tryptophan at 100mgL^{-1} increased significantly the vegetative growth characters (number of leaves and shoots/ plant, number of branches /plants and fresh and dry weight of leaves) except for plant length in the first season and leaves dry weight in the second season, where no significant difference between both concentration of tryptophan (Table 2). These findings were true in both seasons. On the contrary, the lowest values of number of leaves and shoots/plant, number of branches /plants and fresh and dry weight of leaves were recorded in the unsprayed plants. Amino acids can directly or indirectly influences the physiological activities of plant growth and development. El- Shabasi *et al*. (2005) on cucumber and Awad *et al.* (2007) on garlic reported that foliar application of amino acids caused an enhancement in plant growth, yield and its components. Amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000).

Table (2): Effect of nitrogen and amino acids on vegetative growth of snap bean plants in 2008 and 2009 seasons

Treatments		2008 season					2009 season				
		Plant length (cm)	Leaf number /plant	Branch number /plant	Leaves fresh weight (g)	Leaves dry weight (g)	Plant length (cm)	Leaf number	Branch number	Leaves fresh weight (g)	Leaves dry weight (g)
Effect of nitrogen fertilizer level											
35% N		36.2	11.8	6.8	19.1	3.6	39.8	13.4	4.6	34.8	8.8
65% N		41.4	17.7	8.8	38.1	6.8	43.8	16.0	6.5	51.9	10.2
100% N		43.1	23.4	9.2	57.7	8.9	47.2	21.2	7.0	65.9	11.9
LSD at 5%		1.0	3.8	1.5	10.1	1.9	3.1	2.4	1.4	7.6	1.3
Effect of methionine and tryptophan foliar application											
Control		37.3	15.2	7.5	29.5	5.2	38.0	14.0	5.0	32.4	7.4
M1		41.1	16.7	8.2	33.4	5.8	43.2	16.7	6.5	41.9	8.7
M2		40.0	18.2	8.2	40.8	6.3	44.0	17.3	5.8	53.6	10.4
T1		40.7	21.3	9.5	48.5	8.2	47.3	18.3	6.8	68.1	12. 6
T2		42.0	16.8	8.0	39.3	6.6	45.7	18.0	6.0	58.4	12.6
LSD at 5%		1.37	2.2	NS	3.5	1.0	2.3	1.97	NS	6.1	1.7
Effect of interaction											
35% N	Control	32.0	9.0	6.5	13.3	2.1	33.0	10.5	4.0	29.6	5.4
	M1	37.0	11.5	7.0	14.2	2.6	38.0	13.5	4.5	30.2	8.3
	M2	35.0	11.5	6.5	16.3	3. 5	36.0	13.5	4.5	33.9	9.8
	T1	40.0	15.0	7.0	26.6	5.5	47.0	14.0	5.5	41.1	9.6
	T2	37.0	12.0	7.0	25. 5	4.4	45.0	15.5	4.5	39.1	11.0
65% N	Cont.	36.0	15.0	7.5	33.27	5.24	37.00	12.00	4.50	27.86	7.13
	M1	44.0	15.5	8.5	33.29	5.91	45.00	15.50	7.50	48.04	8.17
	M2	41.0	18.0	8.5	34.83	5.87	43.00	17.00	6.50	57.14	9.67
	T1	42.0	22.5	11.0	48.71	9.57	49.00	18.00	7.50	63.34	12.34
	T2	44.0	17.5	8.5	40.56	7.22	45.00	17.50	6.50	63.01	13.59
100% N	Cont.	44.0	21.5	8.5	41.95	8.21	44.00	19.50	6.50	39.72	9.84
	M1	42.4	23.0	9.0	52.76	8.75	46.20	21.00	7.50	47.38	9.53
	M2	44.0	25.0	9.5	71.37	9.57	53.00	21.50	6.50	69.59	11.61
	T1	40.0	26.5	10.5	70.32	9.57	46.00	23.00	7.50	99.89	15.72
	T2	45.0	21.0	8.5	51.96	8.25	47.00	21.00	7.00	72.93	13.09
LSD at 5%		2.5	5.1	1.1	12.46	1.87	3.22	2.15	1.23	11.12	1.46

M1= Methionine (100mgL^{-1}) M2= Methionine (200 mgL^{-1}) T1= Tryptophan (100mgL^{-1}.) T2= Tryptophan (200 mgL^{-1})

3- Effect of the interaction:-

The interaction between nitrogen level and amino acids had a significant effect on the vegetative growth characteristics (Table 2). However, the highest growth of snap bean plants was found with using 100 % N and foliar spray of tryptophan at 100mgL^{-1} in the two seasons, except for plant length, branches number and fresh weight of leaves in the first season and plant length in the second one. On the other hand, the lowest values of growth criteria

were found in the control treatment. These results were true in both seasons (Table 2).

Effect nitrogen and amino acid on yield and quality of snap bean plants:-

1- Effect of nitrogen level:-

Data in Table (3) cleared that the highest yield, pod length and pod weight were recorded by using 100 % N.

On the contrary, the lowest total yield of snap bean and pod weight as well as pod length was found by using 35% N. These results were true in the two seasons. The results are in agreement with that obtained by Abdel-Mawgoud *et al*. (2005); Souza *et al*. (2008) and El-Bassiony *et al*. (2010).

The obtained results may be due to the vital importance of nitrogen for plant growth due to being a part of amino acids, protein, enzymes and chlorophyll molecule (Devlin and Witham, 1986).

2- Effect of amino acids foliar application:-

Data in Table (3) showed that, tryptophan at 100mgL^{-1} increased the total yield of snap bean and pod weight than the unsprayed plants in the two season, except for pod weight in the second season and pod length in the two seasons. Spraying snap bean plants with the two bioregulators enhancement the growth criteria (Table 2) and gave healthy plants with vigour growth which produced more pod weight and consequently resulted in more yield than that unsprayed plants (Table 3). Many studies reported that foliar application of amino acids caused an enhancement in plant growth, yield and its components (El- Shabasi *et al*. 2005) on cucumber and (Awad *et al.* 2007) on garlic.

Table (3): Effect of nitrogen and amino acids on yield and quality of bean pods in 2008 and 2009 seasons.

Treatments		2008 season			2009 season		
		Pod length (cm)	Pod weight (g)	Total yield ton/fed.	Pod length (cm)	Pod weight (g)	Total yield ton/fed.
Effect of nitrogen fertilizer level							
35% N		11.3	3.4	4.24	12.41	3.41	4.25
65% N		12.6	3.9	4.36	12.88	3.87	4.56
100% N		12.7	4.5	4.69	13.91	4.64	4.84
LSD at 5%		0.9	0.1	0.16	NS	NS	0.12
Effect of methionine and tryptophan foliar application							
Control		11.13	3.14	3.76	12.53	3.05	3.82
M1		12.27	3.47	4.05	13.23	3.94	4.20
M2		12.19	3.92	4.57	13.23	4.08	4.60
T1		12.55	4.77	5.28	13.52	4.44	5.32
T2		12.95	4.37	4.50	12.81	4.37	4.79
LSD at 5%		NS	0.17	0.09	NS	NS	0.77
Effect of interaction							
35% N	Control	10.60	2.62	3.43	11.20	2.92	3.60
	M1	11.50	2.94	3.61	13.30	3.23	3.76
	M2	11.20	3.52	4.37	12.30	3.52	4.59
	T1	11.60	4.35	5.56	13.11	3.53	4.94
	T2	11.75	3.74	4.23	12.13	3.84	4.34
65% N	Cont.	11.50	2.84	3.46	12.50	2.74	3.76
	M1	12.50	3.51	3.81	12.50	3.87	4.36
	M2	12.60	3.87	4.37	13.10	3.76	4.33
	T1	12.74	4.63	5.49	13.50	4.59	5.44
	T2	13.60	4.55	4.69	12.80	4.38	4.90
100% N	Cont.	11.30	3.96	4.39	13.90	3.48	4.11
	M1	12.80	3.97	4.74	13.90	4.71	4.49
	M2	12.77	4.38	4.96	14.30	4.95	4.88
	T1	13.30	5.34	4.79	13.95	5.19	5.59
	T2	13.50	4.82	4.59	13.50	4.89	5.13
LSD at 5%		1.32	1.36	1.02	NS	1.24	1.04

M1= Methionine (100mgL^{-1}) M2= Methionine (200 mgL^{-1}) T1= Tryptophan (100mgL^{-1}.) T2= Tryptophan (200 mgL^{-})

3- Effect of the interaction:-

The interaction between nitrogen level and methionine and tryptophan foliar application had a significant effect on the total yield of snap bean and pod weight as well as pod length in both seasons, except for pod weight in the second season. However, the highest growth of snap bean plants was recorded by using 100 %N and foliar spray of tryptophan (100mgL^{-1}) in the two seasons, except for plant length, branch number and fresh weight of leaves in the first season and plant length in the second one. On the other hand, the lowest values of growth were found in the control treatment (Table 3).

Effect of nitrogen fertilizer level and amino acids on photosynthetic pigments content in leaves of snap bean plants:-

1- Effect of nitrogen level:-

Data in Table (4) show that, using 35 % N increasing chlorophyll a, b, carotenoids and total photosynthetic pigments content in the two seasons. Whereas, nitrogen levels had insignificant effect on photosynthetic pigments content in the second season and carotenoids in the first season On the contrary, the lowest values of photosynthetic pigments content were recorded by 100 % N. These results were true and similar in the two seasons.

2- Effect of amino acid foliar application:-

Data in Table (5) showed that the highest values of chlorophyll a found with methionine (200mgL-1) in the first season and by methionine (100mgL-1) in the second season. While the highest values of chlorophyll b was found in control treatment in the first season and by methionine at low concentration (100mgL^{-1}) in the second one. Moreover, the highest values of carotenoids were found by foliar spray of methionine at 200mgL^{-1} in the two seasons.

Table (4): Effect of nitrogen and amino acids on photosynthetic pigments content (mg/g/fw) in leaves of snap bean plant in 2008 and 2009 seasons

Treatments		2008 season				2009 season			
		Chl. a	Chl. b	Carotenoids	Total pigment	Chl. a	Chl b	Carotenoids	Total pigment
Effect of nitrogen fertilizer level									
35% N		0.60	0.23	0.32	1.15	0.75	0.31	0.39	1.45
65% N		0.54	0.20	0.29	1.03	0.70	0.29	0.38	1.37
100% N		0.51	0.16	0.28	0.95	0.77	0.30	0.45	1.53
LSD at 5%		0.04	0.03	NS	0.08	NS	NS	NS	NS
Effect of methionine and tryptophan foliar application									
Control		0.46	0.13	0.27	0.86	0.68	0.26	0.35	1.29
M1		0.57	0.22	0.30	1.09	0.70	0.29	0.39	1.38
M2		0.65	0.22	0.31	1.19	0.84	0.34	0.49	1.67
T1		0.42	0.16	0.24	0.82	0.64	0.27	0.34	1.25
T2		0.65	0.24	0.37	1.26	0.85	0.35	0.46	1.66
LSD at 5%		0.06	0.03	NS	0.14	NS	NS	NS	NS
Effect of interaction									
35% N	Cont.	0.43	0.06	0.29	0.78	0.63	0.19	0.33	1.15
	M1	0.83	0.36	0.41	1.60	0.91	0.38	0.49	1.78
	M2	0.61	0.26	0.29	1.16	0.78	0.34	0.38	1.50
	T1	0.49	0.22	0.27	0.98	0.66	0.31	0.34	1.31
	T2	0.64	0.25	0.34	1.23	0.78	0.34	0.39	1.51
65% N	Cont.	0.47	0.18	0.24	0.89	0.53	0.22	0.27	1.02
	M1	0.49	0.19	0.26	0.94	0.51	0.21	0.31	1.03
	M2	0.66	0.26	0.35	1.27	1.02	0.44	0.52	1.98
	T1	0.38	0.14	0.22	0.74	0.56	0.23	0.29	1.08
	T2	0.68	0.24	0.37	1.29	0.89	0.35	0.51	1.75
100% N	Cont.	0.47	0.16	0.27	0.90	0.89	0.38	0.44	1.71
	M1	0.39	0.12	0.22	0.73	0.69	0.27	0.38	1.34
	M2	0.69	0.15	0.29	1.13	0.72	0.24	0.57	1.53
	T1	0.39	0.12	0.22	0.73	0.69	0.27	0.39	1.35
	T2	0.63	0.24	0.39	1.26	0.88	0.36	0.48	1.72
LSD at 5%		NS	NS	NS	NS	NS	NS	NS	NS

M1= Methionine (100mgL^{-1}) M2= Methionine (200 mgL^{-1}) T1= Tryptophan (100mgL^{-1}.) T2= Tryptophan (200 mgL^{-1})

Spraying snap bean plants with methionine or tryptophan at 100mgL^{-1} recorded the highest value of total photosynthetic pigments (Table 4). The results which recorded by other investigator indicated that amino acids can directly or indirectly influence the physiological activities of the plant. Amino acids help to increase chlorophyll in the plant (Awad *et al.* 2007 and Al-Said *et al.* 2008).

3- Effect of the interaction:-

The interaction between nitrogen level and methionine and tryptophan foliar application had insignificant effect on chlorophyll a, b, carotenoids and total photosynthetic pigments in the two seasons of study (Table 4). This result show that each factor act independent.

Effect of nitrogen and amino acid on free amino acids, phenolic contents in leaves and free amino acids, protein, nitrogen in pod of snap bean plants:-

1- Effect of nitrogen level:-

The results in Table (5) show clearly that using 65 % or 100% N increasing free amino acids in both seasons. While fertigated bean plants with 35 % or 65% N increased the total phenolic compounds in leaves of bean plants in the two seasons. Whereas nitrogen levels had a significant effect on total free amino acids, protein % and nitrogen percentage. The highest values of free amino acids were obtained when bean plants fertigated with 65% nitrogen. In addition, using 100% nitrogen gave the highest values of protein percentage and nitrogen percentage in the two seasons.

Table (5): Effect nitrogen and amino acid on the total free amino acids (FAA), phenolic contents in leaves and free amino acid, protein, nitrogen percentage in pods of snap bean plants in 2008 and 2009 seasons

Treatments		2008 season					2009 season				
		In leaves		In pod			In leaves		In pod		
		FAA	Phenolic	FAA	Protein%	N%	FAA	Phenolic	FAA	Protein%	N%
Effect of nitrogen fertilizer level											
35% N		17.94	12.45	21.44	15.75	2.52	22.74	12.70	22.16	15.88	2.54
65% N		23.43	13.22	27.06	19.25	3.08	25.62	13.41	27.97	18.88	3.02
100% N		24.57	10.76	21.57	20.00	3.20	27.18	10.96	23.60	20.50	3.28
LSD at 5%		1.35	1.16	2.35	1.15	0.18	2.05	1.13	1.22	1.17	0.19
Effect of methionine and tryptophan foliar application											
Control		17.39	11.42	19.42	17.30	2.77	18.71	11.57	21.03	16.88	2.70
M1		20.57	10.84	31.30	21.88	3.50	25.54	10.89	33.18	21.88	3.50
M2		23.52	10.56	26.61	18.55	2.97	25.31	10.83	27.47	18.54	2.97
T1		23.47	12.78	20.56	16.25	2.60	25.82	12.99	21.15	17.09	2.73
T2		24.97	15.12	18.88	17.71	2.83	30.52	15.52	20.05	17.71	2.83
LSD at 5%		1.35	1.23	3.67	1.09	0.17	2.34	1.10	2.14	1.37	0.22
Effect of interaction											
35% N	Control	11.03	9.73	17.02	14.38	2.30	11.19	9.91	17.54	13.75	2.20
	M1	14.69	12.96	36.42	18.13	2.90	23.71	13.07	37.17	18.75	3.00
	M2	25.69	10.34	24.79	16.88	2.70	26.33	10.55	24.89	16.88	2.70
	T1	23.17	11.04	12.97	14.38	2.30	25.45	11.47	13.45	15.00	2.40
	T2	15.11	18.17	15.98	15.00	2.40	27.03	18.52	17.76	15.00	2.40
65% N	Cont.	19.66	15.29	22.82	18.13	2.90	22.00	15.56	23.24	17.50	2.80
	M1	29.11	10.93	35.69	25.00	4.00	31.08	10.94	35.78	23.75	3.80
	M2	21.38	10.06	31.37	19.38	3.10	24.34	10.19	32.83	18.75	3.00
	T1	23.48	16.04	17.46	13.75	2.20	24.65	16.04	18.73	14.38	2.30
	T2	23.54	13.80	27.98	20.00	3.20	26.05	14.34	29.27	20.00	3.20
100% N	Cont.	21.48	9.23	18.43	19.38	3.10	22.94	9.24	22.32	19.38	3.10
	M1	17.90	8.64	21.79	22.50	3.60	21.84	8.65	26.58	23.13	3.70
	M2	23.48	11.27	23.67	19.38	3.10	25.26	11.76	24.69	20.00	3.20
	T1	23.75	11.26	31.26	20.63	3.30	27.37	11.47	31.28	21.88	3.50
	T2	36.25	13.40	12.68	18.13	2.90	38.48	13.69	13.12	18.13	2.90
LSD at 5%		3.52	3.84	2.46	2.26	0.36	3.25	2.76	6.85	1.78	0.28

M1= Methionine (100mgL^{-1}) M2= Methionine (200 mgL^{-1}) T1= Tryptophan (100mgLl^{-1}.) T2= Tryptophan (200 mgL^{-})

2- Effect of amino acids foliar spray:-

Data in Table (5) showed that, the two amino acids foliar spray had a significant effect on all measured characters. Tryptophan (200mgL^{-1}) gave the highest values of free amino acids and phenolic compounds in leaves of bean plants in both seasons. Whereas, using methionine (100mgL^{-1}) increased free amino acids, protein percentage and nitrogen percentage in pod of snap bean plants.

3- Effect of interaction:-

Concerning the interaction between nitrogen levels and amino acid treatments data in Table (5) indicated that using 100% nitrogen fertilizer with tryptophan (200mgL^{-1}) gave the highest values of free amino acids in leaves (36.25 and 38.48 mg g^{-1}dryweight) respectively. Moreover, using 35% nitrogen with tryptophan (200mgL^{-1}) increased the total phenolic in leaves of bean plants in both seasons. Fertigated bean plants with 35% nitrogen plus spraying with Methionine (100mgL^{-1}) increased the free amino acids in pods. Whereas, Methionine (100mgL^{-1}) foliar spray with 65% or 100 % nitrogen fertilizer gave the highest values of protein percentage and nitrogen percentage in pod of bean plants in the two seasons.

4. Conclusion

It can be concluded from the results of this study that using the bioregulator tryptophan (amino acids) as foliar spray at the concentration of 100mgL^{-1} under fertigated with 65% nitrogen fertilizer of snap bean plants resulted in improvement the plant growth, productivity and quality. Accordingly, we can minimize the negative impacts of excessive nitrogen fertilization on soil characteristics and the plants.

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Medicinal Plants of submontane forest in a part of Tarai and Bhawar of Kumaun Himalaya

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Abstract: The medicinal properties of forest vegetation was analyzed in a submontane forest of Tarai and Bhawar of Kumaun adjacent to Kashipur, at (29º 14-43.6)–(29º 19-50.5) E longitude and (79º 03-22.6)–(79º 04-23.2) N latitude at an elevation of 253.4–265.5 meter above the sea level, within the districts of Nainital and Udham Singh Nagar. 29 plants species belonging to 22 family, 26 genera, and 29 species were reported. Of these leaves in 19% cases, roots and whole plants in 16% cases, fruits and bark 13% cases are used. Based on life form 17 phanerophytes, 5 chamaephytes, 4 therophytes, 2 hemicryptophytes and 1 therophyte were recorded.
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Key Words:, Ethnomedicinal, Kumaun Himalaya, Medicinal plant, Submontane forest, Tarai and Bhawar.

1. Introduction

The use of plants in curing and healing is as old as man himself (Hedberg, 1987). All cultures have folk medicine traditions that include the use of plants and plant products. The World Health Organization (WHO) estimates that 4 billion people or 80 percent of the world's population use herbal medicine for some aspect of primary health care. According to Ved Prakash (1998), more than 20,000 species of higher plants are used as medicines in the traditional treatment practices of indigenous cultures living around the world. Investigations on growth performance of medicinal plants have gained adequate attention in India. Ethnomedicinal studies on vegetation in tribal areas have been carried out by Chopra (1980), Sangai (1995), Shylaja *et al.* (1996), Bargali (1997), Chauhan *et al.* (1997), Jamwel and Kaul (1997), Karikanthimath *et al.* (1997), Manian and Gopalkrishnan (1997), Pandey *et al.* (1998), Singh and Singh (1998), Bargali *et al.* (2003), Natrajan and Paulsen (2000), Maruthi *et al.* (2000), Samvastar and Diwanji (2000), Hebbar *el al.* (2004), Vijayan *et al.* 2004, Chhetri *et al.* (2005), Kala (2005), Dobhal *et al.* (2007), Semwal *et al.* 2010 and Joshi (2011).

There are about 1500 to 2000 species with known medicinal worth in India, which support an estimated 5000 indigenous drug manufactures, which make about 2000 preparations in different parts of the country. It is believed that 80% of the raw material requirement is met from the forest only (Chopra, 1994).

Present study provides some information on medicinal property of submontane forest vegetation reported in a part of Tarai and Bhawar of Kumaun Himalaya.

2. Geographical Location

For the present study, the forests of Tarai and Bhawar area of Kumaun Himalaya adjacent to Kashipur were selected. The study sites situated in the foothills of Shivalik mountain of the Outer Himalaya and south-east to Corbett National Park at (29º 14-43.6)–(29º 19-50.5) E longitude and (79º 03-22.6)–(79º 04-23.2) N latitude at an elevation of 253.4–265.5 meter above the sea level, within the districts of Nainital and Udham Singh Nagar and occupies the middle reaches of the river Kosi and Dabaka. (Source: Office of Tarai West Forest Division, Kumaun, Ramnagar, Uttarakhand).

3. Material and Methods

In present study, the information about plants was obtained by frequent field visits, from experience of personals of forest department and the local natives (older household and women). Lists of medicinal plants that are being traditionally used by the local people of area have been prepared. The plants were identified with the help of a plant taxonomist and the forest flora of Kumaun (Osmoston, 1926), Flora Simlensis (Collet, 1971), Flora Nainitalensis (Gupta, 1968) and Flora of Mussoorie (Raizada, 1978).

4. Results

29 plants along with family, common name, vegetation type, plant parts used, active constituents, life form and medicinal uses are described below:

Ø *Acacia catechu* Willd.
Family: Mimosaceae; Common Name: Kattha, Khair; Vegetation Type: Tree; Plant parts: Wood; Constituents: Tannins; Life form: Phanerophyte.

Use: Diarrhoea, cleaning mouth and gums.
Ø *Aegle marmelos* (L.) Correa

Family: Rutaceae; Common Name: Bel; Vegetation Type: Tree; Plant parts: Fruits; Constituents: Carbohydrates, Tannins; Life form: Phanerophyte.
Use: Diarrhoea, dysentery, digestive, appetizer and tonic.

Ø ***Asperagus racemosus* Willd.**
Family: Liliaceae; Common Name: Satavar; Vegetation Type: Herb; Plant parts: Roots; Constituents: Saponins; Life form: Therophyte.
Use: Antioxytocis and galactagogue activity.

Ø ***Bauhinia malabarica* Roxb.**
Family: Caesalpiniaceae; Common Name: Kachnar; Vegetation Type: Tree; Plant parts: Leaves and Bark; Constituents: Tannins; Life form: Phanerophyte.
Use: Vermifuge and antispasmodic.

Ø ***Biophytum sensitivum* Zucc.**
Family: Oxalidaceae; Common Name: Lajalu; Vegetation Type: Herb; Plant parts: Roots and Leaves; Life form: Therophyte.
Use: Diuretic, antipyretic and swelling of body.

Ø ***Boerhaavia diffusa* L.**
Family: Nyctaginaceae; Common Name: Punarnava; Vegetation Type: Herb; Plant parts: whole plant; Constituents: Alkaloids; Life form: Hemicryptophyte.
Use: Liver tonic, diuretic and anti-inflammatory.

Ø ***Bombax ceiba* L.**
Family: Malvaceae; Common Name: Semul; Vegetation Type: Tree; Plant parts: Flower, Gum and Root; Constituents: Tannin, Carbohydrates and Fatty Acids; Life form: Phanerophyte.
Use: Gout and urinary tract infection.

Ø ***Cannabis sativa* L.**
Family: Urticaceae; Common Name: Bhang; Vegetation Type: Shrub; Plant parts: Flowering tops; Constituents: Resins, Carbohydrate and Fatty Acids; Life form: Chamaephyte.
Use: Sedative and antiemetic.

Ø ***Cassia fistula* L.**
Family: Caesalpiniaceae; Common Name: Amaltas; Vegetation Type: Tree; Plant parts: Leaves and Pods; Constituents: Glycosides; Life form: Phanerophyte.
Use: Laxative and skin disorders.

Ø ***Cathranthus roseaus* (L.) G.Don**
Family: Apocynaceae; Common Name: Sadabahar; Vegetation Type: Shrub; Plant parts: Whole Plant; Constituents: Alkaloids; Life form: Chamaephyte.
Use: Antineoplastic agent.

Ø ***Centella asiatica* (L.) Urb.**
Family: Apiaceae; Common Name: Brahmi; Vegetation Type: Herb; Plant parts: Whole Plant; Constituents: Triterpenoid Saponins and Glycosides; Life form: Hemicryptophyte.
Use: Brain tonic, antianxiety and antistress.

Ø ***Cuscuta reflexa* Roxb.**
Family: Convolvulaceae; Common Name: Amar Bael; Vegetation Type: Climber; Plant parts: Whole Plant; Constituents: Cuscutalin and Cuscutin; Life form: Phanerophyte.
Use: Vermifuge and heart tonic.

Ø ***Datura stromonium* L.**
Family: Solanaceae; Common Name: Dhattura; Vegetation Type: Herb; Plant parts: Leaves and Flowering tops; Constituents: Alkaloids; Life form: Chamaephyte.
Use: Spasmolytic. Vomiting agent and motion sickness.

Ø ***Eucalyptus hybrid* L.Herit.**
Family: Myrtaceae; Common Name: Safeda; Vegetation Type: Tree; Plant parts: Leaves; Constituents: Volatile oils; Life form: Phanerophyte.
Use: Antiseptic, diaphoretic and expectorant.

Ø ***Ficus racemosa* L.**
Family: Moraceae; Common Name: Gular; Vegetation Type: Tree; Plant parts: Fruits; Life form: Phanerophyte.
Use: Blood disorders, piles and gonorrhoea.

Ø ***Ficus religiosa* L.**
Family: Myrtaceae; Common Name: Pipal; Vegetation Type: Tree; Plant parts: Bark; Constituents: Tannins; Life form: Phanerophyte.
Use: Laxative and astringent.

Ø ***Holarrhena antidysenterica* Wall.**
Family: Apocynaceae; Common Name: Inderjhon; Vegetation Type: Tree; Plant parts: Bark; Constituents: Alkaloids; Life form: Phanerophyte.
Use: Antidysenteric and febrifuge.

Ø ***Holoptelea integrifolia* Planch.**
Family: Ulmaceae; Common Name: Kanju; Vegetation Type: Tree; Plant parts: Leaves; Constituents: Tannins; Life form: Phanerophyte.
Use: Pyrrhoea and cleaning mouth and gums.

Ø ***Justicia adhatoda* Nees**
Family: Acanthaceae; Common Name: Vasaka; Vegetation Type: Shrub; Plant parts: Leaves; Constituents: Alkaloids; Life form: Chamaephyte.
Use: Expectorant, bronchitis and cough.

Ø ***Murraya koenigii* Spreng.**
Family: Rutaceae; Common Name: Karipatta; Vegetation Type: Shrub; Plant parts: Whole Plant; Constituents: Volatile oils; Life form: Phanerophyte.
Use: Dysentery and antidote in poisoning.

Ø ***Piper nepalense* Miq. (E.)**
Family: Piperaceae; Common Name: Pipali; Vegetation Type: Herb; Plant parts: Fruits; Constituents: Volatile oils; Life form: Therophyte.
Use: Cough and bronchitis.

Ø ***Plumbago zeylanica* L.**
Family: Plumbaginaceae; Common Name: Chitrak; Vegetation Type: Shrub; Plant parts: Root and Bark; Constituents: Plumbagin; Life form: Chamaephyte.
Use: Anoxia, to cure hydrocoel and also used for skin diseases.

Ø ***Rauwolfia serpentina* Benth.**
Family: Apocynaceae; Common Name: Sarpgandha; Vegetation Type: Herb; Plant parts: Roots; Constituents: Alkaloids; Life form: Therophyte.
Use: Malaria.

Ø ***Ricinus communis* L.**
Family: Euphorbiaceae; Common Name: Arandi; Vegetation Type: Tree; Plant parts: Seeds; Constituents: Fixed Oils; Life form: Phanerophyte.
Use: Laxative.

Ø ***Terminalia arjuna* W & A.**
Family: Combretaceae; Common Name: Arjun; Vegetation Type: Tree; Plant parts: Bark; Constituents: Tannins; Life form: Phanerophyte.
Use: Cardiac disease, diuretic and astringent.

Ø ***Terminalia bellerica* Roxb.**
Family: Combretaceae; Common Name: Bahera; Vegetation Type: Tree; Plant parts: Fruits; Constituents: Tannins; Life form: Phanerophyte.
Use: Diarrhoea and dysentery.

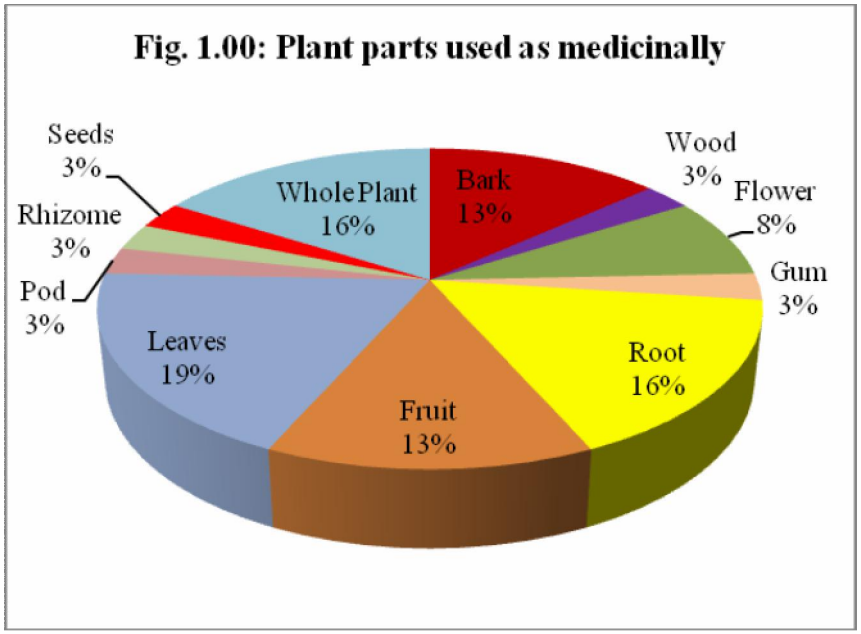
Ø ***Terminalia chebula* Retz.**
Family: Combretaceae; Common Name: Harar; Vegetation Type: Tree; Plant parts: Fruits; Constituents: Tannins; Life form: Phanerophyte.
Use: Diarrhoea and dysentery.

Ø ***Vitex negundo* L.**
Family: Verbenaceae; Common Name: Simalu; Vegetation Type: Shrub; Plant parts: Whole Plant; Constituents: Alkaloids; Life form: Phanerophyte.
Use: Rheumatic arthritis, mental disorder and backache.

Ø ***Zingiber capitatum* Roxb.**
Family: Zingiberaceae; Common Name: Ban Haldi; Vegetation Type: Herb; Plant parts: Roots and Rhizome; Constituents: Alkaloids; Life form: Geophyte.
Use: Antiseptic and used in skin care.

5. Discussion

Based on extensive survey, frequent field visit and interviews it was found that nearly 29 plants species belonging to 22 families, 26 genera, and 29 species are being used by people to care various diseases. Of these leaves in 19% cases, roots and whole plants were used in 16% cases, fruits and bark 13% cases (Fig. 1.00) were used. Based on life form 17 phanerophytes, 5 chamaephytes, 4 therophytes, 2 hemicryptophytes and 1 therophytes were recorded. Most of the species identified as medicinal plants are trees in comparison to herbs and shrubs. Bargali *et al.* (2003) studied 22 medicinal plant species in Jagdalpur district of Chhattisgarh. Chhetri *et al.* (2005) reported that the tribal people of Sikkim and Darjeeling Himalayan region in India utilized 37 species of plants belonging to 28 different families as antidiabetic agents. Dobhal *et al.* (2007) studies 29 species of medicinal plants distributed in 28 genera and 20 families. Vijayan *et al.* (2004) reported different parts of 18 medicinal plants belonging to 14 different families used in the traditional system of medicine collected from Nilgiris were tested for their antiviral activity.



6. Conclusion

The use of plants as medicinally is found in the Rig Veda. Information on the use of medicinal plants is found in books. Excessive use of allopathic medicine, peoples are unaware about the importance of plants as medicinally. Kapoor and Mitra (1979) estimated that about 540 plant species are in use in different formulations in India. Present time younger generation does not take interest in these plants and there is a possibility of losing this knowledge in future. Therefore, this study purposeful for making interest about the use of plants as medicinally likes local tribal and this study will also helpful for new researchers for finding other unknown uses of these plants.

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Mechanism and Modelling for Sorption of Toxic Ion on Cement Kiln Dust

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Abstract: Cement manufacturing is a critically important industry in Egypt. The industrial by-product and waste materials must be managed responsibly to insure a clean and safe environment. Cement kiln dust (CKD) is a significant by-product material of the cement manufacturing process. Cement kiln dust is a waste residue composed chiefly of oxidized, anhydrous, micron – sized particles generated as a by product of the manufacture of Portland cement. The use of cement kiln dust as adsorbent in wastewater treatment has a great attention as cheap material and clay structure. This work will discuss the basic characteristics of CKD physical and chemical properties and regulatory requirements. The batch removal of Cr(VI) from aqueous solution using low-cost adsorbents such as cement kiln dust under different experimental conditions and the influences of initial Cr (VI) ion concentration (50 to 300 mg·l-1) and pH (1 to 4) were investigated in this study. Adsorption of Cr (VI) is highly pH-dependent and the results indicate that the optimum pH for the removal was found to be 1 for CKD. A comparison of kinetic models applied to the adsorption of Cr (VI) ions on the CKD was evaluated for the pseudo first-order, the pseudo second-order, Elovich and intraparticle diffusion kinetic models, respectively. The results showed that the pseudo second-order kinetic model was found to correlate the experimental data well.
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Keywords: CKD; adsorption; Cr (VI); adsorption kinetics; low-cost adsorbents.

1. Introduction:

Chromium is an important industrial metal used in various products and processes. Usually, chromium has been released to the environment via leakage, poor storage or improper disposal practices [1]. Generally, chromium in the environment is found primarily in two oxidation states: hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)). Hexavalent chromium is relatively mobile in the environment and is acutely toxic, mutagenic, teratogenic and carcinogenic. Whilst trivalent chromium has relatively low toxicity and is immobile under moderately alkaline to slightly acidic conditions According to its toxicity, chromium was classified as a primary pollutant and ranked as second among many toxic metals in the environment [2, 3]. A number of treatment methods for the removal of metal ions from aqueous solutions have been reported as: mainly reduction, ion exchange, electrodialysis, electrochemical precipitation, evaporation, solvent extraction, reverse osmosis, chemical precipitation and adsorption.

Most of these methods suffer from drawbacks such as high capital and operational costs or the disposal of the residual metal sludge. In Egypt, production of the different types of cement reached nearly 30 million tons, with 3 millions tons CKD /year in dry lines. Up to twenty-five years ago, cement was produced by the wet process in Egypt. Nevertheless, the on-going shift in the cement industry to the dry method is expected to increase the accumulated dust. The dry process of cement production produces three times more dust than the wet process. CKD represents a mixture of raw feed, partly calcined cement clinker, and condensed volatile salts [4-5]. The chemical composition of CKD is influenced by the size of particles carried away by the kiln gases. The released dust shows considerable amount of alkalis volatilized in the burning zone and condensed on the particles of the dust. Approximately 12% of the kiln feed exits from the kiln with the gas and about 73% of CKD is recycled to the cement making process. For example in Tourah Portland Cement Factory, the production of by-pass kiln dust per day is about 5.3% of the total production of the rotary kiln which is about 9000 ton/day. So the amount of by-pass kiln dust is about 477 ton/day Chemical coagulation, biological treatment and adsorption are the methods for the treatment of wastewater from tanneries for organic substances and heavy metals removal. The present study investigates the possibility of utilization of by-pass cement kiln dust as a adsorbent for removal of Cr (VI) from aqueous solution. A kinetic study was carried out using pH and concentration as parameters [6].

2. Experimental Procedure

Materials

Cement Kiln Dust (CKD)

Cement kiln dust (CKD) is a fine particulate material produced as a result of cement production. Cement kiln dust often consists of clinker particles,

partially calcined materials and alkali compounds, which can be high in lime content. The chemical content of CKD depends upon the raw materials, plant configuration, and the preprocessing type . Cement kiln dust as it is often used as the generic term for dust created in the kiln and collected from the cement manufacturing process. In a wet process, some of the CKD is removed from the kiln as waste dust. In a dry process, dust is collected from the kiln in precipitators. CKD removed from the clinker cooler at the end of the kiln is recalculated in the cyclone and pre-heaters (dry process). Dust collected from the upstream portion of the kiln is removed from the system as by-pass dust. The by-pass dust is removed as a precaution against materials (heavy metals, chlorides, and alkalis) that may cause the clinker to be out of specification limits.

Beneficial uses of "waste" CKD include agricultural liming agent, roadbed stabilization, and waste stabilization CKD has been used for the treatment of sand, expansive clays, and soft or wet soils .Other applications included stabilization of contaminated soil or sludge , pavement filler, and subgrade stabilization. CKD has also been used as an additive in blended cements established that CKD can used to stabilize dune sand with 12% to 50% CKD by sand weight depending upon the application. The stabilized dune sands showed higher compressive strengths with increased CKD content and increased curing temperatures, but the samples failed in freeze-thaw durability testing. For expansive clays, CKD-clay mixture has showed to have comparable engineering properties to fly ash-soil and cement-soil mixtures. The use of CKD in stabilization of clays has showed to improve the unconfined compressive strength and reduce the plasticity index using dust with low LOI. Adding CKD with high LOI resulted in relatively lower unconfined compressive strengths and higher plasticity indices [7-10] indicated that CKD could be used as an alternative to quick lime for sub-grade stabilization in highway construction. The use of CKD as a partial cement replacement in concrete has showed to have adverse effects on strength. Using 80% cement replacement resulted in an 85% decrease in compressive strength as compared with the control. Flexural strength and modulus of rupture also decreased with increasing CKD content. Table 1. Shows the monthly change in CKD chemical composition for different batches in the Tourah Portland Cement Factory. Chemical compositions are varying from batch to batch. CaO contents range from 38% to 73%, while the silicon dioxide (SiO2) ranges from approximately 10% to 20%. The Blaine fineness, or specific surface area, of cement ranges from 300 to 500 m^2/kg as determined by ASTM C 204, Standard Test Method for Fineness of Hydraulic Cement by Air Permeability Apparatus.

Table.1: Monthly change of CKD in chemical composition

Month	Percent by Weight (%)													
	SiO^2	Al_2O_3	Fe_2O_3	CaO	MgO	SO_3	Na_2O	K_2O	TiO_2	P_2O_3	SrO	Mn_2O_3	Cl	lOI
1/06	16.46	5.63	1.69	67.11	1.56	3.37	0.39	3.62	0.25	0.08	0.06	0.02	0.00	34.68
2/06	16.80	5.60	1.56	66.96	1.56	3.39	0.58	3.77	0.24	0.09	0.05	0.02	0.00	34.98
3/06	15.86	5.54	1.79	66.99	1.66	3.79	0.30	3.66	0.24	0.09	0.05	0.03	0.00	34.97
4/06	16.18	5.79	1.66	69.27	1.66	3.30	0.18	2.29	0.26	0/09	0.06	0.02	0.00	34.35
5/06	16.72	5.70	1.95	69.63	1.58	3.14	0.21	2.14	0.26	0.10	0.05	0.02	0.00	34.00
6/06	16.78	5.60	2.03	71.50	1.58	2.51	0.16	1.78	0.25	0.09	0.07	0.02	0.00	34.09
7/06	15.16	5.15	2.07	67.85	1.41	4.05	0.23	4.06	0.23	0.09	0.06	0.02	0.00	35.99
8/06	13.76	5.00	1.92	65.77	1.39	5.38	0.30	6.23	0.21	0.09	0.06	0.02	0.00	35.17
9/06	13.86	4.98	1.96	66.46	1.34	5.32	0.27	5.91	0.23	0.10	0.05	0.02	0.00	35.41
10/06	14.32	5.11	1.98	66.57	1.40	4.52	0.25	5.34	0.23	0.10	0.05	0.02	0.00	35.41
11/06	14.03	4.96	1.93	65.17	1.37	5.19	0.62	6.65	0.22	0.09	0.04	0.02	0.00	NR
12/06	13.39	4.71	1.79	64.00	1.37	6.01	0.42	7.81	0.21	0.09	0.04	0.02	0.00	NR

Fig.1 shows the Particle Size Distribution of CKD1, CKD2, CKD3, CKD4 (batches) and OPC. The CKD1 sample was coarser than OPC on the upper end of particle sizes; between 0% and 60% passing, the CKD1sample and OPC were nearly identical. The CKD4 sample was also coarser than cement. The gap in the distribution curve could possibly be due to dispersion during testing of the sample. The CKD3 and CKD2 samples were finer than cement. For the CKD2 sample, the coarsest 5% of particles was most

likely an artificial result of inadequate particle dispersion during the testing process. The CKD1 and CKD2 samples have otherwise similar distributions. By accounting for the lack of complete dispersion in the CKD2 sample, the maximum particle sizes are approximately 60 µm for the CKD1 and CKD2 samples. The maximum particle size for the CKD3 sample is much larger, approximately 450 µm. OPC has maximum particle sizes of around 100 µm, D of approximately 45 µm, and an average diameter of 15 µm . The D85 of the CKD1 and CKD2 samples are approximately 5 to 8 µm (including the lack of complete dispersion for the CKD1 sample).CKD1 and CKD4 samples is much larger, approximately 70 µm and 170 µm, respectively. The average diameter for the CKD1and CKD2 samples range from 4.7 to 11.9 µm (including the lack of complete dispersion for the CKD2 sample), and the CKD3 sample's average diameter is around 37 µm.

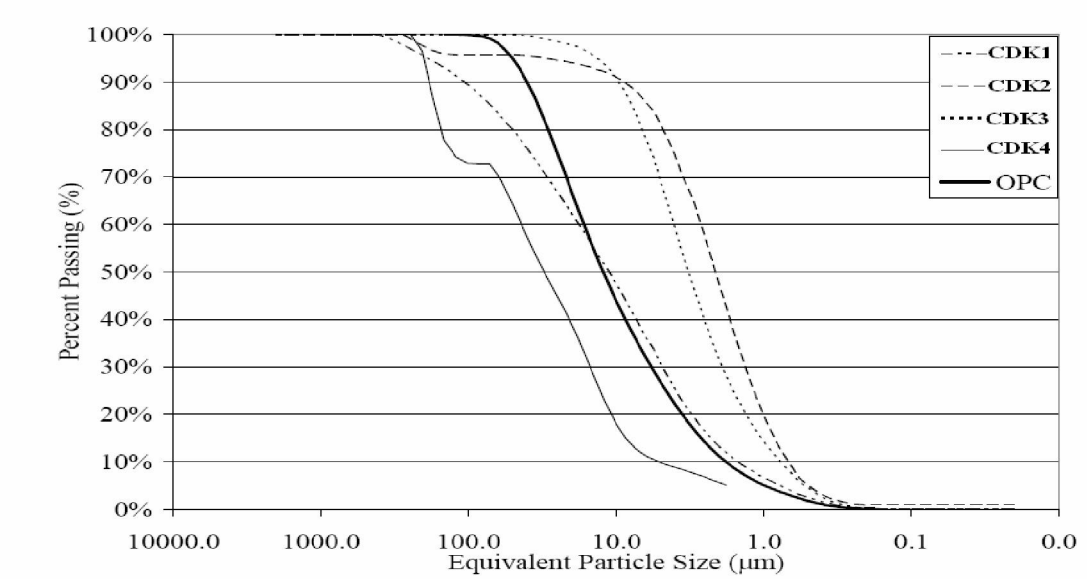

Fig. 1: Particle Size Distribution of CKD1, CKD2, CKD3, CKD4 and OPC

Table 2 indicates the specific surface area of the selected CKD. The high LOI samples (CKD1 and CKD2) were much finer than the low LOI samples,

Table 2: Specific Surface Area of OPC and different CKD

Samples	Specific Surface Area (m^2/ Kg)	D(µm)	D(µm)	D(µm)	D(µm)
OPC	300-500	45	35	13	2
CKD1	3300	13	8	3	0.7
CKD2	3900	30	5	2	0.6
CKD3	1690	200	70	11	1
CKD4	230	200	170	30	5.2

Batch adsorption studies

Batch experiments with CKD1 were conducted to investigate the parametric effects of initial adsorbate concentration on Cr (VI) adsorption. Chromium samples were prepared by dissolving a known quantity of potassium dichromate ($K_2Cr_2O_7$) in double-distilled water and used as a stock solution and diluted to the required initial concentration (range: 20 to 300 mg·L-1). 50 ml of Cr(VI) solution of known concentration (Co) and initial pH was taken in a 100 ml screw-cap conical flask with a required amount of adsorbent and was agitated at a speed of 200 rpm in a thermostatic shaker bath at 25ºC for a specified period of contact time. Then, the solution was filtered through a 0.45 µm membrane filter. The initial pH of the solution was adjusted by using either 0.1 N NaOH or 0.1 N H_2SO_4. Perkin-Elmer UV-visible spectrophotometer (model 550S) was employed with 1,5-diphenylcarbazide in acid medium to determine the remaining concentrations of Cr(VI) in the sample. The filtrate was analysed for the remaining Cr(VI) concentration. The amount of Cr(VI) adsorbed in

mg·g^{-1} at time t was computed by using the following equation:

$$q_t = \frac{(C_o - C_t)V}{m_s} \quad (1)$$

where:
C_o, and C_t are the Cr(VI) concentrations in mg·L^{-1} initially and at a given time t, respectively
V is the volume of the Cr(VI) solutions in ml
m_s is the weight of activated carbon in g.
The percentage of removed Cr(VI) ions (R_{em} %) in solution was calculated using Eq.(2):

$$R_{em}(\%) = \frac{(C_o - C_t)}{C_o} x100 \quad (2)$$

The effect of initial concentration of Cr(VI), contact time and initial pH were investigated by varying any one of the process parameters and keeping the other parameters constant. Adsorption dynamics describes the solute uptake rate and evidently this rate controls the residence time of adsorbate uptake at the solid-solution interface. The kinetics of Cr(VI) adsorption on the CKD were analysed using pseudo first-order [11] pseudo second-order [12], Elovich [13,14] and intraparticle diffusion kinetic models. The conformity between experimental data and the modelpredicted values was expressed by the correlation coefficients (r^2, values close or equal to 1). A relatively high r^2 value indicates that the model successfully describes the kinetics of Cr(VI) adsorption. The pseudo first-order equation [11] is generally expressed as follows:

The pseudo first-order equation

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (3)$$

where:
q_e and qt are the adsorption capacity at equilibrium and at time
t, respectively (mg·g-1),
k_1 is the rate constant of pseudo first-order adsorption (l·min^{-1}).
After integration and applying boundary conditions t = 0 to t = t and
q_t = 0 to q_t = qt, the integrated form of Eq. (3) becomes:

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303} t \quad (4)$$

The values of log (qe - qt) were linearly correlated with t. The plot of log (q_e - q_t) vs. t should give a linear relationship from which k_1 and q_e can be determined from the slope and intercept of the plot, respectively.

The pseudo second-order equation

The pseudo second-order adsorption kinetic rate equation [12]

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (5)$$

where:
k_2 is the rate constant of pseudo second-order adsorption (g·mg-1·min^{-1}). For the boundary conditions t = 0 to t = t and q_t = 0 to q_t = q_t, the integrated form of Eq. (5) becomes:

$$\frac{1}{(q_e - q_t)} = \frac{1}{q_e} + k\,t \quad (6)$$

which is the integrated rate law for a pseudo second-order reaction. Equation (6) can be rearranged to obtain Eq.(7), which has a linear form:

$$\left(\frac{t}{q_t}\right) = \frac{1}{k_2\, q_e^2} + \frac{1}{q_e}(t) \quad (7)$$

if the initial adsorption rate, h (mg·g-1·min-1) is:

$$h = k_2 q_e^2 \quad (8)$$

Then Eqs. (7) and (8) become:

$$\left(\frac{t}{q_t}\right) = \frac{1}{h} + \frac{1}{q_e}(t) \quad (9)$$

The plot of (t/qt) and t of Eq. (7) should give a linear relationship from which q_e and k_2 can be determined from the slope and intercept of the plot, respectively.

The Elovich equation

The Elovich model equation is generally [13, 14]

$$\frac{dq_t}{dt} = \alpha\, exp(-\beta\, q_t) \quad (10)$$

where:
α is the initial adsorption rate (mg·g-1·min-1)
β is the desorption constant (g·mg-1) during any one experiment

To simplify the Elovich equation, [17] assumed α βt >> t and by applying the boundary conditions qt = 0 at t = 0 and qt = qt at t = t Eq.(10) becomes:

$$q_t = \frac{1}{\beta}\, ln(\alpha\,\beta) + \frac{1}{\beta} ln(t) \quad (11)$$

If Cr(VI) adsorption fits the Elovich model, a plot of

qt vs. ln (t) should yield a linear relationship with a slope of (1/β) and an intercept of (1/β) ln(αβ).

The intraparticle diffusion model

The intraparticle diffusion model is expressed as [18, 19]

$$R = k_{id}(t)^{a}$$

A linearised form of the equation is followed by

$$\log R = \log k_{id} + a\log(t)$$

where:
R is the per cent Cr(VI) adsorbed
t is the contact time (h)
a is the gradient of linear plots
k_{id} is the intraparticle diffusion rate constant (h-1) a depicts the adsorption mechanism
k_{id} may be taken as a rate factor, i.e., per cent Cr(VI) adsorbed per unit time.

The values of k_{id} were calculated from the slope of such plots (plots not shown here) and the r^2 values led to the conclusion that the intraparticle diffusion process is the rate-limiting step. Higher values of k_{id} illustrate an enhancement in the rate of adsorption, whereas larger kid values illustrate a better adsorption mechanism, which is related to an improved bonding between Cr(VI) ions and the adsorbent particles .

3. Results and Discussion

Effect of pH

The removal of Cr(VI) by three types of CDK (CDK1, CDK2 and CDK3) at different pHs at an initial Cr(VI) concentration of 100 mg·l-1, a temperature of 25ºC, particle size of 1.00 to 1.25 mm and agitation speed of 200 r·min-1 are shown in Figs. 2 to 4. The adsorption of Cr(VI) occurred in two stages. The first stage was solute uptake i.e. the immediate solute uptake achieved within a few hours, followed by the second stage, i.e. the subsequent uptake of solute, which continued for a long time period. For CKD2, the amount adsorbed increased from 4.21 to 20.98 mg·g-1 as the pH decreased from 4 to 1. While for CKD3, the amount adsorbed increased from 11.44 to 20.98 mg·g-1 as the pH decreased from 4 to 1. For CKD4, the amount adsorbed increased from 14 to19.98 mg·g-1 as the pH decreased from 4 to 1. The variation in adsorption capacity in this pH range is largely due to the influence of pH on the adsorption characteristics of the carbon which indicates that the adsorption capacity of the adsorbent is clearly pH dependent. The optimum pH was observed with 99.9 % Cr(VI) removal at pH 1.0. Chromium exists mostly in two oxidation states which are Cr(VI) and Cr(III) and the stability of these forms is dependent on the pH of the system . It is well known that the dominant form of Cr(VI) at pH 2 is $HCrO_4^-$. Increasing the pH will shift the concentration of $HCrO_4^-$ to other forms, CrO_4^{2-} and $Cr_2O_7^{2-}$. Maximum adsorption at pH 1.0 indicates that it is the $HCrO_4^-$ - form of Cr(VI), which is the predominant species between pH 1 and 4, which is adsorbed preferentially on the adsorbents. Results also show that the adsorption reaction can be approximated with the pseudo second-order kinetic model. The smallest value of correlation coefficient was > 0.991 (Table 3). The rate constants are represented in Table 2. It can be observed that h is generally higher for CDK4 than that of CDK2 and CKD3

Effect of initial chromium ion concentrations

The removal of Cr(VI) by adsorption on different CKD were shows to increase with time and attained a maximum value at 72 h, and thereafter, it remained almost constant. On changing the initial concentration of Cr(VI) solution from 20 to 300 mg·L-1, the amount adsorbed increased from 10.60 mg·g-L (99.99 % removal) to 59.40 mg·g-L (99.0 % removal) at 25 ºC, pH 1.0 from Figs. 5 a, b and c the results indicate that the amount of adsorbate on the solid phase with lower initial concentration of adsorbate was smaller than the amount when higher initial concentrations were used. It was clear that the removal of Cr(VI) was dependent on the concentration of Cr(VI) because the decrease in the initial Cr(VI) concentration increased the amount of Cr(VI) adsorbed. .

The experimental points shown together with the theoretically generated curves Fig. 5 a, b and c reflect the extremely high correlation coefficients shown in Table 4. The data showed good compliance with the pseudo second-order kinetic model (r^2 > 0.989). The values of the rate constants, k_2, were found to increase from 48.10-5 to 58.10-3 (l·mg-L·min-1) with decrease in the initial Cr(VI) ion concentration from 300 to 20 mg·L/l.

The rate constants are represented in Table 3 It can be observed that h is generally higher for CDK4 than that of CKD2 and CKD3

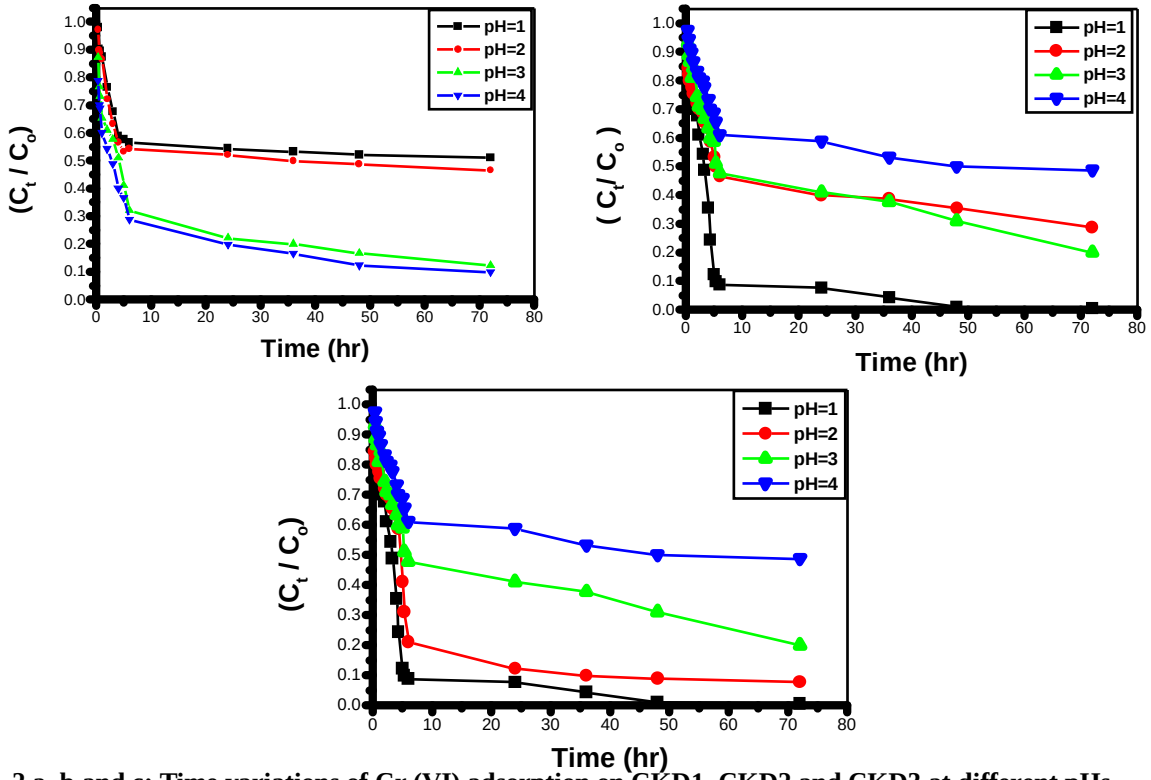

Fig. 2 a, b and c: Time variations of Cr (VI) adsorption on CKD1, CKD2 and CKD3 at different pHs

Table 3: Adsorption kinetic model rate constant for the different CKD at different pHs

Adsorbent	Initial pH	Pseudo first – order		Pseudo second – order			Elovich model			Intraparticle diffusion		
		k_1	r^2	k_2	h	r^2	β	α	r^2	k_{id}	a	R^2
CKD1	1.0	0.226	0.978	0.0091	10.891	0.898	32991	0.131	0.889	29.328	0.422	0.900
	2.0	0.108	0.892	0.0037	6.608	0.986	16704	0.139	0.974	18.934	0.488	0.981
	3.0	0.035	0.898	0.031	1.566	0.983	2421	0.241	0.977	4.633	0.600	0.970
	4.0	0.182	0.8325	0.053	1.066	0.899	4.00	0.643	0.988	2.086	1.008	0.98
CKD2	1.0	0.113	0.977	0.022	27.123	0.988	213.91	0.107	0.982	60.511	0.160	0.963
	2.0	0.106	0.973	0.015	16.503	0.98	75.13	0.188	0.965	41.537	0.300	0.986
	3.0	0.1733	0.966	0.029	12.080	0.988	112.70	0.243	0.948	32.943	0.1.71	0.897
	4.0	0.131	0.966	0.012	4.583	0.989	17.057	0.343	0.977	13647	0.435	0.899
CKD3	1.0	0.087	0.977	0.00122	0.963	0.996	33.923	47.988	0.481	33.676	0.311	0.987
	2.0	0.094	0.887	0.0033	0.881	0.989	16.705	9.876	0.765	16.987	0.499	0.943
	3.0	0.029	0.986	0.027	0.865	0.999	2.341	9.654	0.453	14.987	0.572	0.954
	4.0	0.0498	0.765	0.034	0.765	0.989	3.885	8.6732	0.436	11.997	1.008	0.989

Table6 (a) adsorption kinetic model rate constant for the CKD1 at different initial chromium ion concentrations

C_o(mg/l)	Pseude first – order		Pseude second – order			Elovich model			Intraparticle diffusion		
	k_1	r^2	k_2	h	r^2	β	α	r^2	k_{id}	a	R^2
50	0.324	0.963	0.0591	17.999	0.984	410.537	0.389	0.987	57.200	0.178	0.917
100	0.199	0.989	0.0072	11.771	0.987	33.993	0.122	0.899	37.100	0.411	0.898
200	0.090	0.887	0.0008	9.733	0.898	11.995	0.065	0.875	8.521	0.711	0.984
300	0.078	0.987	0.0007	4.321	0.899	23.011	0.032	0.876	7.3241	0.701	0.985

Table6 (b) adsorption kinetic model rate constant for the CKD2 at different initial chromium ion concentrations

C_o(mg/l)	Pseude first – order		Pseude second – order			Elovich model			Intraparticle diffusion		
	k_1	r^2	k_2	h	r^2	β	α	r^2	k_{id}	a	R^2
50	0.312	0.897	0.0574	19.111	0.896	412.500	0.467	0.984	57.200	0.188	0.887
100	0.199	0.890	0.0091	12.681	0.893	32.971	0.134	0.897	35.180	0.366	0.890
200	0.085	0.972	0.0008	9.764	0.821	13.444	0.067	0.945	8.521	0.732	0.897
300	0.066	0.876	0.0004	5.022	0.889	23.011	0.064	0.987	7.4321	0.537	0.879

Table6 (c) adsorption kinetic model rate constant for the CKD3 at different initial chromium ion concentrations

C_o(mg/l)	Pseude first – order		Pseude second – order			Elovich model			Intraparticle diffusion		
	k_1	r^2	k_2	h	r^2	β	α	r^2	k_{id}	a	R^2
50	0.200	0.888	0.0632	19.029	0.976	408.511	0.501	0.991	57.876	0.196	0.875
100	0.211	0.899	0.0512	11.893	0.899	34.007	0.156	0.897	37.063	0.401	0.900
200	0.092	0.867	0.0005	8.896	0.986	14.006	0.049	0.976	10.122	0.681	0.970
300	0.076	0.923	0.00033	5.0112	0.889	23.001	0.044	0.976	8.0211	0.743	0.986

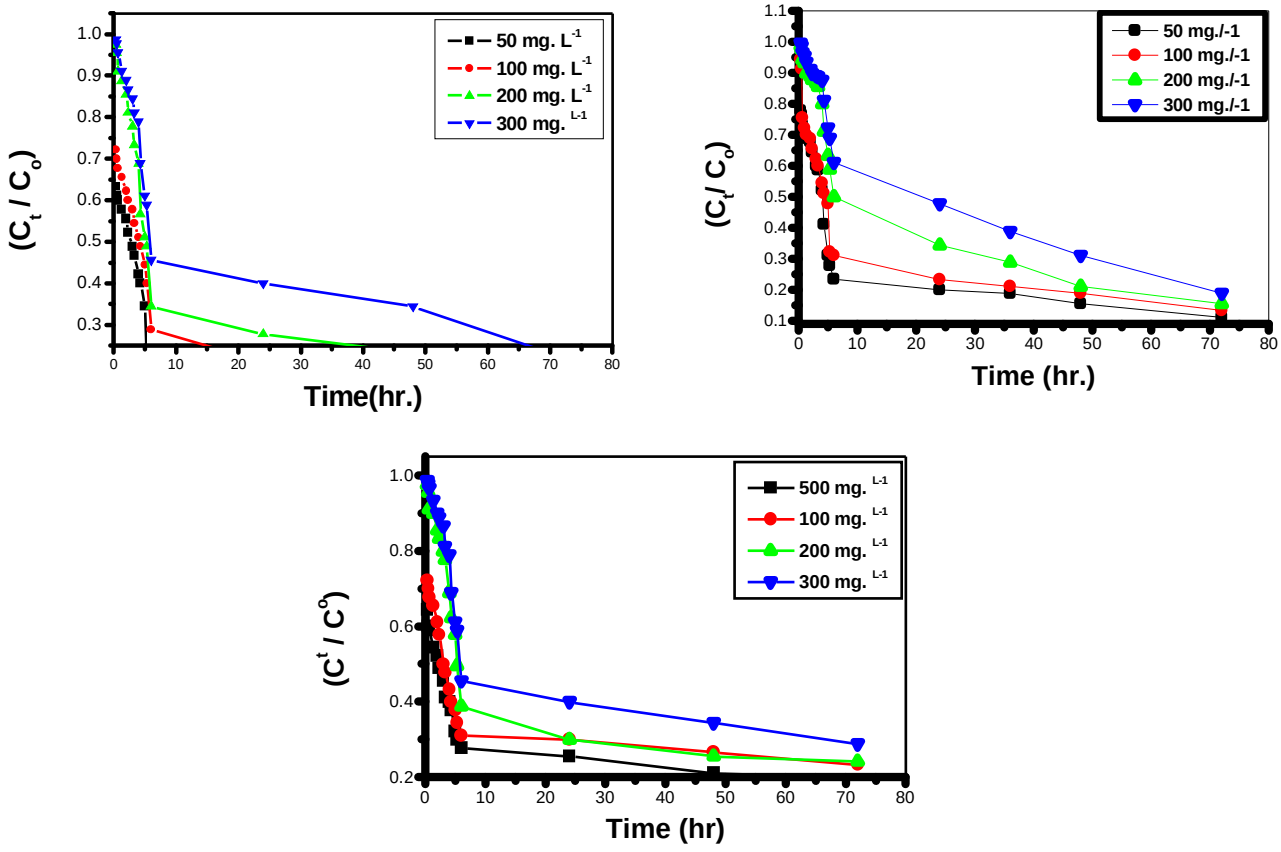

Fig. 5 a, b and c: Time variations of Cr(VI) adsorption on CKD1, CKD2 and CKD3 at different concentrations

4. Conclusion

The volume of by-product materials generated from core-sector industries such as cement, power, steel, and other mining and heavy industries are increasing. The cost of disposal is continuing to grow day-by-day in our society. The growth of by-product materials is inevitable unless new and beneficial use options, which are economically sound and environmentally friendly, are developed and implemented. Cement kiln dust (CKD) is a by-product material of cement manufacturing industry. It is a fine powdery material similar in appearance to Portland cement. The principal constituents of CKD are compounds of lime, silica, and alumina, and iron.

The physical and chemical characteristics of CKD depend on the raw materials used and the method of its collection employed at a particular cement plant. Free lime is found in CKD. The concentration of free lime is generally highest in the coarser particles of CKD captured closest to the kiln. Finer particles of CKD contain higher concentrations of sulfates and alkalis. The primary value of cement kiln dust is its cementitious property. Depending on the concentration of lime (CaO), CKD can be highly cementitious

Our results indicate that cement kiln dust could be recommended as a material for the effective removal of metal pollutants from industrial wastewaters. The results of this study have shown that CKD (a cheap by-product of cement industry) is efficient in the processes of removal of Cr (VI) from aqueous solution. CKD was the most effective, for which the removal reached 99.99 % Cr (VI) at 25ºC. Adsorption of Cr (VI) was highly pH-dependent and the results showed that the optimum pH for the removal was found to be 1, at which Cr (VI) exists mostly as the most easily adsorbed form, $HCrO_4^-$ increases as the initial Cr(VI) concentration and contact time were found to increase the percentage removal of Cr(VI). The kinetics of the Cr(VI) adsorption on the different adsorbents was found to follow a pseudo second-order rate equation.

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The Value of Esterman Binocular Visual Field Testing in Issuing A Driver's License for Glaucoma Patients

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Abstract: Purpose: To identify the relation between monocular visual field in glaucoma patients and binocular visual field (VF) (Esterman VF) and its effect on driving performance in different stages of glaucoma and to investigate whether Esterman disability score (EDS) is suitable for the assessment of mobility difficulty. Objective: Whether the visual efficiency scale in drivers' licensing currently adopted to determine the legal grade of visual disability associated with visual field loss is appropriate or not for the evaluation of disability regarding driving. Patients and Methods: Twenty eight patients recruited from the glaucoma clinic of the Research Institute of Ophthalmology (RIO) with different grades of glaucomatous VF affection were included in the study: mild VF affection: MD <6.00 dB, moderate VF affection: MD 6-12 dB, severe VF affection: >12 dB. Normally sighted control subjects were recruited from the outpatient clinic of the RIO. The glaucoma patients included in the study were follow-up patients of the glaucoma clinic. Detailed ophthalmological examination was performed including corrected and uncorrected visual acuity (VA) measurement using the Landolt VA chart, assessment of the angle of the anterior chamber using the Goldman contact lens for grading, examination of the optic nerve head using the 90 D indirect Volk lens, monocular visual field test using the Automated Humphery VF Analyzer 24-2 strategy and the binocular Esterman VF of the same patient on the same day. The correlation between the EDS and the monocular VF 24-2of each eye and the degree of subjective mobility difficulty was analyzed by statistical formulae. Conclusion: In addition to the currently adopted visual efficiency scale, EDS could be employed for the assessment of mobility difficulty in patients with visual field loss, also to establish new judgment criteria for issuing driver's license.
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Keywords: Esterman Binocular Visual Field; Driver's License; Glaucoma Patient

1. Introduction:

Automobile drivers' licensing is sometimes based partially upon visual field assessment. In most jurisdictions such assessment is the exception rather than the rule, and there are currently no internationally accepted standards. Some authors have suggested that the overall binocular VF is most important in driving and that losses in one eye may well be compensated for if the other eye's overlapping field is still functional, (Johnson and Kelter, 1983).

Usually the least level of visual acuity required for driving is equivalent to 6/12 in each eye according to the Egyptian driving law.

A good field of vision is also required to ascertain whether it meets the requirements for driving. The Driver and Vehicle Licensing Agency (DVLA) state that an adequate field of vision is required by law and a considerable deterioration in the binocular field of vision is a hazardous defect, (Johnson & Keltner, 1983). Drivers with restricted fields may be prone to a higher incidence of side collisions.

The minimum field of vision for safe driving is defined as 'a field of at least 120 degrees on the horizontal, measured using a target equivalent to the white Goldmann III- 4e settings. In addition, there should be no significant defect in the binocular field which encroaches within 20 degrees of fixation above or below the horizontal meridian. Homonymous or bitemporal defects which come close to fixation, whether hemianopic or quadrantanopic, are not accepted as safe for driving.

The Esterman binocular VF test has been used to assess VF disability in motor vehicles license applicants and patients with severe VF loss due to glaucoma. The Esterman scoring system has been adopted by the American Medical Association 1994 as a standard for rating visual disability, (Mills and Drance, 1986).

2. Subjects and Methods

Twenty eight glaucoma patients were included. The male to female ratio was 14 males (50%) and 14 females as shown in table (1). Mean age of subjects was 54.3 ± 16.2 years ranging from 17 to 83 years as shown in table (2).

Patients were categorized according to the results of automated perimetry using Humphrey 24-2 full-threshold VF testing protocol. The VF test mean deviations (MD) provide an overall measure of VF loss in each eye. Patients were divided into:
Mild VF affection: MD <6.00 dB Fig. 5-6,
Moderate VF affection: MD 6-12 dB Fig. 3-4 and
Severe VF affection: >12 dB. Fig. 1-2

Table (3) shows that 7.1% of the patients had mild VF affection, 53.6% had moderate VF affection and 39.3% of the patients had severe VF affection.

Exclusion criteria were: (1) physical or cognitive impairment; (2) an eye disease or condition that may affect vision, including cataracts of greater than mild severity; and (3) failing visual acuity screening tests or a visual field screening test (Humphrey Esterman binocular visual field), (4) complicated and refractory glaucoma.

The Esterman binocular VF test, on the Humphery automated perimeter was performed. It is based on the principal that some regions of the VF are functionally more important than others. The binocular versions of the test presents suprathreshold stimuli equivalent to a III-4e (10dB) target on the Goldmann perimeter at 120 loci throughout the VF, including 150 in the horizontal meridian (75 in each direction) and 100 in the vertical meridian (40 superiorly and 60 inferiorly). More stimuli are presented centrally, inferiorly, and along the horizontal meridian than at other locations as these areas of the VF are thought to be the most important functionally. Points that are missed are retested once before a miss is recorded. The percentage of points seen by the patient comprises the Esterman efficacy score.

The VF requirement typically consists of the ability to see for at least 120 to 140 in the horizontal meridian with both eyes together. The Esterman test can be used to document the extent of the binocular horizontal field for drivers' license requirements. In addition, the Esterman test has been used to assess functional disability in patients with glaucoma, (Mills and Drance, 1986).

The technician moves the chin rest to the far right position, the patient places his/her chin in the chin cup on the left. There is no need to use the trial lens holder or an eye patch; the patient may wear spectacles for the test. The patient moves his/her head to center eye monitor between patient's eyes.

3. Results:

A sample of 28 cases who met eligibility criteria were included. The male to female ratio was 50% as shown in Table (1).

Table (1) Distribution of gender of studied cases (N=28)

	No.	%
Males	14	50.0
Females	14	50.0

The mean age of subjects was 54.3 ±SD 16.2 ranging from 17 to 83 years and the median was 59 years as shown in Table (2).

Table (2) Descriptive statistics of the age of the studied cases (N=28)

	Mean (SD)	Range
Age	54.3 16.2	17-83 years
Median	59 years	

The MD was used to characterize patients' visual fields into three categories (McKean-Cowdin et al., 2007):

1. Mild VF damage: unilateral damage MD ≤ (-)6 dB
2. Moderate VF damage : MD from (-)6 dB – (-)12 dB
3. Sever VF damage: MD ≥ (-)12 dB

Table (3) Distribution of severity of visual field affection among studied patients (N=28)

	No.	%
Mild	2	7.1
Moderate	15	53.6
Severe	11	39.3

This table shows that 7.1% of the patients had mild, 53.6% had moderate and 39.3% of the patients had severe VF affection.

This classification system was based on perimetric test results using Humphrey 24–2 threshold strategy monocular VF test for both eyes

Table (4) Comparison between gender and the mean Esterman Score: (N=28)

	Mean	**SD**	**P**
Males N=14	73.7	30.7	0.2
Females N=14	84.3	17.3	

P>0.05 not significant

There is no statistically significant difference between males and females as regards the mean Esterman score.

There is a higher mean Esterman score among females compared to males but the difference is not statistically significant.

Table (5) Comparison between age of the patients and the mean Esterman Score: (N=28)

Age	Mean	SD	T	P
<=59 years young N=14	79.5	25.8	0.1	0.9
>59 years old N=14	78.5	25.36		

P>0.05 not significant

There is no statistically significant difference between old and young cases as regards the mean Esterman score.

Table (6) Comparison between severity of visual field affection and the mean Esterman score: (N=28)

	Mean	SD	P
Mild	94.5	2.1	
Moderate	90.5	10.4	**0.000****
Severe	**60.5**	30.4	

** P<0.01: highly significant

LSD (least significance difference) shows a significant difference between severe versus mild and moderate VF affection, while no significant difference was found between mild and moderate cases.

There is a lower mean Esterman score among cases with severe vision affection compared to moderate and mild cases and the difference is highly significant statistically.

Table (7) Percentiles of Esterman score among studied patients: (N=28)

	Value
5th	17.9
10th	29.8
15th	42.9
25th	**66.7**
50th	88.5
75th	95.7
90th	99.0
95th	99.5

A percentile (or centile) is the value of a variable below which a certain percent of observations fall. So the 25th percentile is the value (or score) below which 25 percent of the observations may be found.

The 25th percentile is also known as the first quartile (Q_1), the 50th percentile as the median or second quartile (Q_2); the 75th percentile as third quartile (Q_3).

This table shows the median value for Esterman score among all studied patients is (88.5). This table also shows the cut value of Esterman score below which for example we can not issue a driving license is **66.7** (25th percentile of the cases) with good monocular reliability indices (RI).

Table (8) Comparison between severity of visual field affection and the mean right median deviation, left median deviation, right pattern SD, left pattern SD (N=28)

	Mean	SD	F	P
Right median deviation	-0.36	1.4	31.8	**0.000****
Mild	-4.57	2.9		
Moderate	**-22.5**	8.7		
Severe				
Left median deviation	0.32	0.4	30.6	**0.000****
Mild	-5.7	6.3		
Moderate	**-24.3**	6.8		
Severe				
Right pattern SD	1.1	0.1	15.4	**0.000****
Mild	3.5	1.8		
Moderate	**7.6**	2.4		
Severe				
Left pattern SD				
Mild	1.1	0.1	4.9	**0.01***
Moderate	4.2	3.0		
Severe	**7.2**	3.0		

** P<0.01: highly significant

LSD (least significance difference) test showed significant difference between severe versus mild and moderate monocular visual field affection.

There is no significant difference between the mean of the studied parameters of mild cases versus moderate cases.

Table (9) Correlation coefficient between right and left median deviation and Esterman binocular vision score (N=28)

	Right median deviation	Esterman score
Left median deviation	r=0.811 **P=0.000****	R=0.733 **P=0.000****
Right median deviation		R=0.733 **P=0.000****

** P<0.01: highly significant

There is a highly significant positive correlation between left median deviation and right median deviation among studied patients. There is a highly significant positive correlation between left median deviation and Esterman score. There is a highly significant positive correlation between right median deviation and Esterman score.

Table (10) Correlation coefficient between right and left pattern SD and Esterman binocular vision score (N=28)

	Right PSD	Esterman score
Left PSD	r=0.666 **P=0.000****	r=-0.158 P=0.4
Right PSD		r=-0.228 P=0.2

** P<0.01: highly significant

There is a highly significant positive correlation between left PSD and right PSD. There is no significant correlation between left PSD and Esterman score. There is no significant correlation between right PSD and Esterman score.

Table (11) Distribution of low Esterman score among studied cases (cases below cut off value 66.7 is considered as bad Esterman and not pass vision test) (N=28)

	Number	Percent
Pass	21	75.0
Not pass Below cut off value	**7**	**25.0**

This table shows that 7 patients (25%) of the studied patients had a low Esterman score below the cut off value meaning that they are not allowed to be issued a drivers license.

Table (12) Distribution of low Esterman score according to severity of visual field affection among studied patients: (N=28)

	Pass > cut off value No. %	Not pass < cut off value No. %	X2	P
Mild N=2	2 100.0	0 0		
Moderate N=15	14 93.3	**1** **6.7**	**8.4**	**0.01***
Severe N=11	5 45.5	**6** **54.5**		

* P<0.05 significant

One patient from the moderately affected Esterman score patients cannot drive (6.7%), compared to 6 patients from the severely affected (54.5%).

Table (13) Percentiles of Esterman score among cases with severe visual field affection:

Severe cases of visual field affection

N=**11**	Value
5th	17.0
10th	17.4
15th	18.6
25th	31.0
50th	**65.0**
75th	87.0
90th	97.0
95th	99.0

The cut off value for driving in severely affected patients according to the Esterman score was at the 50th (65 value) below which patients should not be allowed to drive

A case of bilateral advanced glaucoma

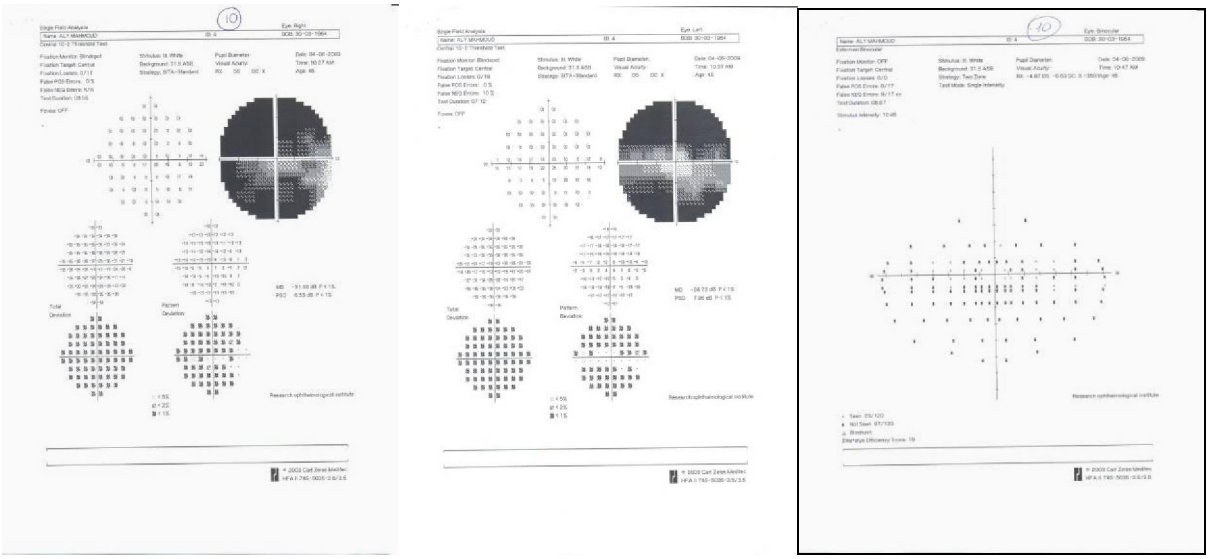

Fig.1 MD of right eye (-) 31.1 MD of left eye (-) 28.72 Esterman score = 23/120

A case of bilateral advanced glaucoma with a good Esterman score

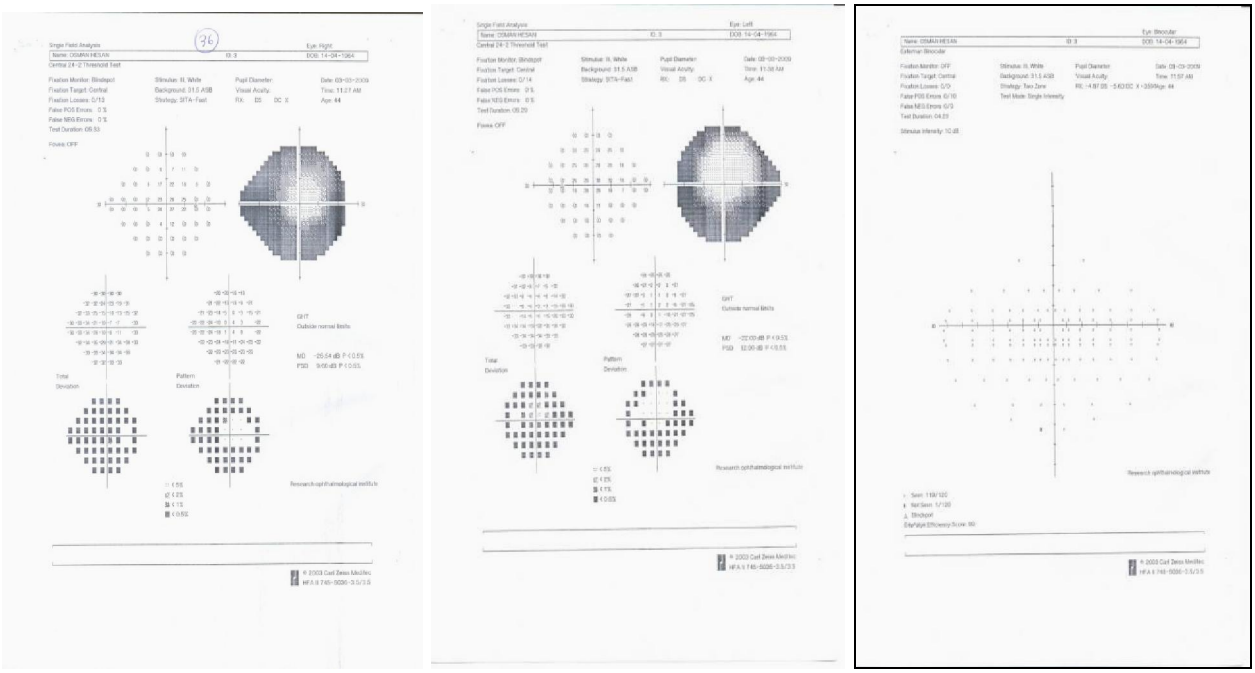

Fig.2 MD of right eye (-) 20.54 MD of left eye (-) 22 Esterman score =119/120

A case of bilateral moderate glaucoma

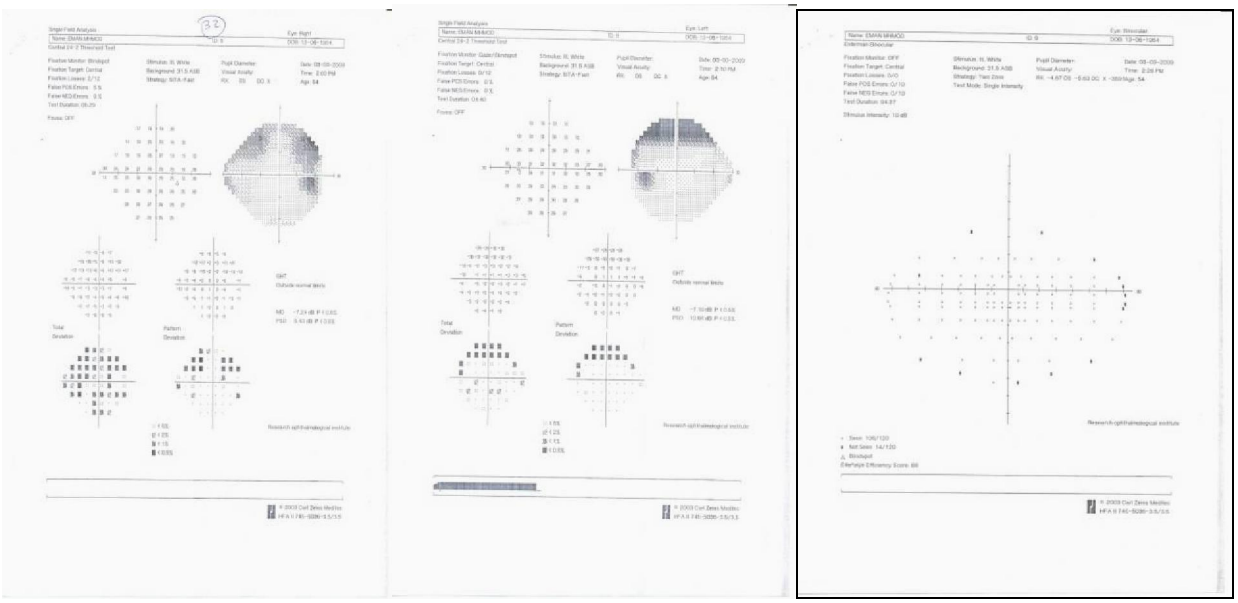

Fig.3 MD of right eye (-) 7.42 MD of left eye (-) 7.10 Esterman score = 106/120

A case of bilateral moderate glaucoma

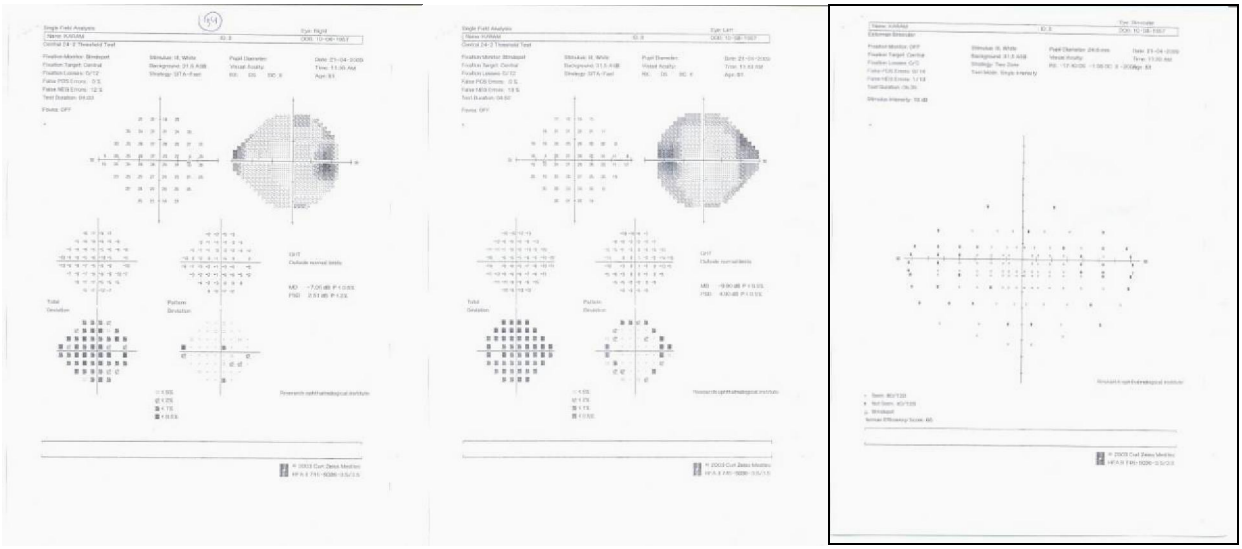

Fig.4 MD of right eye (-) 7.05 MD of left eye (-) 9.90 Esterman score = 80/120

A case of bilateral early glaucoma

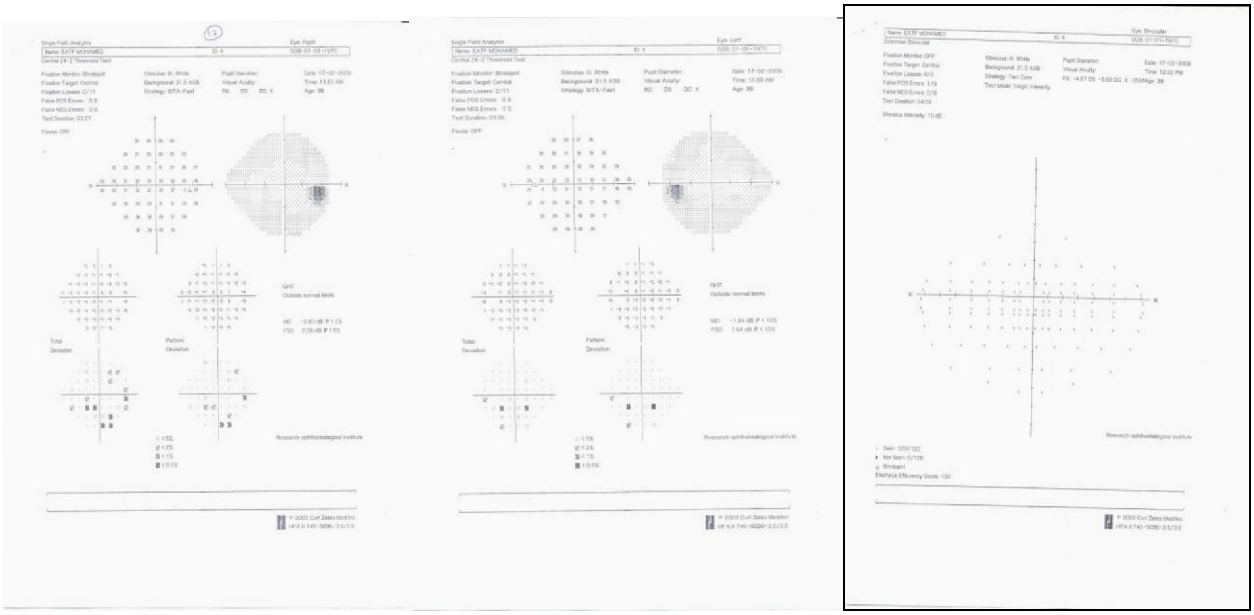

Fig.5 MD of right eye (-) 2.63 MD of left eye (-) 1.84 Esterman score = 120/120

A case of bilateral early glaucoma

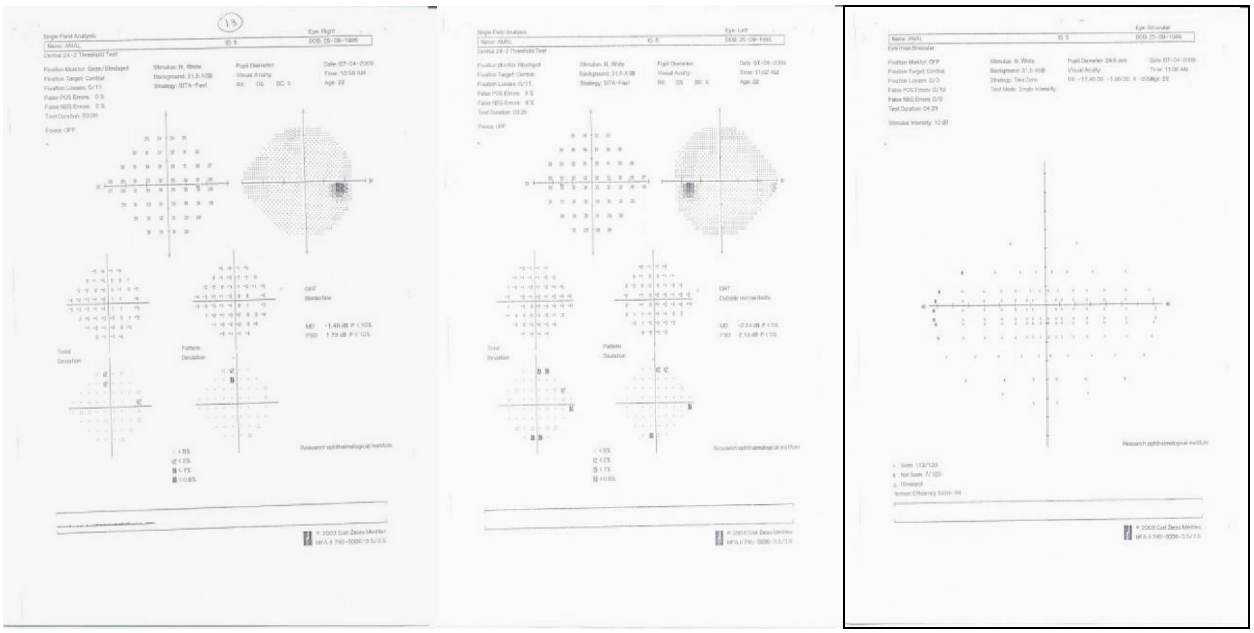

Fig.6 MD of right eye (-) 1.48 MD of left eye (-) 2.24 Esterman score = 113/120

A case with right eye moderate glaucoma affection and left eye severe glaucoma affection

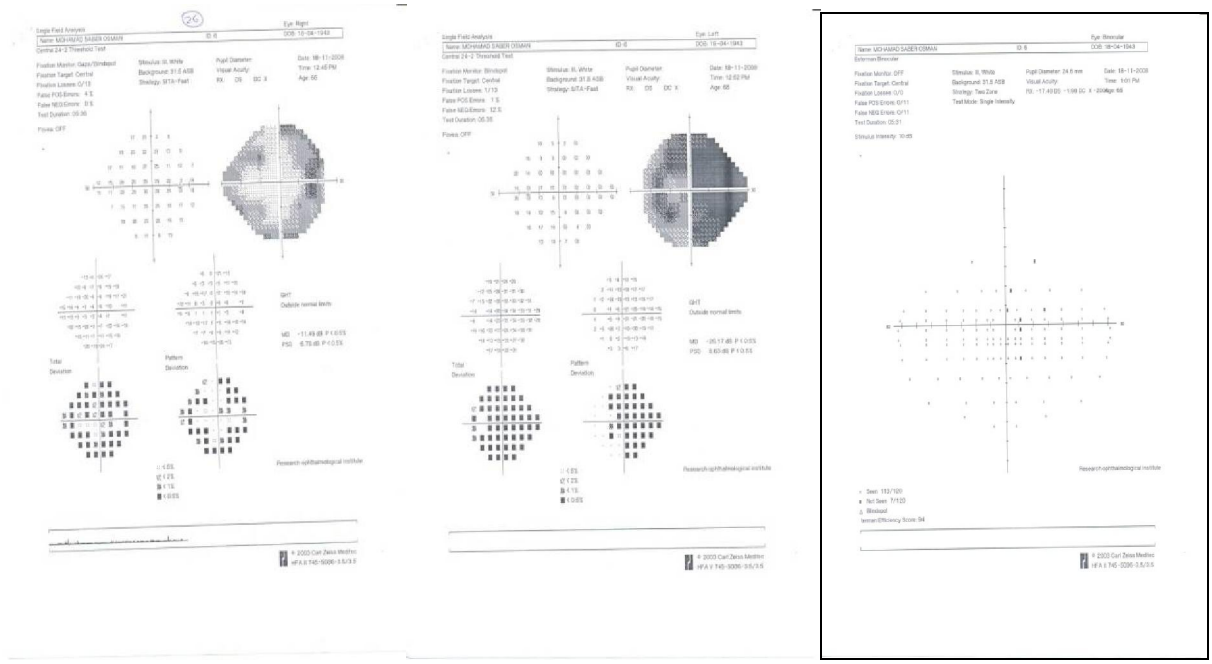

Fig.7 MD of right eye (-) 11.49 MD of left eye (-) 26.17 Esterman score = 113/120

A case with right eye moderate glaucoma affection and left eye mild glaucoma affection

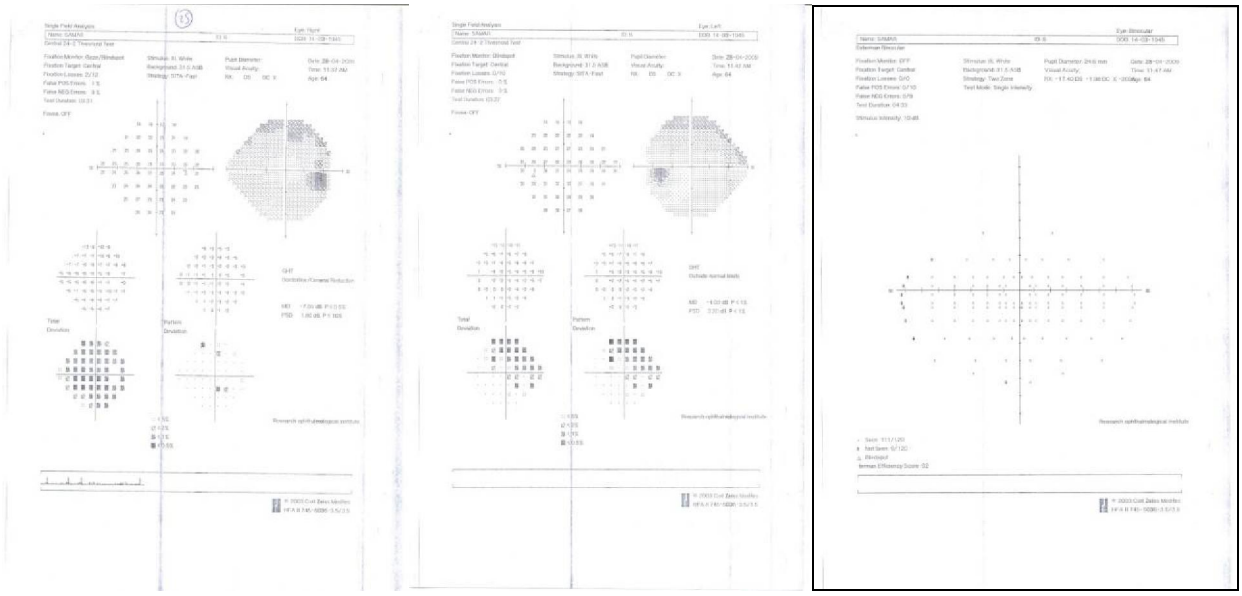

Fig.8 MD of the right eye (-) 7.05 MD of the left eye (-) 4.03 Esterman score = 111/120

4. Discussion:

In the present study we investigated whether the Esterman disabilities score (EDS) is suitable for the assessment of mobility difficulty in patients with visual field loss. Driving endpoints, such as driving cessation or limitation, should be considered as secondary outcomes in evaluating glaucoma treatments.

Glaucoma affects approximately 2% of adults over the age of 40, *(Friedman et al, 2004),* and disease prevalence increases dramatically with age, *(Quigley and Broman, 2006).* Aging of the population worldwide will lead to substantially more individuals with glaucoma in coming years, which may result in dramatically more individuals with glaucoma-related visual disability.

In addition, measurement of disability from glaucoma can define guidelines to increase patient safety while driving, (*Rowe, 2006).*

Patients with glaucoma rate driving as very important in preserving independence. The two most important concerns identified by glaucoma patients were the risk for VF loss leading to an inability to drive and the fear of long-term blindness, *(Bhargava et al, 2006).* Ang and Eke, (2007), found that though most glaucoma patients in their study retained useful vision, almost half (47%) eventually lost vision, resulting in driving ineligibility.

In our study the mean Esterman score was higher among females compared to males but the difference was statistically insignificant as shown in table (4). This coincides with, *(Edwards et al, 2008)* who concluded that gender, although previously found to be predictive of driving cessation, was not a significant risk considering baseline driving. Several cross-sectional studies have indicated that women are more likely than men to cease driving, (*Vance et al, 2006).* Overall, prior research and these results indicate that although older women from contemporary cohorts drive less at baseline, they may not be more likely to cease driving across time. However, they advised to be cautious about over interpreting the conflicting cross-sectional and longitudinal research, as both designs contain specific methodological biases that may make direct evaluation of cross-sectional as compared with longitudinal predictors difficult, *(Anstey, 2002; Hofer, Sliwinski, & Flaherty, 2002).*

No statistical difference was found between age of patients and the mean Esterman score as shown in table (5). Our results coincide with the work of *(Janz et al, 2009),* who found no significant association between driving status and age.

When comparing the severity of VF affection and the mean Esterman score we found a significant difference between severe and moderate VF affection, while no significant difference was found between mild and moderate cases, table (6). There is a lower mean Esterman score among cases with severe visual affection compared to moderate and mild cases and the difference is highly statistically significant, table (6).

McGwin et al, (2005), found that older adults with severe field loss in their worse functioning eye are at risk of involvement in collisions rather than are those with glaucoma who have no field loss.

Nelson-Quigg et al, (2000), noted that in many instances, the appearance of the binocular visual field of patients with glaucoma was better than expected on the basis of observation of the monocular visual fields alone which coincide with our study. This is in part because glaucomatous visual field loss only occasionally overlaps for corresponding locations in the two eyes, the degree of overlap is often partial, and the degree of sensitivity loss is often asymmetric between the two eyes. A method of generating an accurate representation of the binocular visual field from monocular visual field data may be useful for clinicians in assessing whether patients are likely to encounter difficulties with driving, mobility skills, and other everyday tasks.

Percentiles of Esterman score among our studied patients are shown in table (7). A percentile is a value that represents a percentage position in a list (range) of data. This table also shows that the driving endpoint value of Esterman score was 66.7, below which for example, we can not issue a driving license (25^{th} percentile of all cases) with good monocular reliability indices (RI).

Our study is different from and additive to others in that, we could determine the percentile for mild, moderate and severe glaucoma below which patients are advised not to drive being 25^{th} percentile for mild and moderate glaucoma patients and 65^{th} for advanced glaucoma VF changes, tables (8 -10) reveal that in glaucoma with the binocular VF affection, patients can drive if below 25^{th} percentile, table (11) shows the distribution of low Esterman score among studied cases (the value 66.7 is considered as bad Esterman below which glaucoma patients cannot be allowed to pass the vision test), 7 patients (25%) of the studied groups had that score.

Table (12) shows the distribution of low Esterman score according to severity of visual field affection among studied patients, one patient from the moderately affected Esterman score cannot drive (6.7%), compared to 6 patients from the severely affected (54.5%).

The increased accident incidence in the advanced glaucoma group indicates that glaucoma patients were not able to compensate for their visual field loss during driving. These findings are

consistent with a study of the peripheral visual fields by Johnson and Keltner (1983) conducted over 10,000 driver's license applicants in California, who found that individuals with binocular peripheral field loss had twice the accident rates as compared to a control group with normal visual fields. Our results showed, that for safe driving in severely affected patients with advanced VF losses OU according to the Esterman score, was at the 50th percentile (65 value), below which patients should not be allowed to drive, table (13). Therefore, binocular VF is very important for the decision of driving, since the monocular VF even if advanced in both eyes may be misleading, Fig. 7-8.

5. Conclusion

Patients with glaucoma have greater difficulty in performing safe driving tasks with progressive VFs, particularly when bilateral damage is present. Using the binocular VF examination has proven importance in assessing the driving ability of glaucoma patients before issuing a driver`s license, even if the monocular VF of each eye is severely affected, which will definitely improve their quality of life. We advise adding the binocular VF test among the numerous driving licenses tests conducted at traffic offices. It will be important to conduct further studies, that directly assess the on field driving and mobility of glaucoma patients.

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Histopathological and Enzyme Changes in *Clarias gariepinus* (Burchell 1822) Exposed to Nitrite at Different Water Temperatures

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Abstract: Nitrite is a natural component of the nitrogen cycle in ecosystems.lt is an intermediate in the oxidation of ammonium to nitrate.The elevation of ambient nitrite concentration is a potential problem for freshwater fish. This study was designed to investigate the effect of different water temperatures on the toxic effect of nitrite in a freshwater fish. Sixty *Clarias gariepinus* (300 ± 1.30g), were exposed to nitrite at different water temperatures (27^0C and 35^0C) for 48hours. Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and total protein levels were assayed in the gill, liver and tissue (skin) of the fish. The statistical analysis was performed using the Statistical Analysis (SPSS 11.0 for Windows). Statistical differences were determined by one-way analysis of variance (ANOVA) and paired-sample t test. ALT and ALP increased significantly ($P<0.05$) in nitrite-intoxicated fish at 35^0C compared to the value obtained at 27^0C in the organs while a significant decrease ($P<0.05$) was observed for the enzyme AST at 35^0C compared to 27^0C. Protein level in all the tissues showed a significant decrease in nitrite-intoxicated fish at higher temperature. The histopathological changes observed in the gills of nitrite-treated fish at 35^0C were that of congestion and vacuolization while the liver showed generalized fatty35^0C were that of congestion and vacuolization while the liver showed generalized fatty degeneration, congestion of central veins and multifocal necrosis. Moderate hydropic degeneration of the epidermal layer was observed in the skin tissue. These results revealed that high temperature can increase the toxic action of nitrite in fish.

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Keywords: Nitrite , *Clarias gariepinus*, enzymes , proteins, histopathology, Temperature, toxicity.

Introduction

Nitrite is a natural component of the nitrogen cycle in ecosystems and its presence in the environment is a potential problem due to its well-documented toxicity to animals (Watenpaugh 1985; Lewis and Morris 1986; Williams 1997 and Jensen 2003). It is an intermediate in the oxidation of ammonium to nitrate. Nitrite is a well-known toxicant for fish as well as a disrupter of multiple physiological functions including ion regulatory, respiratory, cardiovascular, endocrine and excretory processes (Kroupova et al., 2005).

Nitrite accumulates in tissues such as gills, liver, brain and muscle (Margiocco et al., 1983). According to Casillas et al., (1983), enzyme activities are considered as sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water and the presence of toxicants. These biochemical methods has been advocated to serve as an early warning of potentially damaging changes in stressed fish.

Alanine aminotransferase (ALT) is one of the enzymes that catalyze the reactions of transmination of alanine, glutamic and aspartic acids. In conjunction with a co-enzyme, it couple the protein, carbohydrate and fat metabolism and tricarboxylic acid cycle under altered physiological, pathological and induced environmental stress conditions (Murugesan et al., 1999). Aspartate aminotranferase (AST) is also an enzyme found in liver, muscle and heart tissues. An increased level of AST has been associated with heart attack, liver function problems and injury or disease to the muscles.

Alkaline phosphatase (ALP) on the other hand, is a brush border enzyme, which splits various phosphorus esters at an alkaline pH. It is well known that phosphatases are involved in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzymes, secretory activity and transport to phosphorylated intermediates across the cell membranes (Vijayavel et al. 2006). Inhibition and induction of these biomarkers is a good approach to measure potential impacts of pollutants on environmental organisms (El-Shehawi et al. 2007).

There had been various reports on the toxic effect on Nitrite in fish. Kroupova et al. (2006) focused on the haematological and biochemical changes associated

with nitrite intoxication in Common Carp (*Cyprinus carpio* L.) at different water temperature. Velmurugan et al.(2007) also investigated the changes on some tissue enzymes of *Clarias gariepinus* fingerlings exposed to sublethal concentrations of Cadmium Chloride, a heavy metal but there is little information on the histopathological and enzyme changes of *C. gariepinus* exposed to nitrite at higher temperatures. This study is quite necessary as *C. gariepinus* is a hardy fish and is the most cultured species in Nigeria and the temperature during the growing season of this fish in Nigeria vary between 27^0C and 35^0C.

Materials and methods

Experimental Fish

Sixty pieces C. *gariepinus* of mean weight 300±1.30g (mean ±SD) and mean total length of 60.0±1.0cm were used for the study. The fish were collected in the morning between 8 – 9a.m. They were obtained from Bowen fish farm and maintained for 2 weeks in 4 circular plastic tanks (300L) with dechlorinated tap water. Four days before the start of the experiment, the fish where divided into four groups and acclimated to 27^0C and 35^0C. During acclimation and experiment period, fish were not fed.

Experimental procedure and fish sampling

The test was performed in a semi-static assay for 48 hours.12 fish were kept in 4 thermostat-controlled water bath, each containing 40 liters of test solution. The nitrite concentration was obtained by adding $NaNO_3$ to dechlorinated tap water. The dose of nitrite represented the median lethal concentration (LC_{50}) for *Clarias gariepinus* at a similar chloride water concentration and similar relative weight of fish according to Ajani (2006).

During the acclimation and experimental period, the basic chemical indices of water taken were acid neutralization capacity 42mg/l, total ammonia 60mg/l, phosphate 32mg/l, hardness 69.2mg/l, chloride 19.0mg/l, Oxygen saturation of the water 8.0mg/l and the pH 7.4 using HACH freshwater aquaculture test kit(FF – 1A).Four groups each containing 8 specimens of four-month old *C. gariepinus* were exposed to nitrite at different water temperature (27^0C and 35^0 C).

Nitrite and chloride content were checked twice during the test and the measured values did not differ from the nominal value by more than 7 percent. After treatment, both the experimental and control fishes were killed after 48hours. Gill, liver and tissue were removed out of each fish and frozen at -20^0C until analysis.

Biochemical Examinations

Tissues were homogenized in 3 vol (v/w) of 10mM Tris-HCI, 0.25M sucrose**.** Adequate measures were taken to minimize pain or discomfort.

After treatment, both the experimental and control fishes were sacrificed after 48hours. Gill, liver and tissue were obtained and frozen at -20^0C until analysis.

Tissues were homogenized in 3 vol (v/w) of 10mM Tris-HCI, 0.25M sucrose buffer (pH 7.4) at 4^0C, using a homogenizer equipped with a Teflon pestle. Homogenates were centrifuged at 1600 x g for 20minutes at 4^0C to remove cell debris. The resulting supernatant was collected and used in the estimation of AST, ALT, ALP and protein.

Measurement of enzyme activities and protein were performed using semiautoanalyser [Microlab-200 (Merck)]. The protein content of the enzyme source was estimated by the Biuret method (Gormall et al., 1949), using bovine serum albumin as standard. The protein values are expressed mg/g.

Histopathology

Samples from the gill, liver and tissue obtained were collected in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin for histological examination using x 40 of light microscope.

Statistical Analysis

The statistical analysis was performed using the Statistical Analysis (SPSS 11.0 for Windows). Statistical differences were determined by one-way analysis of variance (ANOVA) and paired-sample t test. The significance of test results was ascertained at $P<0.05$.

RESULTS

Significant changes of ALT, AST, ALP and protein were observed in nitrite-intoxicated fish at 35^0C when compared to that at 27^0C. They are presented in tables I-III.

The protein levels in the nitrite-intoxicated fish at higher temperature (35^0C) decreased significantly ($P<.05$) to that at lower temperature (27^0C) in the tissue, liver and gill. However, drastic decline in the levels was observed in the tissue.

ALT and ALP increased significantly ($P<0.05$) in nitrite-treated tissue at 35^0C compared to that at 27^0C while a significant decrease was observed for the enzyme AST at 35^0C compared to 27^0C.

The value obtained for ALT and ALP at 35^0C nitrite-intoxicated fish is 3 folds and more than 6 folds when compared to the value obtained at 27^0C in the liver of *C. gariepinus*. The highest increase in ALT and ALP found in the liver of *C. gariepinus* reflects the level of damage nitrite can inflict on fish at higher temperature.

Table 1: Enzyme activities and protein levels in tissue (skin) of *C. gariepinus* after 48-h nitrite exposure

Enzyme metabolite	27^0C		35^0C	
	Nitrite-free	Nitrite	Nitrite-free	Nitrite
ALT(mg/l)	54.10 ± 8.05^b	38.31 ± 6.02^a	52.62 ± 9.01^b	54.63 ± 7.01^b
AST(mg/l)	362.00 ± 14.01^c	181.0 ± 10.11^b	338.10 ± 8.62^c	146.0 ± 8.62^a
ALP(mg/l)	18.08 ± 11.21^c	1.00 ± 9.21^a	16.10 ± 11.81^c	11.30 ± 10.12^b
Protein(mg/l)	4.80 ± 0.50^c	4.00 ± 0.04^b	3.51 ± 0.01^b	1.63 ± 0.15^a

The values are expressed as means ± SD for n = 3.
Mean followed by different superscript in the same row are significantly different (P<0.05)

Table 2: Enzyme activities and protein levels in liver of *C. gariepinus* after 48-h nitrite exposure

Enzyme metabolite	27^0C		35^0C	
	Nitrite-free	Nitrite	Nitrite-free	Nitrite
ALT(mg/l)	60.32 ± 7.10^b	40.36 ± 3.18^a	56.31 ± 8.21^b	120.00 ± 3.01^c
AST(mg/l)	368.38 ± 1.10^c	200.81 ± 5.31^a	340.00 ± 3.87^c	232.43 ± 4.01^b
ALP(mg/l)	21.00 ± 0.51^b	3.01 ± 2.43^a	18.16 ± 6.00^b	20.61 ± 2.80^b
Protein(mg/l)	4.61 ± 3.10^c	3.95 ± 2.41^b	3.40 ± 0.35^b	2.03 ± 0.08^a

The values are expressed as means ± SD for n = 3.
Mean followed by different superscript in the same row are significantly different (P<0.05)

Table 3: Enzyme activities and protein levels in gills of *C. gariepinus* after 48-h nitrite exposure

Enzyme metabolite	27^0C		35^0C	
	Nitrite-free	Nitrite	Nitrite-free	Nitrite
ALT(mg/l)	52.08 ± 6.01^b	39.00 ± 4.02^a	53.00 ± 6.02^b	65.30 ± 4.81^b
AST(mg/l)	261.00 ± 8.11^b	248.00 ± 8.61^b	244.01 ± 7.11^b	198.00 ± 6.31^a
ALP(mg/l)	20.18 ± 3.00^b	3.00 ± 0.04^a	15.06 ± 8.17^b	16.00 ± 6.04^b
Protein(mg/l)	3.81 ± 0.00^b	3.28 ± 2.10^b	3.21 ± 0.81^b	2.00 ± 1.00^a

The values are expressed as means ± SD for n = 3.
Mean followed by different superscript in the same row are significantly different (P<0.05)

Histopathology

The photomicrograph of the nitrite-intoxicated gill,liver and skin of *C.gariepinus* at 27^0C and 35^0C are reflected in figures 1-6.

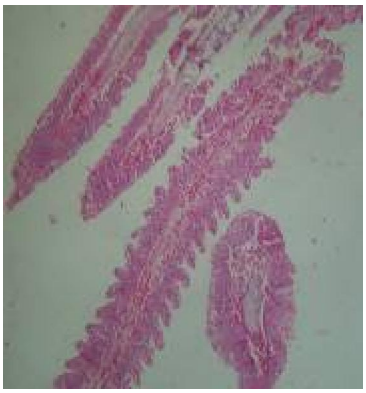

Figure 1: The photomicrograph of the gills of nitrite-intoxicated fish at 35^0C showing congestion and vacuolisation.HXE 40

Figure 2: The photomicrograph of the gills of nitrite-intoxicated fish at 27^0C .HXE 40

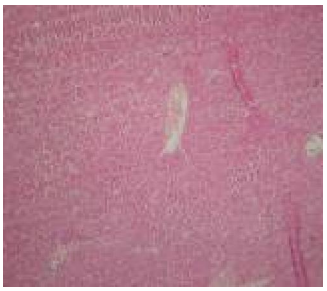

Figure 3: The photomicrograph of the liver of nitrite –intoxicated fish at 35^0C showing generalised fatty degeneration, congestion of central veins and multifocal necrosis. HXE 40

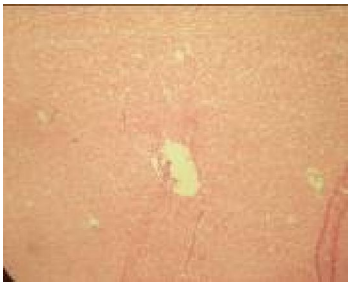

Figure 4: The photomicrograph of the liver of nitrite –intoxicated fish at 27^0C showing no congestion. HXE 40

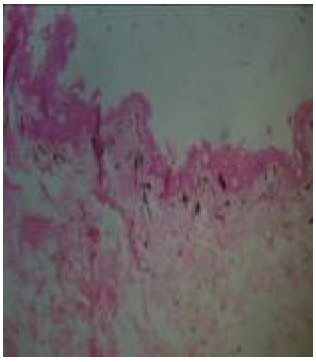

Figure 5: The photomicrograph of the skin of nitrite-intoxicated fish at 35^0 C showing moderate hydropic degeneration of the epidermal layer.HXE 40

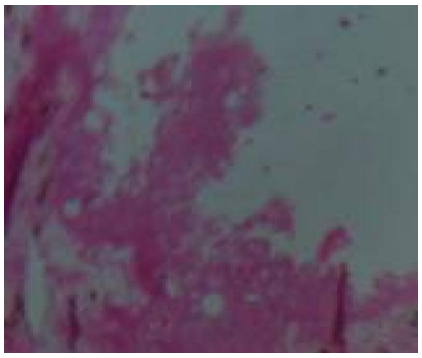

Figure 6: The photomicrograph of the skin of nitrite-intoxicated fish at 27^0C showing no degeneration of the epidermal layer HXE 40

DISCUSSION

The response to pollution is reflected as changes in some enzyme activities, especially key enzymes of biotransformation systems of organisms which can be used as biomarkers that are sensitive to pollution, of note are ALT, ALP and AST. These biomarkers, therefore, provide a tool for specific early warning sign for aquatic pollution also in fish species (Sirmac and Braunbeck 2000). ALT is an enzyme frequently used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscle and gills (De la Torre et al. 1999; 2000). This enzyme is known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental conditions (Nichol and Rosen 1963; Knox and Greengard 1965; Victor 1985; and Velmurugan et al. 2007).

Significant ($P<0.05$) elevation in the level of AST and ALT in the liver, gill, tissue of the fish in this study is similar to that obtained in Cadmium Chloride toxicity (Velmurugan et al. 2007) as this response as been reported to be stress induced since nitrite generates ketoacid-like: ketoglutarate and oxaloacetate necessary to meet the excess energy demand under the toxic situation. The elevations in ALT activity were also observed in *C. carpio* and *Oreochromis niloticus* exposed to cadmium (De La Torre et al. 2000; De Smet and Blust 2001; Almeida et al. 2002).

ALP is a membrane-bound enzyme related to the transport of various metabolites (Lin et al 1976; Wahwon et al. 1992). Various membrane functions such as permeability, the activity of bound enzymes and hormone receptors and the efficiency of transport systems are controlled by membrane dynamics (Shinitzky,1984). ALP is an integral enzyme known to be intimately associated with the hydrophobic core of

the intestinal microvillus membrane (Brasitus et al.1979). The enzyme AST activity at higher temperature in nitrite water increased when compared with lower temperature (27^0C).

The increase in ALP activity at higher temperature in nitrite water supports earlier findings. Sastry and Subhadra (1985) recorded elevation in ALP in ovary and muscles of freshwater catfish, *Heteropneustes fossilis* exposed to cadmium. Also, the ALP activity showed an increased in all the organs liver, gills, gut and gonads examined in Rosy barb (*Puntius conchonius*) exposed to mercuric chloride (Gill et al. 1990).

Accumulation of the environmental pollutants and toxicants has been shown to cause alterations in the activities of many enzymes concerning cellular energy metabolism (Sebert et al. 1993).

The decrease in protein content of fish at 35^0C nitrite water indicates the physiological adaptability of the fish to compensate for the stressful condition. Protein catabolism must have occurred to compensate for the high energy demand in order to adjust/overcome the toxic stress of nitrite.Sancho et al. (1997) corroborated this. Also, the utilization of protein in cell repair and organization cause the depletion in the tissues at 35^0C nitrite water. Bradbury et al. (1987) pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthesis.

Histopathological studies of fish exposed to pollutants revealed that fish organs were efficient indicators of water quality (Cardoso et al. 1996; Barlas 1999; Cengiz et al. 2001). The gills are important organs in fish for respiration, osmotic regulation, acid base balance and nitrogenous waste excretion (Heath 1987). Michael et al. (1987) also observed hyperplasia and hypertrophy in the gills of *Clarias lazera* chronically exposed to nitrite.

From this study, the histopathological changes observed in the gills of nitrite – treated fish at 35^0C showed congestion and vacuolization. This is well in line with earlier findings of Svobodova et al. (2005b). They recorded hyperplasia, vacuolization and elevated number of chloride cells as the main histological lesions that occurred in the gills of nitrite treated carp (*Cyprinus carpio*).

The high demand on the liver due to the stressful condition is which and is what is revealed in the histopathology. The pathology of the liver of nitrite – intoxicated fish at 35^0C was that of generalized fatty degeneration, congestion of central veins and multifical necrosis. The main functions of the liver are to process nutrients from food, make bile, remove toxins from the body and build proteins. According to Daniel (2009), interference with these important functions can lead to poor health.

The tissue of the skin of nitrite-intoxicated fish at 35^0C showed moderate hydropic degeneration of the epidermal layer while at 27^0C, there was no significant lesion.

CONCLUSION

Nitrite has deleterious effect in *C. gariepinus* fish at higher temperature (35^0C). Significant alterations in the levels of AST, ALP, ALT and protein in different tissues of this fish reflect this. Thus, nitrite build-up in culture systems should attract prompt attention.

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Enzyme Mediated Amido Black Decolourization by Soil borne RS-II Strain Isolated from an Industrial Town.

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Abstract: Total 10 strains of microorganisms were isolated from the soils exposed to dyeing industry effluent, in and around Baddi. (HP). The isolate, RS-II, tentatively identified as *Galactomyces* sp. showed maximum amido black (azo dye) decolourization activity (72.08%), on primary screening. However, this isolate exhibited 81.43% decolourization of amido black, under optimal conditions of pH (8.0) and temperature (37^0C). The decolourization activity was found to be pH and temperature dependant, and mediated by enzymatic step. The SDS-PAGE gel electrophoresis results spotted a 66 Kd band. The purification of crude enzyme was carried out by Ion Exchange Chromatography. The activity of the pure fraction (eluted CM sepharose) was recorded as 5.5 moles/min/ml. The study highlights that RS-II has an adequate potential to decolourize the amido black dye, and the pure fraction of enzyme has even higher potential to do so. The findings could be a safe and viable solution for bioremediation of azo dye containing effluents, and could be an effective gateway to evolve more advanced and effective strategies based on the use of pure or immobilized enzymes.
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Key words: decolourization**,** amido black**,** azo dye, bioremediation**,** SDS-PAGE**,** *Galactomyces* sp.

1. Introduction

Synthetic dyes offer a vast range of new colours to food and textile industry. Many synthetic dyes are used in textile, paper, leather, ceramics, cosmetics, foods processing and ink industries (Buitron *et al*., 2004). The majority of these dyes are azo dyes, that are characterized by the presence of -N = N group. Azo dyes account for approximately 60-70% of all dyes, used in food and textile manufacture. The worldwide production of these dyes has been estimated at 4,50,000 tons/year, and almost 50,000 tons/year, are lost in effluent during application and manufacture. About 2-50% of these dyes are lost as waste effluents, at the time of production and application (Sokman *et al*., 2001; Olukanni *et al*., 2009). It has been reported that 90% of the reactive textile dyes, entering activated sludge sewage treatment plants, pass through unchanged, and are discharged into rivers (Pierce, 1994). Some of these dyes are toxic and may be hazardous to human health (Saue *et al*., 2002). Dyeing factory effluent, that alters the colour and quality of the water bodies, has also proved to be hazardous to aquatic organisms. The effluents containing azo dyes, adversely affect water resources, soil fertility, plant growth, aquatic organisms and ecosystem integrity. They can be lethal, genotoxic, mutagenic and carcinogenic to the aquatic organisms and animals. The toxic compounds, present in dye effluents, enter the aquatic organisms, and reach man through the food chain. Thus, causing various physiological disorders like hypertension, sporadic fever, renal damage, cramps, etc. The bioaccumulation of these toxicants depends on availability and persistence in water and food, and physiochemical properties of the toxicants (Jalandoni-Buan *et al*., 2010).

The dyes are not readily degradable under natural conditions. Moreover, the conventional waste water treatment systems are not effective for their removal. These dyes are mainly metabolized by bacteria to colorless aromatic amines by azoreductases. These amines are further degraded aerobically by bacteria. Some bacteria can degrade azo dyes both aerobically and anaerobically. Extensive work has been carried out on the problems associated with the discharge of dye effluent from industries. It has been documented that the safe method for azo dye biodegradation is combined aerobic treatment (Mabrouk and Yusef, 2008; Olukanni *et al*., 2009). Microorganisms use their enzymatic machinery to decolourize synthetic dyes. Different enzymes such as azoreductases, lignin and manganese peroxidase and laccases are involved in microbial decolourization of dyes (Naidu *et al*., 2003; Ghasemi *et al*., 2010; Suwannawong *et al*., 2010).

Evaluation of optimal enzyme activity, time profile and expression profile are useful to target the enzyme mediated biotransformation process of dyes. Thus,

screening of microflora with effective decolourization, and know how about the enzyme involved, could evolve plausible bioremediation strategies for removal of azo dyes from effluents.

The present work focuses on isolation and screening of indigenous microbial strains for their ability to decolorize azo dyes aerobically, and optimizes the pH and temperature, required for efficient decolourization. Further, an effort has also been made to purify and characterize the enzyme to ascertain its the role in decolourization process.

2. Materials and Methods

2.1 Collection of Samples

The soil samples were collected in sterile plastic bottles under aseptic conditions, in duplicate, at random, from three different sites, in and around Baddi, Distt. Solan (H.P), India. Baddi is an industrial town located in Himachal Pradesh, India ($30^0$57' 31.08" N, 76^0 47' 17.87"E) at 1375 ft. above sea level.

2.2 Enrichment and Isolation of Amido Black Decolourizing Microorganism

10 different isolates of microorganisms were obtained using standard methods of cultivation.

Mineral salts medium with 1% amido black was used for the enrichment of Amido Black decolourizing bacteria (Cohen-bazire, 1954). The inoculated culture flasks were incubated at 37^0C in shaker (REMI-CIS-24BL) at 120 rpm. The decolorizing cultures were further enriched by transferring aliquots of enriched cultures into fresh media, and processed as done earlier. This step was repeated once more.

The organisms were isolated from the final enriched culture, on nutrient agar, using streak plate method. The isolates were purified and maintained for further investigations. The pure cultures were identified on the basis of morphological and biochemical characteristics.

2.3 Screening for Decolourization Activity

10 ppm amido black dye solution in distilled water was scanned spectrophotometrically (Systronics 2202) to find out maximum absorbance (λ_{max}) for amido black dye. Decolourization experiments were performed in 100 ml conical flasks, containing 50 ml nutrient broth with 50 ppm amido black. Three flasks, each for an isolate, were inoculated with approximately 20 mg dry cell mass (Biomass measured using OD 600), and were incubated at 37^0C. The decolorization was measured spectrophotometrically at λ_{max} (617.60 nm). Three ml sample was collected at 72 h incubation, and centrifuged (4000 x g for 15 min) to exclude biomass. Percentage decolourization was calculated as per method documented (Olukanni *et al.*, 2006).

$$\text{Decolourization (\%)} = \frac{A_0 - At}{A_0} \times 100$$

Where,

A_0 = Absorbance of the blank (dye solution)

At = Absorbance of the treated dyes solution at specific time.

2.4 Percentage Decolourization at Different pH and Temperature

Nutrient broth supplemented with 50 ppm amido black was prepared, and was dispensed in different conical flasks, each containing 50 ml. The pH of the broth in different flasks, was adjusted from 2.0 to 11.0 using NaOH or HCl. Then, added approximately 20 mg dry cell mass of RS-II to each flask. The experiments were performed in triplicate. The flasks were incubated at 37^0C for 24 hrs. The absorbance was recorded to calculate percentage decolourization. The percentage decolourization by RS-II was also determined at different temperatures (20^0C, 25^0C, 30^0C, 37^0C, 40^0C, 45^0C and 50^0C) to find out optimum temperature required for maximum decolorization.

2.5 Protein Isolation and Precipitation

For the isolation of proteins, 24 h old culture broth (with 50 ppm amido black) was centrifuged (8000 x g at 4^0C for 10 min). The pellet was re-dissolved in 50 ml. sonication buffer (10 mM Tris HCl, 5 mM EDTA, 5% (v/v) ethanol, 1mM sodium azide, 1mM DTT). Then, vortexed it for 10 minutes, and sonicated at 10% maximum amplitude, 5 sec pulse interval for 5 minutes at 4^0C in sonicator (Misonix, New York). The sonicated products were then centrifuged at 8000 x g for 10 minute at 4^0C, to remove the cell debris. Equal volumes of 70% ammonium sulphate (ice cold) were added to the supernatant. Then, centrifuged the contents at 12000 x g for 20 minutes at 4^0C. The supernatant was discarded and the pellet was washed twice with PBS (pH 7.0) by centrifugation at similar conditions. Protein concentration was determined by Biuret method.

The protein sample pellet obtained by ammonium sulphate precipitation method was mixed with 30 ul of sample loading buffer. Samples were loaded in the wells of the gel. The samples were subjected to SDS-PAGE (Laemmli, 1970). Then, the protein bands were visualized using silver stain.

The purification of the crude protein was carried out by Ion Exchange chromatography. The activity of all fractions of ion exchange chromatography was measured at 617.60 nm.

$$\text{Enzyme activity} = \frac{\mu\text{mol of substrate hydrolysed}}{\text{Minutes X ml. of sample}}$$

The biochemical and physical characteristics of solid wastes (e.g., constituents, pH, and moisture) and operating conditions of solid waste composting (e. g., carbon to nitrogen ratio, aeration rate, reaction temperature and pressure) impose significant effects on an ecological succession of microorganisms (Vallini, 1993; Huang, 2000). Although relationships between these factors have been stressed, it is often difficult to synthesize such a large volume of materials. Generally, the factors that affect composting processes, such as temperature and oxygen availability, are controlled to maintain a relatively better growth environment for microorganisms during the process of composting. Analytical and numerical modeling of the composting process could be used as a tool to analyze composting system performance under different operating scenarios.

3. Results

10 different microbial isolates were screened for amido black (azo) dye decolorization. Among all isolates, the maximum decolourisation was observed in the flask inoculated with RS-II; followed by that of RS-V. The isolates viz. RS-I, III, IV, VI showed almost same results. 90% of all the isolates were found to decolorize azo dyes.

Table 1. Percentage decolourization of Azo dye by RS-II at different pH

pH				**Absorbance at 617.60 nm**		**Decolorization (%)**
Initial		**Final**				
Control	Test	Control	(Test pH ± S.D.)	Control	(Test ±S.D)	
2.00	2.00	2.00	1.76 ± 0.2828	0.114	0.084 ± 0.0021	26.31
3.00	3.00	3.00	2.94 ± 0.1273	0.436	0.245 ± 0.0041	43.80
4.00	4.00	4.00	3.91 ± 0.0424	0.557	0.214 ± 0.0035	61.57
5.00	5.00	5.00	5.09 ± 0.0848	1.232	0.428 ± 0.0318	65.21
6.00	6.00	6.00	7.72 ± 0.1273	1.469	0.449 ± 0.0883	69.43
7.00	7.00	7.00	7.98± 0.0070	1.519	0.394 ± 0.0487	74.06
8.00	8.00	8.00	8.19 ± 0.0848	1.814	0.336 ±0.0487	81.43
9.00	9.00	9.00	8.36 ± 0.2404	1.735	0.330 ± 0.0367	80.97
10.00	10.00	10.00	9.67 ± 0.0141	0.587	0.490 ± 0.1605	16.52
11.00	11.00	11.00	9.99 ± 0.0212	1.159	1.045 ± 0.0049	9.83

Secondary screening of isolates for percentage azo dye decolourization was also carried out. (Table.1). It was observed that RS-II had maximum decolourization (72.08%) of amido black followed by RS-V (69.49%) at λ_{max} (617.60 nm) (Figure. 1).

The influence of different pH on percentage decolorization of amido black was also recorded. Maximum decolourization activity (81.43%) was observed at pH 8.0. (Table.1). It was also observed that the decolorization increased from 26.31% to 81.43% with increasing pH (from 2.0 to 8.0). Further increase in pH of the media led to decline in decolourization percentage with minimum activity (9.83%) at pH. 11. (Figure. 2).

The influence of different temperatures on percentage decolourization of amido black was studied at optimized pH 8.0. (Table 2).

Maximum activity (81.43%) was recorded at 37°C. This value was almost similar to data obtained on effect of pH earlier. The percentage decolourization showed increasing trend with rise in temperatures till 37^0C. Further, rise in temperature, beyond 37^0C, led to decline in decolourization ability of the strain.(Figure. 3).

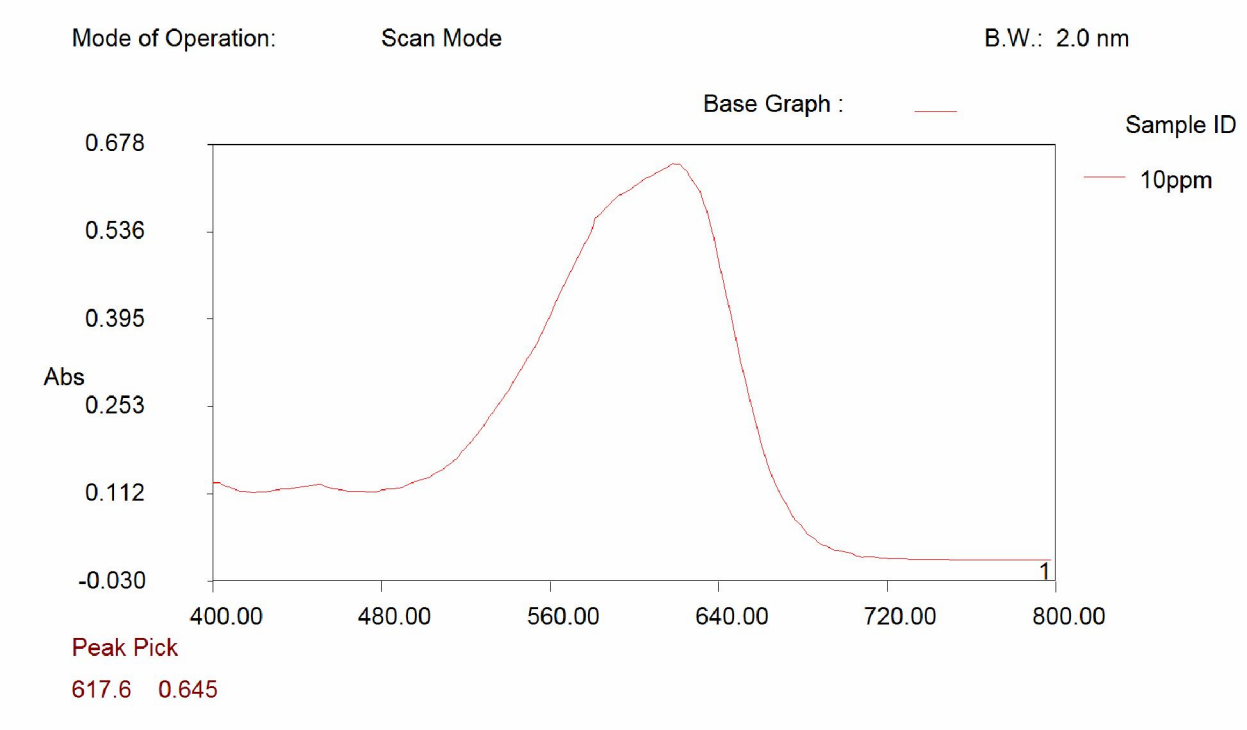

Figure 1. λmax for Amido Black Azo dye (400-800 nm on UV-VIS spectrophotometer)

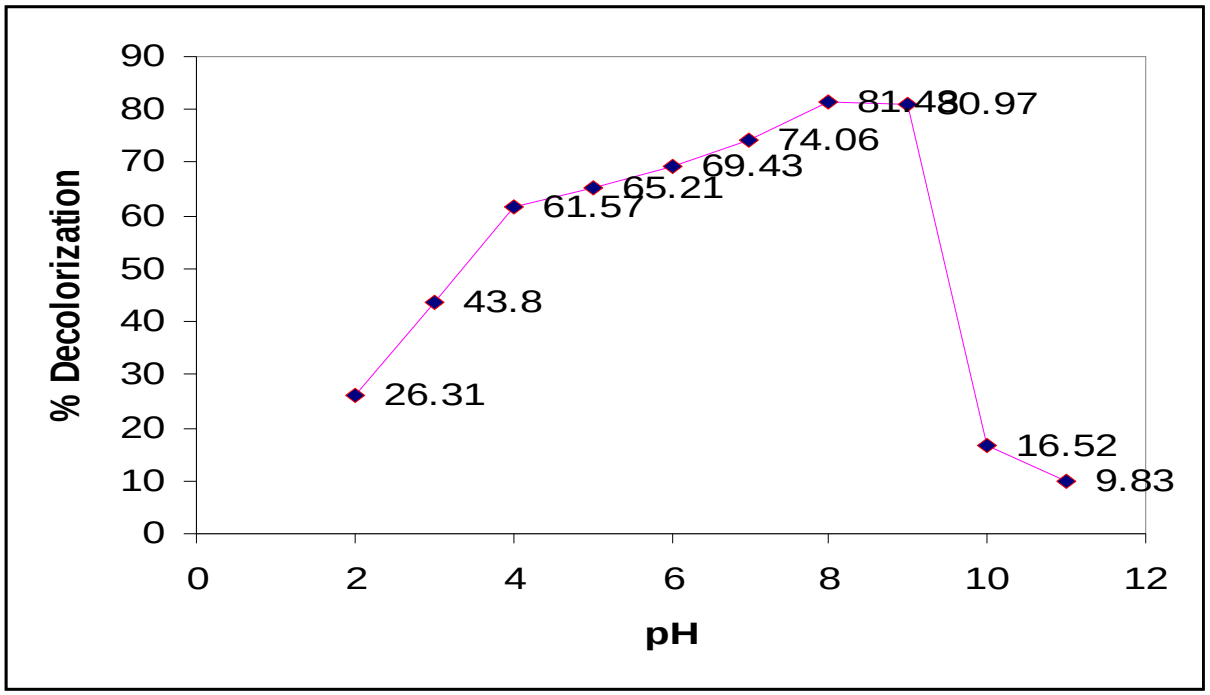

Figure 2. Plot of pH Vs Decolourization % of Azo Dye by RS-II

Table 2. Percentage Decolourisation of Azo Dye by RS-II at Different Temperatures

Tempera-ture (° C)	pH				Absorbance (617.60 nm)		Decolourization (%)
	Initial		Final				
	Control	Test	Control	Test pH±S.D.	Control	TEST± S.D.	
20° C	8.00	8.00	8.00	8.31 ± 0.0212	1.706	1.211±0.3471	8.70
25° C	8.00	8.00	8.00	8.35 ± 0.0777	1.471	1.343±0.0841	17.52
30° C	8.00	8.00	8.00	8.37 ± 0.9899	1.883	1.553±0.0452	29.01
37° C	8.00	8.00	8.00	8.30 ± 0.0707	2.160	0.401±0.0339	81.43
40° C	8.00	8.00	8.00	8.52±0.0707	1.721	1.013±0.2814	41.13
45° C	8.00	8.00	8.00	7.70±0.1414	1.976	1.831±0.3316	18.45
50° C	8.00	8.00	8.00	7.45±0.0494	1.897	1.547±0.875	7.33

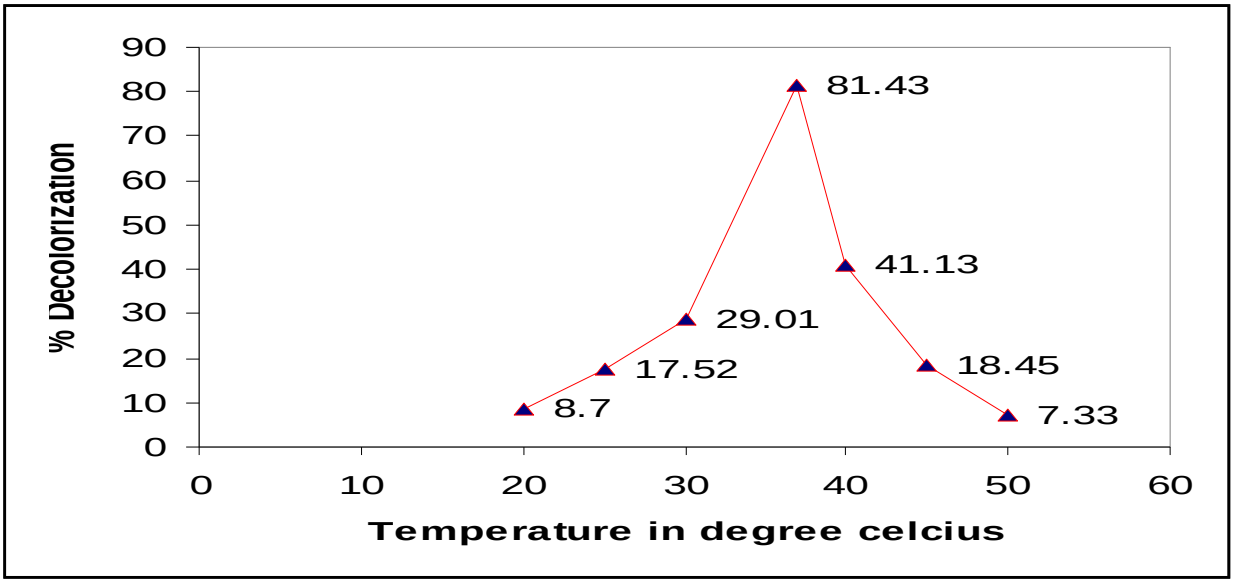

Figure 3. Temperature Vs Decolourization % of Azo dye by RS-II

The protein estimation using SDS PAGE showed two bands (25Kd and 66 Kd). The crude protein was purified using Ion Exchange chromatography. The activity of all the fractions of ion exchange chromatography was determined. The enzyme responsible for decolourization of amido black azo dye was found to effectively bound to cationic exchanger i.e. CM Sepherose. Thus, indicating that the enzyme responsible for amido black decolourisation is a positively charged protein. The enzyme activity of the eluted CM fraction (66Kd) was found out to be 5.5 moles/min/ ml.

4. Discussion

All the isolates showed different decolourization ability for amido black. RS-II strain exhibited highest decolourization at 37^0C. The optimal conditions for decolourization of amido black by this isolate were found to be, pH 8.0 and temperature 37^0C. Since, each microbial strain and its enzymes are highly specific to

pH and temperature, so decrease or increase in decolourization extent of dye might be due to variation in pH and temperature. A large number of workers have investigated the effect of pH and temperature on decolourization of dyes by microorganisms (Raghukumar, 2008; Jadhav *et al*., 2008; Shedbalkar *et al*., 2008; Saratale *et al*., 2009). It is documented that behaviour of each strain varies for dye decolourization with variation in pH (Nosheen *et al*., 2010). The work on azo dye and bacterial combinations to determine optimal conditions for maximum decolourisation efficiency has been documented (Maier *et al*., 2004; Olukanni *et al*., 2009). The authors have concluded that the significant suppression of decolourizing activity at different pH and temperatures, might be due to loss of cell viability or deactivation of enzymes responsible for decolourization. The favourable pH and temperature for dye decolourization by different strains has been reported as 7.0-8.0, and 30-37^{0}C respectively. These findings are similar to our observations.

The decolourizing process of microorganisms is reported to be mediated by different enzymes viz. azoreductases, laccases, peroxidases (Morozova *et al*., 2007; Suwannwong *et al*., 2010). During our studies, two protein bands (25 Kd and 66 Kd) were noticed on SDS PAGE. Earlier, a 66.2 Kd spot on SDS PAGE gel electrophoresis has been observed on azo dye decolourization by *Phanerochaete chrysosporium* (Ghasemi *et al*., 2010). It has been reported that most commonly fungal laccases lie between 50-130 Kd, and that type of dye has no significant effect on extracellular enzyme production (Morozova *et al*., 2010).

Further purification of the protein fraction by Ion Exchange Chromatography and determination of enzymatic activity exhibited significant activity for azo dye decolourization. Thus, it is obvious that the isolate RS-II harbors active enzymatic machinery for amido black decolourization and could be a useful bioremediation tool for treatment of dyeing industry effluent.

5. Conclusion

The study led us to conclude that the isolate has adequate potential to decolourize the azo dye, and that the pure fraction of enzyme has even higher potential to do so. This finding could be a safe and viable solution to bioremediation of azo dye containing effluents and can be an effective gateway to evolve more advanced and effective strategies based on use of pure or immobilized enzymes.

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The Ends of the Earth
(The Four Corners of the Earth)

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Abstract: Old and New Testament Bible Stories told us The Creation Story; and God used Jews stories to teach us that we should obey God Jesus, walked the earth in the form of a man, he lived only near Jerusalem. But God did not only live in Jerusalem. God was everywhere! The Bible mentioned "the Ends of the Earth" many times. The Queen of the South came from the Ends of the Earth (*Matthew12:42*). When The Queen of the South would rise at the Judgment, more stories of "the Ends of the Earth" would be given. This article briefly introduced where "the Ends of the Earth" were; who lived in "the Ends of the Earth"; The Brief histories and legends of "The Ends of the Earth". Indexing references were listed at the end of the paper in alphabetical order.
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Keywords: Bible; Creation; God; Jews; Jerusalem; End; Earth

Old and New Testament Bible Stories had told us The Creation Story; and God used Jews stories to teach us that when the Jews obeyed God, they did well. When they disobeyed, the consequences were bad.
Jesus, walked the earth in the form of a man, he lived only near Jerusalem.
But God did not only live in Jerusalem. God was everywhere!

When Noah was 601 years old, the water had dried up from the earth (*Genesis8-13*). From Noah to Peleg, the whole world had one language and a common speech, they settled in Shinar (Babylonia) (*Genesis11-1*). Since Peleg, the world was divided (*Genesis 10-25*).and the Lord confused the language of the whole world. From there the Lord scattered them over the face of the whole earth (*Genesis 11-8*).

The Bible mentioned "the Ends of the Earth" many times. "The Ends of the Earth" also called "The Four Corners of the Earth" (The Easternmost Place; The Westernmost Place; The Southernmost Place; The Northernmost Place). Here were 58 times that The Bible uses the phrase "the ends of the earth".
(http://peacepink.ning.com/group/prayersgroup/forum/topics/the-ends-of-the-earth-from-the)
(1) In Psalm 65:5 the ends of the earth were parallel to the farthest sea.
(2) Isaiah 41:5 which said, "The islands have seen it and fear; the ends of the earth tremble. They approach and come forward;" Here islands were considered to be synonymous with, or near the ends of the earth. The earth was surrounded by water, but there were islands at the edge of the world where the sky met the sea.
(3) Revelations 7:1 and 20:8 mentioned the four corners of the earth.

When The Queen of the South would rise at the Judgment (*Matthew 12:42)*, more stories of "the Ends of the Earth" would be given.

William McGaaghey (<Five Epochs of Civilization: World History as Emerging in Five Civilizations>) thought that there were five Epochs of Civilization: Ancient Babylon (4000BCE-2250BCE), Ancient Egypt (before 3500BCE), Ancient Greece (3000BCE-1100BCE), Ancient India (before 2000BCE), Ancient China (before 2000).

Four civilizations were near Jerusalem. Only ancient China was from the East End of the Earth--far away from Jerusalem. It was the Promised Land where The Queen of the South would be born.

Mr. Carleton S. Coon had divided humanity into five races:
*Caucasoid race: White race (**高加索人种**)*
*Mongoloid race: Yellow race (**蒙古人种**)*
*Negroid race: Black race (**尼格罗人种**)*
*Capoid race: (Most live in South Africa) (**开普人种**)*
*Australoid race: (dark skin) (**澳洲土著人种**)*

I) The descendants of Shem (*Genesis 10:21-32*) spread out over all the Continent of Asia to North and East. They eventually reached the East Corner and the North Corner of the Earth. They were partial Caucasoid race (White) and partial Mongoloid Race (yellow).

II) The descendants of Japheth (*Genesis10:2-5*) were

maritime people, they were good at navigation. They spread out all over the Continent of Europe to West. They also spread out to North (Slavs in Russia), India and south Asia where were the tents of Shem (*Genesis 9-27 .....may Japheth live in the tents of Shem....*). They spread out to America (*Genesis 9-27 May God extend the territory of Japheth....*). They eventually reached the West Corner of the Earth.
The descendants of Japheth were Caucasoid race (White).

III) The descendants of Ham (*Genesis 10:6-20*)
(1) Nimrod (Ham-Cush-Nimrod) was a mighty warrior, He built his kingdom: In *Shinar* (Babylonia): (Babylon, Erech, Akkad, Calneh), and In *Assyria*: (Nineveh, Rehoboth Ir, Calah, Resen)
The Bible had recorded a history of all kingdoms of Nimrod from its rise to fall.
(2) The descendants of Ham spread out to South to Africa. They also spread out over South Asia, India, Thailand, Malaysia, Indonesia, and Through Malay Archipelago (a bridge prepared by God); they arrived in Australia and New Zealand and small islands of The South Pacific Ocean.
They eventually reached the South Corner of the Earth.
The descendants of Ham were Negroid race, Capoid race, and Australoid race. They had the dark skin color.

This article briefly introduced where "the Ends of the Earth" were; who lived in "the Ends of the Earth"; The Brief histories and legends of "The Ends of the Earth".

Through stories of the Ends of the Earth, it should bring more confidence that Bible prophesied the development of human history; Prophecies of the Bible were fully fulfilled; we had the same God; we were all brothers and sisters in God's big family.

The East Corner of the Earth (The Easternmost Place)

Where was "The East Corner of the Earth"?
Weihai Prefecture (in Shandong Province) was the Easternmost prefecture of China (Before year 1987, Yantai was the Easternmost prefecture of China. Weihai was a county of Yantai prefecture).
There was a place named "End of Land" (North latitude: between 36 ~ 38, East Longitude: between122-124), it was the easternmost place in China. "End of Land" originally came from Qin Shi Huang (259 BC –210 BC). He ordered his Chancellor Li Si to erect a monument with "End of Land, East Gate of Qin".

Who lived in"The East Corner of the Earth" ?
The descendants of Shem (*Genesis 10:21-32*) spread out over all the Continent of Asia to North and East. They eventually reached the East Corner.

The descendants of Shem were partial Caucasoid race (White) and partial Mongoloid Race (yellow);

Jesus was the descendant of Shem.
The Queen of the South was also the descendant of Shem.
Noah-Shem-….Dong-Yi Ethnic Groups - (Ancient country: Lai (?-567BC) in Shandong Peninsula) - Liu Bang (256BC-195BC)-…..… - the Queen of the South.

The Brief History and Brief legends
Since Peleg, the world was divided (*Genesis 10-25*).and the Lord confused the language of the whole world. From there the Lord scattered them over the face of the whole earth (*Genesis 11-8*).
The descendants of Shem spread out over all the Continent of Asia to North and East. The journeys for them to "the East Corner" and "The North Corner" were not very long.

Jesus Christ was the descendant of Jacob (Israel) and Shem.
In Old Testament Bible Stories, God used Jews stories to teach us that when the Jews obeyed God, they did well. When they disobeyed, the consequences were bad.
In New Testament Bible Stories God told us stories of Jesus and his 12 disciples (12 apostles) who lived near Jerusalem.

The Queen of the South was also the descendant of Shem.
Noah-Shem-….Dong-Yi Ethnic Groups - (Ancient country: Lai (?-567BC) in Shandong Peninsula) - Liu Bang (256BC-195BC)-…..… - the Queen of the South.

William McGaaghey (<Five Epochs of Civilization: World History as Emerging in Five Civilizations>) thought that there were five Epochs of Civilization: Ancient Babylon (4000BCE-2250BCE), Ancient Egypt (before 3500BCE), Ancient Greece (3000BCE-1100BCE), Ancient India (before 2000BCE), Ancient China (before 2000).
Four civilizations were near Jerusalem. Only Ancient China was in the East End of the Earth; Dong-Yi Culture was the root of Ancient China culture. Dong-Yi (Shandong Peninsula) was the Promised Land where The Queen of the South would be born.
The Queen of the South rose at the Judgment at the South. She lived at least two Ends of the Earth. (She came from the East End of the Earth, and rose at the South End of the Earth.)

The earliest ancestors of The Queen of the South came

from the Middle East.
They had been to East End of the Earth and North End of the Earth; they also had gone to America and West End of the Earth. They were called Dong-Yi Ethnic groups in ancient China.

According to 'Shan Hai Jing", there were many oldest ethnic groups in Neolithic China, Di-Jun, Zhuan-Xu, Shao-Hao, Huang-Di, Yan-Di, etc. Millet was their staple food.
Before Dong-Yi Ethnic Groups came to East China, Emperor Di-Jun Ethnic Groups and Emperor Shao Hao Ethnic Groups had already lived there.
Dong-Yi Ethnic Groups were the first groups who brought wheat to China and planted wheat in China. Wheat cultivating history was much later than Millet history in Neolithic China.
Dong-Yi Ethnic Groups built the most advanced culture (Dong-Yi Culture) in Neolithic China. Emperor Di-Jun Ethnic Groups learned Dong-Yi Culture; inherited, carried forward and spread Dong-Yi Culture. Later Di-Qiang culture also had elements of Dong-Yi Culture.
Dong-Yi Ethnic Groups had clear Caucasoid race characteristics. They also intermarried with other Oldest Ethnic Groups.

(1) Dong-Yi Ethnic Groups were the first groups who planted wheat in China.

Before Dong-Yi Ethnic Groups came to East China, China did not have wheat.
According to "Shan Hai Jing", there were many oldest ethnic groups in Neolithic China Such as: Di-Jun, Zhuan-Xu, Shao-Hao. Millet was their staple food. Other Oldest ethnic groups ate meat. Such as: Huang-Di, Yan-Di
Millet cultivating history was much earlier than wheat history. 11000 years ago, mankind in China started to cultivate millet. Many China archaeological sites had found cultivated millet: Ci Shan of Hebei Province (10300-7500 years ago); Da Di wan Culture (8200 years ago); Jian Village in Lin Tond of Shanxi Province (5500～5000 years ago).

Some scientists thought that Ancient China did not have objective conditions for wild species hybridizing naturally and evolving to Triticum aestivum L (wheat). China Wheat was from Middle East. Dong-Yi Ethnic Groups brought wheat to East China.
Many China archaeological sites had found cultivated wheat: Shan County of Henan Province(7000 years ago); Jiaozuo County of Henan Province(4000 years ago); Tianshui County of Gansu Province(4800years ago); Min Le County of Gansu Province (Wheat and Barley, 5000 years ago);
Wheat had been widely cultivated in Shandong during Long Shan culture (4350-3950 years ago).

(2) **Dong-Yi Culture was the most advance culture in Neolithic China.**

'Shan Hai Jing' had some records that Doing-Yi Ethnic Groups worshipped Birds and they could predict weather or good and bad luck. Emperor Di-Jun Ethnic Groups built friendly relations with Dong-Yi Ethnic Groups. Di-Jun Ethnic Groups even regarded Dong-Yi Ethnic Groups as half human and half spirit. They learned Dong-Yi Culture; inherited, carried forward and spread Dong-Yi Culture.

The writing system of Dong-Yi was one of the oldest writing systems in Neolithic China. It was an important source of the Shang Oracle bone script.
Dong-Yi Ethnic Groups were inventor of arrows. (Zuo Zhuan, Shuowen Jiezi had similar records about this).
Dong-Yi Ethnic Groups were the earliest users of copper and iron in Neolithic China. They also had high skill in making pottery.
Dong-Yi Ethnic Groups were starter of etiquette. Set of etiquette in Long Shan Culture had shown social stratification and formation of the nation.

(3) Dong-Yi Ethnic groups were regarded as minority by oldest China ethnic groups

Even Dong-Yi Ethnic Groups had built friendly relations with Other East China Ethnic Groups in their early history. They were always regarded as minority by Other China ethnic groups.
Dong-Yi Country became main hostile country of Shang Dynasty (1600BC-1046BC) and Zhou Dynasty (1046BC- 256BC).
The last country of Dong-Yi Ethnic Groups was ancient "Lai" in Shandong Peninsula. Country Qi eliminated "Lai" completely in BC567. Lai King was killed; Lai capital was burned; Lai culture was destroyed; Lai people were moved to Ni County (Teng Zhou of Shandong). (There was a village named Lai village).
Pei county of Jiangsu, where Liu Bang (256BC-195BC) was born, was only 25-30km from Ni County. There were many Dong-Yi people in Pei County at Liu Bang's time. (Liu Bang's mother possibly came from Dong-Yi Ethnic Groups)
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Since the descendant of Shem spread out over China, they lived in the God Promised Land. Ancient China was one of Five Epochs of Civilization and kept a long history of a unified country since Qin (22BC-207BC) and Han Dynasty (202BC-220AC).

The descendants of Shem also have gone across the Bering Strait, and spread out over America.
God promised to extend Japheth's territory (*Genesis9-27*). America was the Promised Land of

Japhethites. After Japhethites arrived to America (The first Europeans arrived to America in 1542), the descendants of Shem had to give the West Corner land to Japhethities.

In history, there were other places regarded as East Corner, and became very strong states. Such as: Japan, But they were all abandoned by God because of their brutality, greed and the persecution to other ethnic groups.

Archaeologists discovered Caucasoid race characteristics in Ancient Shandong

Scientists discovered Ancient Residents of Shandong Peninsula and East China (including parts of Henan, Hebei or Jiangsu) came from Middle East.
And People had clear Caucasoid race characteristics in Ancient Shandong.

In archaeological sites of Beizhuang in Changdao, Shandong (about 6500 years ago), archaeologists discovered a pottery mask which had Caucasoid race characteristics.

Archaeologists and Scientists of Molecular genetics paleontology had discovered Caucasoid race characteristics (HV genes) from DNA which extracted from corpse bones in ancient tombs of Linzi in Shandong and archaeological sites of Da Wen Kou in Shandong (about 6000 years ago), and archaeological sites of Beizhuang in Changdao, Shandong(about 6500 years ago).

Guo Mo-Rue discovered that Dong-yi Ethnic groups (during the period of Da Wen Kou Culture) had nice whiskers and a beard, aquiline nose, bearing some resemblance to Caucasoid race in appearance.

According to historical records：Confucius (551BC-479BC) had clear Caucasoid race characteristics; Emperor Gaozu of Han Liu Bang (256BC-195BC) had high nose, high forehead, high brow-bone, nice whiskers and a beard, bearing some resemblance to Caucasoid race in appearance.

According to "Shan Hai Jing", "Emperor Jun gave birth to Emperor Hong, Emperor Hong gave birth to white people ". There were white people in Emperor Jun's tribes.

Scientific research indicated incontestably that local residents in Shandong where Confucian originated had Caucasoid race characteristics since *the Neolithic Age* until late *Spring and Autumn Period (770BC-476BC).*
Since Qin Dynasty (221BC-207BC), Emperor encouraged heterogeneous marriages.
After The Han Dynasty (202BC-220AC), the members of the ethnic majority in China, the "people of Han, (Han Race)" are named.
In China history, Emperors had encouraged large-scale migration, and as a result, there were more heterogeneous marriages.
After Sui Dynasty (581AC -618AC) and Tang Dynasty (618AC-907AC), Han Race had more Mongolian racial characteristics.

Isaiah 49:12: "Behold, these shall come from far: and, lo, these from the north and from the west; and these from the land of Sinim." (A lot of people believed "Sinim was Qin Dynasty in China")

Religions' history in China

The earliest Chinese myths were Pangu opening up the Universe and Nuwa repairing the Heavens and stopping the Flood. Today, there are still some people worship Pangu and Nuwa.
According to the Bible, the universe was formless and empty and darkness. It was God who created universe (the heavens and the earth).
According to China Pangu Legend, the universe was formless. It was Pangu who started the universe. From China Nüwa's story, we knew there was a big flood. This flood was exactly the same flood of Noah (*Genesis 6*). The waters were from the heavens.
(http://peacepink.ning.com/profiles/blogs/the-beginninggenesis-and-pangu)

In ancient China, there were not religions, people from different groups worshipped different totems. Human Beings only believed in one God and kings were chosen by God to lead all people.

Taoism had grown to a religion since Late East Han Dynasty (25AC-220AC). After late East Han Dynasty, Buddhism came to China. Islam came to China since Yuan Dynasty (1206AC-1368AC). After Ming Dynasty (1368AC-1644AC), some people started to believe in Christian.

Many groups in ancient China chose bird as their totem. Such as: people in Shandong Peninsula before Zhou Dynasty (1050BC-256BC); And Shang Dynasty (1600BC-1046BC) ("*God Ordered Xuan Bird to Give Birth to Shang*" "Shi Jing")
From bird worship, ancient China started to worship Chinese phoenix/phoenix, queen/king of all birds, it was a symbol of good luck.

But later Dragon worship had grown to the most important role, and even to a totem of whole China. Emperors of China called themselves: "Son of God, The Real Dragon". The dragon became a symbol of

power, strength, and good luck.

From Bible, we knew that Dragon was Son of God. Satan was an angel in the Heaven; He could *roam through the earth and go back and forth in it." (Job 1-7).*
But Satan turned himself to a bad one. *And there was war in heaven. Michael and his angels fought against the dragon, and the dragon and his angels fought back. …He was hurled to the earth, and his angels with him. (Revelation 12:7).*
The war happened after Liu Bang (256BC-195BC) was born. After Satan and his angels were cast down from heaven, God sent Jesus Christ to the earth to save the humanity. Jesus Christ had seen Satan fall like lightning from heaven. (*Luke 10:18 Jesus sent out the 72 and told them "I saw Satan fall like lightning from heaven".)*

Satan was a deceiver. When Liu Bang (256BC-195BC) (First Emperor of Han Dynasty (206BC-220AC)) was born; Satan stood aside Liu's mother and deceived mankind for pretending to be the God.
(http://peacepink.ning.com/profiles/blogs/the-woman-revelation12)
Satan also worked hard to build Dragon worship in Asia. People started to believe that Fuxi and Nüwa had an image of half people and half snake (or Dragon) since Han Dynasty (206BC-220 AD).
(http://peacepink.ning.com/profiles/blogs/the-beginninggenesis-and-pangu)

God could not let mankind in China worship dragon as their God forever. But since last century many countries who believed in Jesus Christ had attacked China, it was hard to let mankind in China to believe a religion which its believers had persecuted them. God chose Mao Ze Dong to be leader of Chinese Communist Party to win wars in China and destroyed all religions in China. God prepared mankind in China to wait for the right religion.

Economic reforms in China began in 1978, involved the decollectivization of agriculture, the opening up of the country to foreign investment, permission for entrepreneurs to start up businesses; involved the privatization and contracting out of much state-owned industry and the lifting of price controls, protectionist policies, and regulations, although state monopolies in sectors such as banking and petroleum remained.

The opening up of the country let mankind of China had the opportunity to learn more about the Bible.

The North Corner of the Earth (The Northernmost Place)

Where was "The North Corner of the Earth"?
Even from the history, we could not find any written record about where The North Corner was; it must be near Arctic Circle. Eskimos (Mongoloid Race) had lived in Siberia 18000 years ago.

Who lived in "The North Corner of the Earth"?
The descendants of Shem (*Genesis 10:21-32*) spread out over all the Continent of Asia to North and East. They eventually reached the North Corner of the Earth. The descendants of Shem were partial Mongoloid Race (yellow).

The descendants of Japheth also spread out to North (Slavs in Russia) where the tents of Shem were. (*Genesis 9-27*) "*May God extend the territory of Japheth; may Japheth live in the tents of Shem, and may Canaan be his slave*".

The Brief History and Brief legends
Eskimos (Mongoloid Race) or Esquimaux were indigenous peoples who had traditionally inhabited the circumpolar region from eastern Siberia (Russia), across Alaska (United States), Canada, and Greenland. Their artifacts found in Siberia going back to perhaps 18,000 years ago. They might have been in Alaska as far back as 10,000 to 12,000 years or more. (http://en.wikipedia.org/wiki/Eskimo)

The history of Russia begins with that of the East Slavs. The first East Slavic state, Kievan Rus', adopted Christianity from the Byzantine Empire in 988, beginning the synthesis of Byzantine and Slavic cultures that defined Russian culture for the next millennium. Kievan Rus' ultimately disintegrated as a state, finally succumbing to Mongol invaders in the 1230s. During this time several regional magnates, in particular Novgorod and Pskov, fought to inherit the cultural and political legacy of Kievan Rus'.
Slavs were Caucasoid race (White) and they were from east Europe. They were the descendants of Japheth.

Since gaining its independence with the collapse of the Soviet Union at the end of 1991, Russia has faced serious challenges in its efforts to forge a political system to follow nearly 75 years of Soviet rule. A new constitution, creating a strong presidency, was approved by referendum in December 1993.

The West Corner of the Earth (The Westernmost Place)

Where was "The West Corner of the Earth"?
Current West Corner of the Earth located in North America (North latitude: between 36 ~ 38, East Longitude: between122-124).

Who lived in"The West Corner of the Earth"?

The descendants of Japheth (*Genesis10:2-5*) were maritime people, they were good in navigation. They spread out all over the Continent of Europe to West. They also spread out to North (Slavs in Russia), India and south Asia where were tents of Shem (*Genesis 9-27 .....may Japheth live in the tents of Shem....*). They spread out to America (*Genesis 9-27 May God extend the territory of Japheth....*). They eventually reached the West Corner of the Earth.

The descendants of Japheth were Caucasoid race (White).

The Brief History and Brief legends

Since Peleg, the world was divided (*Genesis 10-25*). The descendants of Japheth spread out to the west and lived all Coastal areas of Europe. They were maritime people. God gave them good ability of navigation; they could live in Europe where was full of waters and around by seas.

Comparing with Asia and Africa (plus Australia and New Zealand), Europe was a very small place. God promised that the descendants of Japheth might live in the tents of Shem. (*Genesis 9-27 may Japheth live in the tents of Shem, and may Canaan be his slave.*)

Some descendants of Japheth spread out to India and south Asia. Some descendants of Japheth spread out to North. (Slavs in Russia).

The family of Japheth was essentially what we called the Aryans. The Japhethites, or Aryans, split into two groups in earlier history. One group settled in India and the other group in Europe. Together they formed what was known as the "Indo-European" family of nations.

Ancient India (before 2000BCE) was the path where descendants of Ham went to South End of the Earth. Early Indian Caste System proved that Canaan was Japhethites' slaves and Japhethites might live in the tents of Shem.

God promised to extend Japheth's territory *(Genesis9-27 May God extend the territory of Japheth)*. America was the Promised Land for the descendants of Japheth. The first Europeans arrived in America in 1542.

Ancient Greece (3000BCE-1100BCE) was mother of Western Culture.

Roots of the Western civilization may be traced back to 9000 BC. Western culture in its strictly European geographical range began with the Greeks, was enlarged and strengthened by the Romans.

At first, West End of the Earth was in Europe. When Japhethites moved to their extending land: America, West End of Earth was in America.

The descendants of Shem also crossed the Bering Strait, and spread out over America. After Japhethites arrived to America, The descendants of Shem had to give the West Corner land to Japhethities.

The Maya was a proof that America was God Promised Land for Japhethities.

The Maya was a Mesoamerican civilization, noted for the only known fully developed written language of the pre-Columbian Americas, as well as its art, architecture, and mathematical and astronomical systems. Initially established during the Pre-Classic period (2000BC to 250AD), according to the Mesoamerican chronology, many Maya cities reached their highest state development during the Classic period (250AD to 900AD), and continued throughout the Post-Classic period until the arrival of the Spanish. At its peak, it was one of the most densely populated and culturally dynamic societies in the world.

For reasons that are still debated, the Maya centers of the southern lowlands went into decline during the 8th and 9th centuries and were abandoned shortly thereafter. This decline was coupled with a cessation of monumental inscriptions and large-scale architectural construction. Although there is no universally accepted theory to explain this collapse, current theories give either non-ecological or ecological explanations. (http://en.wikipedia.org/wiki/Maya_civilization)

The Maya were the descendants of Shem; The Maya collapsed and gave the West Corner land (America) to Japhethities.

Religions' history in Europe and America

(I) Ancient Greek and Roman Religion

The origins of Greek history has been lost in pre-history, the only knowledge that has remained is that the Greeks assimilated religions of pre-Greek inhabitants of the Peninsula.

Ancient Greek religion was polytheistic, consisting of the worship of many Gods. The Greeks believed that the Gods would offer protection and guide their city-states. The Greeks also placed an immense faith in the idea that an afterlife awaited them after their death. They firmly believed that this life was not the only reality in which the soul lives, but after this lifetime, a whole new and different one awaited them.

Early Roman religion was based on spirits. The Romans did not build great mythologies like the Greeks; rather they believed everything had a spirit. These spirits were thought to influence for good or evil, daily life. The Romans therefore had to keep them

happy through worship and sacrifice. If the rituals and sacrifices were performed properly the Romans believed the gods would be happy and help them.

(II) Christian

Jesus Christ shed his blood for Christians' final victory in Roman Empire. Constantine (272AC-337AC) reversed the persecutions of his predecessor, Diocletian, and issued the Edict of Milan in 313, which proclaimed religious tolerance of Christians throughout the empire.

The Second Temple was constructed around 19BC by Herod the Great. The Romans destroyed Jerusalem and The Second Temple in 70AC under Titus. West Wall remaining prophesied that Japhethites (who lived in the West of the world) would became main group of Christians.

The South Corner of the Earth (The Southernmost Place)

Where was "The South Corner of the Earth"?

Australia (The Latin name: terraaustralis means South Land)
Melbourne is the southernmost city of Australia.
New Zealand is the most south land.

Who lived in"The South Corner of the Earth" ?

The descendants of Ham (*Genesis10:6-9*) had a history of all kingdoms of Nimrod from its rise to fall, The descendants of Ham spread out all over the Continents of Africa and South Asia to Australia and New Zealand. They eventually reached South Corner of the Earth.

The descendants of Ham were Negroid race, Capoid race, and Australoid race. They all have the dark skin color.

The descendants of Ham spread out all over the continent of Africa.
They spread out over India, South Asia, Thailand, Malaysia, and Indonesia; Through Malay Archipelago (a God prepared bridge); they spread out over Australia and New Zealand. They also spread out over small islands of The South Pacific Ocean.

The Brief History and Brief legends

The Bible had recorded a history of all kingdoms of Nimrod from its rise to fall. Nimrod was a mighty warrior, He built his kingdom:
(1) In Shinar (Babylonia): Babylon, Erech, Akkad, Calneh,
Where is Shinar? (Map: http://bibleatlas.org/shinar.htm) *Genesis 11:2*, states that Shinar was a plain settled after the flood, where mankind still speaking one language, built the Tower of Babel. In *Genesis 14:1, 9* Shinar was the land ruled by king Amraphel. "Shinar" was further mentioned in *Joshua 7:21*; *Isaiah 11:11*; *Daniel 1:2*; and *Zechariah 5:11*, as a general synonym for Babylonia.
(2) In Assyria: Nineveh, Rehoboth Ir, Calah, Resen (Map of Assyria: http://bibleatlas.org/areapages/assyria.htm)

Ancient Egypt (before 3500BCE) was mother of African Culture.

Noah had cursed Canaan. (*Genesis 9-25*) *He said: "Cursed be Canaan! The lowest of slaves will he be to his brothers." 9-26 He also said "Blessed be the Lord, the God of Shem! May Canaan be the slave of Shem. 9-27 May God extend the territory of Japheth; may Japheth live in the tents of Shem, and may Canaan be his slave".*

We could find evidences that some descendants of Canaan had been slaves.
In archaeological sites of Shang Dynasty (1600BC-1046BC) tombs in China, there were Negroid race slaves Immolated; Dark skin color was the lower class in early Indian Caste System; Some Africans had been slaves in world history.

No more curses for the descendants of Canaan after Jesus Christ shed is blood. The descendants of Canaan had been saved by Jesus Christ.

Jesus Christ shed His blood on the cross to pay the penalty for YOUR sin! "*Who his own self bare OUR SINS in his own body on the tree," (1 Peter 2:24) ". . .Unto him that loved us, and washed us from OUR SINS in his own BLOOD," (Revelation 1:5b)*

In conclusion, it was very clear that "The Ends of the Earth" were very important phrase of the Bible.
God was everywhere! Even Bible did not give too many details stories of "the Ends of the Earth"; they were very important places of God mentioned in the Bible.

Since Peleg, the world was divided (*Genesis 10-25*). With the help of God, Human Beings had built different cultures. Cultural diversities made our world rich and colorful.

Ancient Babylon (4000BCE-2250BCE), Ancient Egypt (before 3500BCE), Ancient Greece (3000BCE-1100BCE) were root Cultures of The world.

Ancient India (before 2000BCE) was the path where descendants of Ham went to South End of the Earth. Early Indian Caste System proved that Canaan was

slaves of Japhethites and Japhethites might live in the tents of Shem. (*Genesis 9-27 may Japheth live in the tents of Shem, and may Canaan be his slave.*)

Dong-Yi Culture was root of Ancient China (before 2000) Culture.
Dong-Yi (Shandong Peninsula) was the Promised Land where The Queen of the South would be born.

Today, we had huge population on the earth, if we checked our genes; we could find genes from different races. All nations were not divided by races.
God blessed (Pure, Not Evil) Noah and his sons and his descendants to be fruitful and increase in number and fill the earth.
If mankind kept doing sinful things and violating the laws of God, Judgment would come soon.

Adam's son Cain killed his brother Abel, and God punished Cain. (*Genesis 4*)
From this story, we learned that God wanted us mutual supervise and took care of each other; and wanted us do good to all people, *especially to those who belong to the family of believers. (Galatians 6:9)*

4/17/2011

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Increase the efficiency of adult education with the proper use of learning styles

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Abstract: Students, in fact all individuals, are most effective when they are taught in their personal learning style. In fact, there are three major types of learners: visual, auditory, and tactile/kinesthetic. While most individuals without disabilities can learn using any one of these styles, most people have one for which they show a stronger affinity. There are many tests available to help you and your students discover your best learning style. Generally speaking, however, if you are someone who is more likely to think in pictures, prefer to meet with someone in person, and are more likely to want visual diagrams when completing a project you have tendencies towards visual learning. Similarly, if you are more likely to think in terms of sounds, prefer to speak on the phone with someone, and want verbal instructions then you tend towards auditory learning. Finally, if you are more likely to think in terms of moving images like mini-movies in your mind, prefer to participate in an activity when you meet to speak with someone, and tend to jump right into a project without reading directions you tend towards tactile/kinesthetic learning.
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Keywords: learning styles, adult learning

Introduction:

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular population) with a particular means (e.g., adult basic education) to achieve the goal.

Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. "Literacy initiatives" often are defined in terms of the needs of a particular target group. These may be parents of young children, youth who have dropped out of high school without earning a high school diploma, welfare recipients, persons with limited English-speaking ability, incarcerated adults, or adults in the workforce.
2. Other literacy initiatives are defined in terms of a particular educational service, strategy, or means to address a target population's literacy problems. "Adult basic education" and "family literacy" are examples. These initiatives are often defined in terms of a particular configuration of services for the target population (e.g., assessment and information and counseling services).
3. The term "lifelong learning" is often associated with "literacy." Lifelong learning is a means to the goal of maintaining necessary levels of literacy throughout one's lifetime. The goal of lifelong learning has implications for both individual adult's learning behavior as well as education policy and the design of the education system.

Goal six of the National Education Goals illustrates a broadly stated goal that incorporates expectations about both adult literacy and the kinds of policies and services that should be in place to improve literacy. Goal six, "Adult Literacy and Lifelong Learning," states that, "By the year 2000, every adult will be literate and possess the knowledge and skills necessary to compete in a global economy and exercise the rights and responsibilities of citizenship." The objectives related to this goal touch on several of the common elements of definitions listed above, for example:

- Different dimensions of literacy (e.g., academic and workplace skills),
- The level of education attainment (e.g., increasing the number of persons who complete postsecondary degrees),
- The needs of target groups (e.g., parents, minorities, or part-time learners),
- The need to increase the availability of particular educational services, strategies or means (e.g., accessibility of libraries to part-time learners or opportunities for parental involvement), and
- The importance of lifelong learning, both in the learning behavior of individuals and in the educational

system's responsiveness to the needs of adult learners.

adult who is able to recognize their needs. He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge.

Several definitions of adult education has been done Community

- Adult Education is a⎤in the following examples are given of them. conscious effort by public institutions or voluntary organizations to promote community awareness comes action.
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- Adult Education is a process in which people who⎤and education is presented. somehow been cut course they consciously to change or advance their skills in information and do organized activities.
- Adult education includes all formal and informal training and volunteer after⎤ school, which by experienced educators and aware of the system.

Educational materials on adult education with daily life, needs, goals, aspirations and past experiences of adults and their relationship helps to results learned in life and career are used.

Characteristics of adult education:

flexibility in time:

In the past, usually one of the obstacles in the way of learning and development of adult education was being inflexible and time courses were programs. But now most countries have to consider that the speed limit of time and learning ability and facilities must be adults. Flexibility in time means that not only should the time classes and programs for adults is appropriate, but necessary facilities should be provided for independent study.

Flexibility in the location:

One of the aspects of flexible space is that individuals can, regardless of their residence to the study and advancing their knowledge and skills pay. For example, adults in remote villages should like people who live in the city use of educational programs. After flexibility in other places is that the issue of specificity of location is not considered primarily educational.

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To combine education and job responsibilities:

Adults should be able to work during that time engaged in training classes take them. In other words, their presence in the class should be considered part of their work. This means that low-literate or illiterate working people who are allowed to work an hour of your daily spending surpassed participation in educational programs.

Understanding and Using Learning Styles

Students, in fact all individuals, are most effective when they are taught in their personal learning style. In fact, there are three major types of learners: visual, auditory, and tactile/kinesthetic. While most individuals without disabilities can learn using any one of these styles, most people have one for which they show a stronger affinity.

A Look at the Three Learning Styles

1- Visual Learners - Visual learners are those who generally think in terms of pictures. They often prefer to see things written down in a handout, text or on the overhead. They find maps, graphs, charts, and other visual learning tools to be extremely effective. They remember things best by seeing something written.

2- Auditory Learners - Auditory learners are those who generally learn best by listening. They typically like to learn through lectures, discussions, and reading aloud. They remember best through hearing or saying items aloud.

3- Kinesthetic Learners- Kinesthetic, also called tactile, learners are those who learn best through touching, feeling, and experiencing that which they are trying to learn. They remember best by writing or physically manipulating the information.

Learning Style Assessments:

There are many tests available to help you and your students discover your best learning style. Generally speaking, however, if you are someone who is more likely to think in pictures, prefer to meet with someone in person, and are more likely to want visual diagrams when completing a project you have tendencies towards visual learning. Similarly, if you are more likely to think in terms of sounds, prefer to speak on the phone with someone, and want verbal instructions then you tend towards auditory learning. Finally, if you are more likely to think in terms of moving images like mini-movies in your mind, prefer to participate in an activity when you meet to speak with someone, and tend to jump right into a project without reading directions you tend towards tactile/kinesthetic learning.

How to Effectively Use Learning Styles in Class:

In the best of all possible worlds, you would incorporate all three learning styles into each of your lessons. However, this is just not possible in the real world of teaching. In truth, it is often not hard to include both auditory and visual learning styles in your lessons. For example, you can have instructions written on the board and say them out loud. However, it is not always as easy to include the tactile/kinesthetic learning style into your lessons. The sad truth is that many students have this as their strongest learning style. It is best to not force the issue but instead find natural places to include kinesthetic learning. If your class warrants it, you could include simulations, role-playing, debates, or the use of manipulatives.

Concerns When Incorporating Learning Styles

Though rarer today then in the past, some teachers discount the importance of learning styles. They continue to teach in their one major method without trying to vary instructional methods. This is a mistake that will lead to less learning in the classroom.

On the other hand, many students and to a lesser degree some teachers make the mistake of thinking that they cannot learn using methods that are not focused on their learning style. This is also a huge mistake that in the end will result in less learning. If teachers do not help their students find ways to be successful learning information presented in any style, they are not helping them succeed in the future. The fact is that students will be faced with many different styles of teaching during the educational career. Only by finding ways to adapt and learn using other styles, will students end up succeeding.

Examples of ways that students can adapt:

- Kinesthetic learners would include writing down information that they are to learn.
- Visual learners could create word webs, venn diagrams, or other visual presentations of information.
- Auditory learners could read a passage out loud from their textbook or from handouts

1- Kinesthetic Learners:

A Look at Kinesthetic Learners:

Kinesthetic learners typically learn best by doing. They are naturally good at physical activities like sports and dance. They enjoy learning through hands-on methods. They typically like how-to guides and action-adventure stories. They might pace while on the phone or take breaks from studying to get up and move around. Some kinesthetic learners seem fidgety, having a hard time sitting still in class.

Key Learning Methods for Kinesthetic Learners:

Kinesthetic learners learn best through doing including manipulating items, simulations and role plays, and other methods that physically involve them in the learning process. They enjoy and learn well from experimenting and first hand experience. Further, they learn best when activities are varied during a class period.

Ways to Adapt Lessons for Kinesthetic Learners:

Vary instruction not only from day-to-day but also within a single class period. Provide students with as many opportunities as your curriculum warrants to complete hands-on work. Allow students to role-play to gain further understanding of key concepts. Provide students with the opportunity to work in small

discussion groups as they study materials. If possible, plan a field trip that can help reinforce key concepts. Allow students to stretch partially through the class if they seem to become restless.

2- Auditory Learners

A Look at Auditory Learners:

Auditory learners learn best by listening and talking aloud. They typically notice and remember sounds. They are good at remembering things that they hear. They are also good with words and language. They often read to themselves as they study. They are also often distracted by noise and sounds.

Key Learning Methods for Auditory Learners:

Auditory learners learn best through hearing the information. They often need to read the written word aloud to help them remember key points. Verbal repetition is an effective means of study for auditory learners.

Ways to Adapt Lessons for Auditory Learners:

Provide students with oral along with written instructions for assignments. Include whole group discussion in your class. Provide students with videos to complement the written text. Allow time for students to read out loud or talk through problems they might be having. Provide breaks from silent reading periods. Also, realize that those who are strong in auditory learning typically take longer to read a passage.

3- Visual Learners

A Look at Visual Learners:

A typical visual learner uses visualization techniques to remember things. They often have a good sense of direction because they visualize maps and directions in their mind. Many prefer to read information in a textbook or on the whiteboard rather than listen to the teacher lecture. They also enjoy doodling and drawing. Visual learners typically use sight words in their everyday terminology. For example, they might say "Let's take a look at this." or "Let's look at this from a different perspective." They remember details including colors and spatial arrangements.

Key Learning Methods for Visual Learners:

Visual learners learn best by seeing what they are being taught. Visual learners typically prefer images, maps, graphs, and other visual representations over other forms of instruction. They will find that if they include images, mind maps, lists, and other visual techniques in their notes then they will have a better chance of remembering key information.

Ways to Adapt Lessons for Visual Learners:

Including diagrams, mind maps, word webs, visuals, and other forms of graphic organizers will help visual learners get the most from your instruction. Teach students to use highlighters when going through their notes and to create flashcards when studying for tests and learning information. Try not to give only oral instructions before requiring students to complete an assignment. Further, stay away from lecture without accompanying notes and/or visuals.

Conclusion:

Material often set different types of materials and educational content in books and pamphlets, books, training guides, trainers, equipment auxiliary audio, visual and material are included such that during actual teaching sessions, are used in the transmission and content but also to achieve the goals of making education programs are important.

Additional material for the next stage of learning often means to be expected when developing your learning skills Learners to increase awareness and enjoyment of reading and studying to operate.

To improve the quality of life, learning materials should reinforce the skills they acquired previous. This material should have access to information and provide new technology. should also have to make learning more fun. Additional materials should provide opportunities for literacy skills to read and to strengthen their cognitive awareness.

Track materials (continued) which increased literacy skills and knowledge gained is also effective in enriching learning environment for learners are important. Participatory materials to ensure the participation of learners in the learning process and codification are included out of class activities, dialogue, role playing, etc.

In traditional programs that the principles of psychology and curriculum planning, less attention is the form of

content presentation ie codification and providing books, original format and have the dominant form, while for adult content that could have valuable experience in addition to writing, other ways also be provided Affect the selection of pictures and images related to the concepts and content produced by including them.

Learning activities such as activities outside the classroom, dialogue, role playing and ... Another type of content is presented. Duties are placed on the learner, a resource for developing knowledge, skills and insights he considered.

Curriculum content only from the training provided to learners or not, but put together their learning through activities that can inform or does, skills and attitude to achieve. In this case, apart from learning that the assays taught learners directly to sustainable and effective learning occurs in his.

Another way of providing content that is educational activities outside the learning environment possible for learning more and better enables adult learners. For example, hits, field trip experiences for learners or transfer is provided, develop knowledge, insight and skills they will.

To ensure that science curriculum and educational aspects, according to community needs and audiences, application form is provided or not, the content selection criteria should be considered. These criteria is being include knowledge, effectiveness, flexibility, diversity, relevance and practical learning

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Learning styles in education: with emphasis on adult education

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Abstract: Though rarer today then in the past, some teachers discount the importance of learning styles. They continue to teach in their one major method without trying to vary instructional methods. This is a mistake that will lead to less learning in the classroom. On the other hand, many students and to a lesser degree some teachers make the mistake of thinking that they cannot learn using methods that are not focused on their learning style. This is also a huge mistake that in the end will result in less learning. If teachers do not help their students find ways to be successful learning information presented in any style, they are not helping them succeed in the future. The fact is that students will be faced with many different styles of teaching during the educational career. Only by finding ways to adapt and learn using other styles, will students end up succeeding.
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Introduction:

adult who is able to recognize their needs. He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge.

To be successful, the Commonwealth's strategies must energize and gain the commitment of all the state's political, education, business, and civic leaders. No strategy will succeed unless it engages leaders in each community and county to identify needs and develop programs and services appropriate to the community's unique circumstances. The most serious challenge will be to motivate low-skilled, under-educated adults within the working age population to seek further education. Simply expanding the number of providers and programs will not necessarily increase demand from the populations and communities where the needs are greatest. Deepseated social, economic and cultural barriers—many dating back generations—lead people to undervalue education. In addition, in many counties it is difficult for people to see a direct relationship between better education and better-paying jobs. Either there are no jobs available or many existing employers do little to emphasize the connection between better education and the possibilities for getting a job, keeping a job, or earning a higher wage. For many, getting more education and earning a high school diploma or a college degree has little positive meaning.

Several definitions of adult education has been done Community

- Adult Education is a in the following examples are given of them. conscious effort by public institutions or voluntary organizations to promote community awareness comes action.
- adult education teaching is typically specific age group above the legal age⎦ limits as formal and informal, voluntary and at different levels of time, place
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Educational materials on adult education with daily life, needs, goals, aspirations and past experiences of adults and their relationship helps to results learned in life and career are used.

in developed countries, adult education is a form of informal education for people above 24 years is presented. In fact, a means of expanding knowledge, skills and abilities of adults. In these countries, adult education helps adults to variable conditions of political, social, economic and cultural adjustment, and pay to fix their shortcomings.

In developing countries and backward because the

problems in primary education, lack of resources and facilities, poverty, social existence, economic and cultural concept of adult education is different. In such countries the concept of adult education, literacy education is.

Concept of adult education in revolutionary countries, is a combination of these two concepts. Changes in these countries due to social, political and cultural revolution, resulting from, literacy and continuing education necessary to find because of the revolution, there is cultural poverty on the other hand the implementation of development plans and the need for skilled personnel are expert. General adult education system based on economic conditions - social and cultural community is different and each specific goals will follow. General objectives of adult education and literacy in two categories is divided into professional education.

Adult characteristics:

to understand the characteristics of adult learners, their mental and physical condition should be considered in the following referred to some of them.

Operating speed:

slow reaction in adults is natural that necessarily means reducing the logic and practice skills, not due to weakness and increased awareness of natural forces and their skills.

Consciousness:

no stimulus and incentives encouraging, despite inhibiting stimuli, slow transfer rate, mental, and weak inhibitors of natural forces (mostly visual and auditory) are factors that slow reaction affect individual mental and cognitive activities, but never able to understand, understanding and learning ability (which varies with the speed of learning) is not relevant.

Health:

what is most age, longer duration is necessary to be heard by listening issue. Why is that when elderly people and old could not hear well, their confidence and vulnerable to the possibility that negative beliefs about their find, they are great. Visual abilities can be like other people, usually decreases with age.

Background of knowledge - skills and beliefs of adults:

adults, social experiences, many have already learned different values and beliefs in their pronouns have stabilized, so changes in the new act very cautiously. The idea of such a manner that skill and applying them older and longer life is, Similar resistance to accept new ideas will be more and more severe. Thus, the adult criteria for the built and paid for their ideas and beliefs that are forming. Because of these criteria and the beliefs that they are afraid of failure, Therefore, to prevent it, sometimes against the resistance of new phenomena are only the material taught and its face that make reinforced concrete and tangible interference situation is.

Characteristics of adult education:

flexibility in time:

In the past, usually one of the obstacles in the way of learning and development of adult education was being inflexible and time courses were programs. But now most countries have to consider that the speed limit of time and learning ability and facilities must be adults. Flexibility in time means that not only should the time classes and programs for adults is appropriate, but necessary facilities should be provided for independent study.

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The Three Different Learning Styles:

Style 1: Visual

Fleming states that visual learners have a preference for seeing material in order to learn it.

- **Strengths of the visual learner:** Instinctively follows directions, can easily visualize objects, has a great sense of balance and alignment, is an excellent organizer

- **Best ways to learn:** Studying notes on overhead slides, reading diagrams and handouts, following a PowerPoint presentation, reading from a textbook, studying alone
- How do you know if you're a visual learner?

Style 2: Auditory

With this different learning style, students have to hear information to absorb it.

- **Strengths of the auditory learner:** Understanding subtle changes in tone in a person's voice, writing responses to lectures, oral exams, story-telling, solving difficult problems, working in groups
- **Best ways to learn:** Participating vocally in class, making tapes of class notes and listening to them, reading assignments out loud, studying with a partner or group
- How do you know if you're an auditory learner?

Different Learning Style 3: Kinesthetic

Kinesthetic learners tend to want to move while learning.

- **Strengths of the kinesthetic learner:** Great hand-eye coordination, quick reception, excellent experimenters, good at sports, art, drama, high levels of energy
- **Best ways to learn:** experiments, acting out a play, standing, moving, or doodling during lectures, studying while performing an athletic activity like bouncing a ball or shooting hoops

How do you know if you're a kinesthetic learner?

Conclusion:

Some research findings that can be a learning process for the Guidelines for training operations are applied, is given below:

1- - Preparation for adults to learn how much he depends on previous learning. Knowledge that has accumulated because of an ability to absorb new information more person is. Past educational experience features a diverse group of adult learners, the starting point of any activity on the diversity training is emphasized.

2- intrinsic motivation, learning a deeper and make them sustainable. When the need is met directly by the learning itself, what is learned, but is complementary learning. Creating a training activity in adult learning needs, learning ensures stable

3- Positive reinforcement (reward) learning to reinforce the negative (punishment) is more effective. Many adults because of negative experiences at the beginning of schooling, are weak and afraid. Feeling of success in adult learning for continuous learning and adult participation is essential.

4- To maximize learning, information must be provided an organized manner. Entries can be simple or complex can be arranged around related concepts are organized. Starting point for organizing content knowledge for adults and adults is linked to past experiences

5- Learning, especially regarding skills development, will be added frequently.

6 - Duties and meaningful content than meaningless subjects are learned more easily and are later forgotten. This issue, especially for older adult learners is true. Challenges of adult learning facilitators by the way that content was significantly associated with the experiences and needs of learners is.

7- Passive than active participation in learning activities, learning increases. Adult educators are allowed to participate actively in India, a stable and meaningful learning to help

8- Environmental factors affect the learning. Tangible things such as noise, crowded places, temperature, light and ... Learning process can be prevented. Other factors such as stress, ridicule, pressure, fatigue and low health can also reduce learning.

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Understanding and Using Learning Styles

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Abstract: There are many tests available to help you and your students discover your best learning style. Generally speaking, however, if you are someone who is more likely to think in pictures, prefer to meet with someone in person, and are more likely to want visual diagrams when completing a project you have tendencies towards visual learning. Similarly, if you are more likely to think in terms of sounds, prefer to speak on the phone with someone, and want verbal instructions then you tend towards auditory learning. Finally, if you are more likely to think in terms of moving images like mini-movies in your mind, prefer to participate in an activity when you meet to speak with someone, and tend to jump right into a project without reading directions you tend towards tactile/kinesthetic learning.
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Keywords: learning styles, adult learning

Introduction:

As indicated earlier, a strength of adult education in Kentucky is the dedication of the many teachers often serving under difficult conditions, without adequate support, and often with compensation and benefits less than teachers in the public schools. Testimony before the task force characterized the work of adult educators as "missionary" work. Recognizing the seriousness of the adult literacy issue in Kentucky, it should be a major concern that the Commonwealth does not have a comprehensive approach to the professional preparation, development, and support of adult educators.

The challenge for Kentucky will be to move from a system that still depends on teachers with limited training in working with adults, to one in which professional competence in working with adults is a basic requirement. Any strategy to make this transition must involve both professional development and support for the teachers now in the field as well as a new system for a new generation of adult educators.

To be successful, the Commonwealth's strategies must energize and gain the commitment of all the state's political, education, business, and civic leaders. No strategy will succeed unless it engages leaders in each community and county to identify needs and develop programs and services appropriate to the community's unique circumstances. The most serious challenge will be to motivate low-skilled, under-educated adults within the working age population to seek further education. Simply expanding the number of providers and programs will not necessarily increase demand from the populations and communities where the needs are greatest. Deepseated social, economic and cultural barriers—many dating back generations—lead people to undervalue education. In addition, in many counties it is difficult for people to see a direct relationship between better education and better-paying jobs. Either there are no jobs available or many existing employers do little to emphasize the connection between better education and the possibilities for getting a job, keeping a job, or earning a higher wage. For many, getting more education and earning a high school diploma or a college degree has little positive meaning.

Only the negative consequences are obvious: getting more education often means leaving one's family and community for jobs and opportunities for advancement somewhere else. The future of Kentucky depends on uplifting the quality of life and economy of all of Kentucky. The social and economic costs of neglect of large parts of the state will drag down the rest of the state and seriously hinder its capacity to compete in the global economy.

Much like strategies to curb epidemic, strategies to reduce illiteracy and raise the educational attainment of Kentucky's population must include both short-term efforts to face the immediate crises as well as long-term strategies to get at the underlying causes. Short-term crises include the imperative to keep helping welfare clients make the transition from welfare to work within the constraints of federal and state mandates and the need to train workers for immediate employer demands. Long-term prevention must address the underlying, persistent problems of the state's economic structure as well as the low awareness--if not appreciation--among segments of the population of the vital connection among education, employment, and improved standards of living.

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions

have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular population) with a particular means (e.g., adult basic education) to achieve the goal.

Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. "Literacy" refers to the knowledge, skills, and competencies of individuals. The federal Adult Education and Family Literacy Act (Title II of the Workforce Investment Act)1 defines literacy as "an individual's ability to read, write, speak in English, compute and solve problems, at levels of proficiency necessary to function on the job, in the family of the individual, and in society." Literacy is often defined in terms of specific domains such as "basic academic skills," "workplace skills," "life skills," "parenting skills," or skills

necessary to exercise one's rights and responsibilities for citizenship. Different dimensions of literacy are often categorized by terms that cluster several dimensions of literacy important for different clients. Examples include workplace literacy (combining both basic academic skills and workplace skills), and family literacy (combining basic academic skills and other skills essential for successful parenting).

2. "Education attainment" usually refers to the numbers of years of schooling completed or the level of credential (e.g., high school diploma or associate degree) an individual has obtained. Despite concerns about the meaning of credentials, there is a strong correlation between educational attainment and literacy.

3. "Literacy initiatives" often are defined in terms of the needs of a particular target group. These may be parents of young children, youth who have dropped out of high school without earning a high school diploma, welfare recipients, persons with limited English-speaking ability, incarcerated adults, or adults in the workforce.

4. Other literacy initiatives are defined in terms of a particular educational service, strategy, or means to address a target population's literacy problems. "Adult basic education" and "family literacy" are examples. These initiatives are often defined in terms of a particular configuration of services for the target population (e.g., assessment and information and counseling services).

5. The term "lifelong learning" is often associated with "literacy." Lifelong learning is a means to the goal of maintaining necessary levels of literacy throughout one's lifetime. The goal of lifelong learning has implications for both individual adult's learning behavior as well as education policy and the design of the education system.

Goal six of the National Education Goals illustrates a broadly stated goal that incorporates expectations about both adult literacy and the kinds of policies and services that should be in place to improve literacy. Goal six, "Adult Literacy and Lifelong Learning," states that, "By the year 2000, every adult will be literate and possess the knowledge and skills necessary to compete in a global economy and exercise the rights and responsibilities of citizenship." The objectives related to this goal touch on several of the common elements of definitions listed above, for example:

- Different dimensions of literacy (e.g., academic and workplace skills),
- The level of education attainment (e.g., increasing the number of persons who complete postsecondary degrees),
- The needs of target groups (e.g., parents, minorities, or part-time learners),
- The need to increase the availability of particular educational services, strategies or means (e.g., accessibility of libraries to part-time learners or opportunities for parental involvement), and
- The importance of lifelong learning, both in the learning behavior of individuals and in the educational system's responsiveness to the needs of adult learners.

Literacy goals include:

- Providing primary education in childhood that adults were deprived
- raising awareness for adults;
- knowledge bases and adults about their cultural heritage;
- increase confidence in adults.

Professional education goals include:

- Equipped with the necessary skills to adults living;
- providing the necessary manpower for the country's goals;
- achieving social equality and equity and eliminate the existing differences between different classes.

Adult characteristics:

to understand the characteristics of adult learners, their mental and physical condition should be considered in the following referred to some of them.

Operating speed:

slow reaction in adults is natural that necessarily means reducing the logic and practice skills, not due to weakness and increased awareness of natural forces and their skills.

Consciousness:

no stimulus and incentives encouraging, despite inhibiting stimuli, slow transfer rate, mental, and weak inhibitors of natural forces (mostly visual and auditory)

are factors that slow reaction affect individual mental and cognitive activities, but never able to understand, understanding and learning ability (which varies with the speed of learning) is not relevant.

Health:

what is most age, longer duration is necessary to be heard by listening issue. Why is that when elderly people and old could not hear well, their confidence and vulnerable to the possibility that negative beliefs about their find, they are great. Visual abilities can be like other people, usually decreases with age.

Background of knowledge - skills and beliefs of adults:

adults, social experiences, many have already learned different values and beliefs in their pronouns have stabilized, so changes in the new act very cautiously. The idea of such a manner that skill and applying them older and longer life is, Similar resistance to accept new ideas will be more and more severe. Thus, the adult criteria for the built and paid for their ideas and beliefs that are forming. Because of these criteria and the beliefs that they are afraid of failure, Therefore, to prevent it, sometimes against the resistance of new phenomena are only the material taught and its face that make reinforced concrete and tangible interference situation is.

Characteristics of adult education:

flexibility in time:

In the past, usually one of the obstacles in the way of learning and development of adult education was being inflexible and time courses were programs. But now most countries have to consider that the speed limit of time and learning ability and facilities must be adults. Flexibility in time means that not only should the time classes and programs for adults is appropriate, but necessary facilities should be provided for independent study.

Flexibility in the location:

One of the aspects of flexible space is that individuals can, regardless of their residence to the study and advancing their knowledge and skills pay. For example, adults in remote villages should like people who live in the city use of educational programs. After flexibility in other places is that the issue of specificity of location is not considered primarily educational.

Flexibility in age:

Educational opportunities for certain age should not use it for all regardless of their age, is possible. In fact, educational programs must use people of different ages to prepare.

Flexibility in admission:

No adult should not only be deprived of education because of the necessary conditions for admission in the class does. Of course this is not such a person without academic records to participate in university classes is accepted, Adoption order is that the adults in educational programs at different levels, according to the possibility of using the opportunity that is provided must be based on the experience and knowledge and their knowledge is.

To combine education and job responsibilities:

Adults should be able to work during that time engaged in training classes take them. In other words, their presence in the class should be considered part of their work. This means that low-literate or illiterate working people who are allowed to work an hour of your daily spending surpassed participation in educational programs.

The Three Different Learning Styles:

Style 1: Visual

Fleming states that visual learners have a preference for seeing material in order to learn it.

- **Strengths of the visual learner:** Instinctively follows directions, can easily visualize objects, has a great sense of balance and alignment, is an excellent organizer
- **Best ways to learn:** Studying notes on overhead slides, reading diagrams and handouts, following a PowerPoint presentation, reading from a textbook, studying alone
- How do you know if you're a visual learner?

Style 2: Auditory

With this different learning style, students have to hear information to absorb it.

- **Strengths of the auditory learner:** Understanding subtle changes in tone in a person's voice, writing responses to lectures, oral exams, story-telling, solving difficult problems, working in groups
- **Best ways to learn:** Participating vocally in class, making tapes of class notes and listening to them, reading assignments out loud, studying with a partner or group
- How do you know if you're an auditory learner?

Different Learning Style 3: Kinesthetic

Kinesthetic learners tend to want to move while learning.

- **Strengths of the kinesthetic learner:** Great hand-eye coordination, quick reception, excellent experimenters, good at sports, art, drama, high levels of energy
- **Best ways to learn:** experiments, acting out a play, standing, moving, or doodling during lectures, studying while performing an athletic activity like bouncing a ball or shooting hoops

How do you know if you're a kinesthetic learner?

Conclusion:

Beyond the issues relating directly to DAEL(Department of Adult Education and Literacy), the task force heard a number of concerns about the Commonwealth's overall approach to adult literacy.

- Lack of coherent statewide leadership and coordination among multiple complementary initiatives aimed at the same problem.
- Lack of continuity in state leadership. Cited in particular was the difficulty sustaining a high level commitment to the issue long enough to make a difference because of changes in priorities of the state's political leaders. A high level of turnover in the leadership of the Department of Adult Education and Literacy has also contributed to the instability.
- Tendency to think of adult education as a separate categorical program rather than a strategy that cuts across the mission and responsibility of multiple Commonwealth programs and initiatives (e.g., early childhood education, welfare reform, economic development, and corrections).
- Multiple uncoordinated categorical federal initiatives that tend to drive (and fragment) policy for an overall state effort that is largely funded by Kentucky.
- A tendency to commingle and confuse different functions. The most important distinction is between functions focused on the needs of clients (adult learners, employers, communities, regions, and the Commonwealth as a whole) and functions associated with the operations and performance of providers. It is important that each of these functions receive attention, yet the tendency is for one (e.g., overseeing a network of providers) to drive out attention to overall system strategy.
- Inadequate coordination of services to meet the needs of individual adults, communities, employers, and regions is hindered by:

- Vertical financing and regulatory relationships between separate federal and state programs and local providers and administrative units. These vertical relationships can hinder the horizontal coordination of services for individual adult learners, communities, and employers.

- Turf wars among providers, local politics, and long-standing conflicts among neighboring counties.

- Inadequate links with and leverage of other public and private initiatives and investments to reach the target population. Major sources of help include employers, postsecondary education, and workforce development.
- Lack of a state financing policy and strategy for provider performance incentives and collaboration, and tax and other employer incentives for leverage of non-state resources.
- Lack of programmatic and administrative flexibility to meet the rapidly changing needs of adult learners, employers, regional economies, and communities.

Some research findings that can be a learning process for the Guidelines for training operations are applied, is given below:

1- - Preparation for adults to learn how much he depends on previous learning. Knowledge that has accumulated because of an ability to absorb new information more person is. Past educational experience features a diverse group of adult learners, the starting point of any activity on the diversity training is emphasized.

2- intrinsic motivation, learning a deeper and make them sustainable. When the need is met directly by the learning itself, what is learned, but is complementary learning. Creating a training activity in adult learning needs, learning ensures stable

3- Positive reinforcement (reward) learning to reinforce the negative (punishment) is more effective. Many adults because of negative experiences at the beginning of schooling, are weak and afraid. Feeling of success in adult learning for continuous learning and adult participation is essential.

4- To maximize learning, information must be provided an organized manner. Entries can be simple or complex can be arranged around related concepts are organized. Starting point for organizing content knowledge for adults and adults is linked to past experiences

5- Learning, especially regarding skills development, will be added frequently.

6 - Duties and meaningful content than meaningless subjects are learned more easily and are later forgotten. This issue, especially for older adult learners is true. Challenges of adult learning facilitators by the way that content was significantly associated with the experiences and needs of learners is.

7- Passive than active participation in learning activities, learning increases. Adult educators are allowed to participate actively in India, a stable and meaningful learning to help

8- Environmental factors affect the learning. Tangible things such as noise, crowded places, temperature, light and ... Learning process can be prevented. Other factors such as stress, ridicule, pressure, fatigue and low health can also reduce learning.

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Farmers's Perception of Sugar cane Production and Marketing Problems in Qena and Asswan Governorates, Egypt

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Abstract: The main objective of this paper was to know farmers' perception and evaluation of problems facing sugar cane growers in Qena and Asswan governorates, Egypt. An empirical investigation was carried out to identify and assess problems facing sugarcane growers in six villages in these two governorates (four villages in Qena and two villages in Asswan). The identification of sugarcane problems was based on data gathered from nine focus groups held with farmers in three villages and problems identified in the previous research. Twenty seven production problems and nineteen marketing problems were identified. The assessment of these identified problems was based on survey data collected by means of personal interview using questionnaires from a random sample of 262 farmers in the other three villages (Two villages in Qena and One village in Asswan). Sample members were asked to state whether each problem existed, the degree of its importance, and efforts devoted to solve it. Different methods and techniques were used for problems assessment. These are: importance, achievement, the discrepancy between importance and achievement, Borich model, Delta N, and the Modified Delta N method. Results showed that most of the identified problems were perceived by farmers were evaluated as important or very important problems. Differences among farmers in the three villages in the two governorates were examined. Problems were rank ordered according to the results of different assessment methods and techniques. Ranking results showed spatial differences among farmers in the two governorates. The extension system should be aware of such problems and differences and plan its programmes and activities based on them.

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Keywords: Sugar cane Problems, assessment methods, Modified data N, Egypt.

1. Introduction:

Sugar cane is one of the main agricultural products in Egypt. It is grown mainly in middle and Upper Egypt. But most of the area under sugarcane in Egypt (over 72 %) is grown in Qena and Asswan governorates. Also over 72 % of sugarcane production is produced in these two governorates (Abdel-Maksoud and Elshrabassee, 2007).

In order to increase the productivity of agricultural crops and achieve agricultural development in Egypt, there must exist: an effective research system which is capable to produce the new technology, and an effective agricultural extension system which is capable to diffuse the new technology among farmers and encourage them for their adoption. Effective extension programmes and activities should be planned on the basis of clients' problems and needs. Therefore, modern sophisticated methods of problems and needs determination and assessment are essential for effective extension programmes.

Problems and needs assessment refers to the process of identifying problems and needs and placing them in some order of priority. There are several approaches, quantitative and qualitative methods and techniques for problems and needs assessment. The most frequently used methods and techniques were reviewed and described by Abdel-Maksoud (2008 and 2010). The quantitative methods for needs assessment are based on measuring individuals' perception and evaluation of their knowledge, skills or abilities, attitudes, achievement, and the degree of importance of particular items. Data are gathered and processed, and items are ranked according to individuals' evaluation of their level of knowledge, skills, and the degree of importance of each item. They can be ranked also according to the discrepancy between importance and knowledge and the distribution of respondents on two dimensions (importance x knowledge or importance x achievement).

In 1980, Borich developed the following equation for training needs assessment:

Training need = (Importance – Knowledge) Average Importance

In 1984, Misanchuk developed Delta N statistic (Misanchuk, 1984). This method was employed by Pigg et al. (1995). Delta N can be computed by the following Formula:

$$\text{Delta N} = 1 - \frac{\sum_{i=1}^{R}\sum_{j=1}^{C} Wij\ Pij}{\sum_{i=1}^{R}\sum_{j=1}^{C} Wij\ Pi\ Pj}$$

Where Wij is the error weight for cell (i,j), Pij is the probability of a randomly sampled observation falling into cell (i,j), and Pi and Pj are the expected marginal probabilities for rows and columns respectively.

The method of computation of Delta N involves establishing cell values following the "proportionate reduction in error" approach developed for the analysis of cross-classified ordinal data. This approach predicts the probability of occurrence of certain combinations of joint distribution. The method is well explained in detail by Misanchuk (1984 and 1987). Suggested values for error weights for Delta N computation are shown in Appendix (1).

A modified Delta N method was developed by Abdel-Maksoud to avoid some drawbacks of Delta N and simplify its computation (Abdel-Maksoud, 2010). The modified Delta N can be computed by the following equation:

$$\text{The Modified Delta N} = 1 - \sum_{i=1}^{R}\sum_{j=1}^{C} Wij\ Pij$$

To understand the computation method of Delta N and the modified Delta N, the reader is referred to Abdel-Maksoud, 2010.

In addition to the above methods and techniques, there are several other methods which can be used for data collection and problems and needs assessment. Among these methods are: Delphi Technique, Scaled comparison, Key informants and supervisors interviews, Focus groups, Nominal groups, Observation (Formal and Informal personal observation), Meeting with individuals, and Informal group methods (Abdel-Maksoud, 2008). It is recommended to apply more than one method to assess problems and needs.

Objectives:

The main objectives of this paper were to:

1. Identify production and marketing problems facing sugarcane growers in Qena and Asswan governorates, Egypt.
2. Assess the identified problems using different methods and techniques of needs assessment.
3. Examine spatial differentials of perceived production and marketing problems among farmers in different villages i Qena and Asswan governorates.

Methodology:

In order to achieve the above objectives, an empirical research was conducted in three districts in Qena and Asswan governorates (two districts in Qena and one district in Asswan). Two villages were selected from the villages in each district. Focus groups of farmers were organized and held in one of these two selected villages, and a survey on a sample of farmers was conducted in the other village. The three districts, the six villages and the sample of farmers were randomly selected.

Nine focus groups were held in the three villages (three focus groups in each village). The total number of farmers participated in these focus groups was 91 farmers. Data gathered from members of these focus groups were used to identify production and marketing problems facing sugarcane growers in the research area. Nineteen production problems and sixteen marketing problems were identified by members of these focus groups. But the total number of problems included in this research was 27 production problems and 19 marketing problems as some other problems of which were identified in previous research (Abdel-Maksoud &,Elshrabassee, 2007 and Abdel-Maksoud, 2008) were added.

The total number of completed questionnaires from farmers was 262. Data were collected from sample members by means of personal interview using a questionnaire form prepared for this purpose. Table (1) gives some information of the cultivated area, the area of sugarcane, the number of farmers who participated in the focus groups, and the number of completed questionnaires from farmers in each village.

To assess the identified sugarcane production and marketing problems, farmers' evaluation of the degree of importance of each, their evaluation of evaluation of achievements or efforts devoted to solve each problem, the discrepancy between importance and achievement, Borich model, Delta N method, and the Modified Delta N method were applied. The top ten problems in each village were determined according to ranking results of Delta N and the Modified Delta N methods.

Results and Discussion:

Results of this research can be presented as follows:

First: Characteristics of farmers included in the

research:

1. Members of focus groups:

The total number of farmers who participated in focus groups was 91 farmers. Their ages ranged from 20 years to over 60 years and their level of education varied and included some illiterate farmers, some others who were able to read and write, and others who held preparatory, diploma of secondary, or a university degree. Most members were mainly farmers, others have taken agriculture as a secondary occupation. Their agricultural land holdings, and the area they grew of sugarcane ranged from less than one feddan to more than five feddans.

Table (1): Information concerning the research area

Govrernorate	District	Village	Cultivated area (feddan)	Area of Sugarcane (fedd.)	Total Number of farmers	Number of sugarcane growers	Method of data collection	Number Farmers paticipated
Qena	Qena	Elshaikh Eissa	875	337	630	300	Focus groups	32
		Alashraaf	4073	1011	750	270	Questionnaire	71
	Nagaa Hammady	Alraeiciah	2150	2058	2636	1600	Focus groups	27
		Alhefnawiah	769	672	800	745	Questionnaire	91
Asswan	Komombo	Alsabeil	4372	2600	1617	1507	Focus groups	31
		Kagog	904	322	301	301	Questionnaire	100

Source: Agricultural co-operative associations of the three villages.

2. Sample members:

Their ages ranged from less than 30 years to more than 70 years, and the majority of them were married living in families consisting of five to eight members or more. Their level of education varied from illiterate (15 %) to holding a university degree (6.5 %). But a large proportion of them (45.4 %) knew how to read and write. Agriculture was the principal occupation for most sample members (59.5 %), and over one quarter of them (26 %) were governmental employees. Most sample members (55.7 %) did not have any secondary occupation. Most of them (56 %) had less than two feddans of agricultural land, and 22 % of them had from five to ten feddans or more. Nearly three quarters of them grew less than two feddans of sugarcane, and 12.6 % of them grew five to ten feddans or more (Table 2).

Second: Identification of problems:

As stated before, the total number of problems included in this research was 27 production problems and 19 marketing problems. Table (3) includes a list of these identified production and marketing problems.

Third: Assessment of the identified problems:

In order to assess the identified problems in this research, six assessment methods and techniques were adopted. These are: importance, achievement, the discrepancy between importance and achievement, Borich model, Delta N method, ad the modified Delta N method. The adoption of these assessment methods has revealed to the following results:

1. Most assessment methods adopted gave similar ranking for most problems included in this research (Table 4).
2. There is a complete and positive correlation between Delta N and the Modified Delta N methods. Ranking results according to these two methods were used to determine the top ten problems in the three villages included in this research.
3. Marketing problems have dominated the top priorities of the identified problems.

   Among the top ten problems appeared in the three villages, there were seven marketing problems and three production problems in two villages (Alashraaf and Alhefnawiah villages in Qena governorate), and eight marketing problems and only two production problems in the village of Asswan governorate (Kagog). These problems were problems number: 45, 39, 37, 33, 42, 14, 41, 46,10, and 13 in Qena villages (Alashraaf and Alhefnawiah), ad problems number 41, 37, 39, 40, 42, 21, 43, 10, 45, and 44 in Asswan village (Kagog) (Table 4).

Table (2) Distribution of sample members according to their characteristics

Characteristics	Alhefnawiah	Alashraaf	Qagog	Total	%
1. Age:					
- Less than 30 years	6	-	-	6	2.3
- 30 -	16	7	7	30	11.4
- 40 -	22	19	30	71	27.1
- 50 -	9	18	23	50	19.1
- 60 -	21	16	30	67	25.6
- 70 or more	17	11	10	38	14.5
2. Marital status:					
- Single	2	1	2	5	1.9
- Married	85	65	94	244	93.1
- Widow	4	5	4	13	5.0
3. Family size:					
- Less than 5	38	12	22	72	27.5
- 5 – 7	43	32	56	131	50.0
- 8 or more	10	27	22	59	22.5
4. Education:					
- Illiterate	18	19	2	39	14.9
- Read & Write	32	21	66	119	45.4
- Primary and Preparatory	-	8	3	11	4.2
- Secondary	19	19	22	60	22.9
- Above average	8	3	5	16	6.1
- University	14	1	2	17	6.5
5. Principal occupation:					
- Farmer	45	54	57	156	59.5
- Employee	30	13	25	68	26.0
- Merchant	8	1	6	15	5.7
- Worker	8	3	12	23	8.8
6. Secondary occupation:					
- None	44	53	49	146	55.7
- Farmer	46	17	43	106	40.5
- Merchant	-	1	5	6	2.3
- Others	1	-	3	4	1.5
7. Agricultural land holding:					
- Less than one feddan	14	4	38	56	21.4
- 1 -	56	7	54	117	34.7
- 3 -	11	16	1	28	10.7
- 5 -	9	27	5	41	15.6
- 10 feddans or more	1	17	2	20	7.6
8. Area of sugarcane:					
- Less than one feddan	18	9	71	98	37.4
- 1 -	58	16	23	97	37.0
- 3 -	10	21	3	34	13.0
- 5 -	4	16	2	22	8.4
- 10 feddans or more	1	9	1	11	4.2

Source: Questionnaire forms

4. Low price of product and inaccuracy of weigh (problems number 37 and 39) were among the top three problems in the three villages.

5. Ranking results were similar in the two villages of Qena governorate, but there were obvious differences between the ranks in Qena villages and

Asswan village. While problems number: 13, 14, 33, and 46 appeared among the top ten problems in Qena villages and did not appear among the top ten problems in Asswan village, problems number: 21, 40, 43, and 44 appeared among the top ten problems in Asswan village and did not appear among the top ten problems in Qena villages.

6. In spite of similarities between the problems appeared among the top ten problems in the three villages, there were obvious differences in their ranking in the two governorates.

Table (3): Identified sugarcane production and marketing problems

Production problems	Marketing Problems
1. Shortage and high costs of fertilizers	28. Shortage and high wages of labour
2. Shortage and high costs of labour	29. Shortage of and irregular transportation means
3. Differences in planting dates	30. High costs of transportation
4. Non-growing in aggregates	31. Frequent accidents by tractors
5. Non-adoption of soil assessment	32. Unsystematic cutting
6. Non-adoption of subsoil ploughing	33. High costs of cutting
7. Non-adoption of levelling by laser	34. Delay of cutting
8. Non-adoption of recommended furrowing rate	35. Long period of cutting
9. Differences in crop rotation	36. Long period of non-irrigation of crop
10. Non-availability of new varieties	37. Low price of product
11. late planting	38. Steal of product during transportation
12. Shortage of irrigation water	39. Inaccuracy of weigh
13. High costs of irrigation	40. Long distance from factory
14. High costs of petroleum Products	41. The contract is controlled by the company
15. Over application of nitrate fertilizers	42. Delay of getting the value of product
16. Non-availability of phosphate fertilizers	43. High interest rate of loans
17. Spread of insects & diseases	44. High added expenses to loans
18. Spread of weeds	45. High ratio of defects
19. Over irrigation	46. Misuse of discounts from the value of product.
20. Non-cleaning of irrigation and drainage canals	
21. Small and fragmented holdings	
22. Non-availability of harvesting Machines	
23. Low productivity of C9 variety	
24. Non-availability of calcium sulphate	
25. Shortage of insecticides	
26. Weak extension services	
27. Spread of mice	

Source: Focus groups and previous research.

Table (4) Ranks of sugarcane problems in the three villages according to the results of different assessment methods*

Problem	Alashraaf village						Alhefnawiah village						Kagog village					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1	15.5	25	24.5	24	24	24	7.5	24.5	16	14	24	24	4	28	21	17	24	24
2	11	22.5	19	17	18	18	4.5	13	7	7	18	18	9.5	20	16	15	17	17
3	38.5	40	41	41	40	40	31.5	24.5	26	26	40	40	22	38	37	37	37	37
4	40.5	44.5	43	43	44	44	44	27	33	35	44	44	38	35	34	33	35	35
5	33	11	13	14	15	15	46	6	19	21	15	15	43.5	14	23	26	22	22
6	21	36	34	34	37	37	27	18	21	20	37	37	35	19	25	27	25	25
7	36.5	33	33	33	34	34	24.5	12	15	16	34	34	31.5	25.5	27.5	29	26	26
8	33	41	40	40	41	41	37	34	41	42	41	41	30	44	41	41	43	43
9	30	31.5	30	30	31	31	45	29	37	38	31	31	42	42	42	42	42	42
10	24.5	3	8	9	9	9	39	1	3	4	9	9	12	9	7	7	8	8
11	27.5	34.5	35	35	35	35	33.5	36	43	43	35	35	21	40	38	38	38	38
12	19	17	18	19	17	17	19.5	44	42	41	17	17	26	46	45	45	46	46
13	11	9	10	10	10	10	17	40	35.5	36	10	10	38	41	40	40	40	40
14	9	4.5	6	6	6	6	22	5	5	6	6	6	45	18	33	35	33	33
15	44	42	42	42	42	42	21	46	46	46	42	42	33.5	39	39	39	39	39
16	30	26	27	27	27	27	31.5	22	23	23	27	27	33.5	45	43	43	41	41
17	13.5	18.5	17	16	16	16	14	14	12.5	13	16	16	9.5	30	27.5	25	28	28
18	13.5	16	15	15	14	14	11	17	10	12	14	14	7.5	30	26	24	29	29
19	42.5	44.5	44	44	43	43	42	37	44	44	43	43	38	43	44	44	44	44
20	38.5	38	38	38	38	38	17	32	28	29	38	38	31.5	37	36	36	36	36

Table (4) Continued: Ranks of sugarcane problems in the three villages according to the results of different assessment methods

Problem	Alashraaf village						Alhefnawiah village						Kagog village					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
21	27.5	14	16	18	19	19	43	7	18	19	19	19	7.5	6.5	6	6	6	6
22	21	12	12	12	13	13	33.5	15.5	22	22	13	13	36	13	15	20	13	13
23	30	24	24.5	25	26	26	23	28	27	25	26	26	13.5	15	13	11	14	14
24	42.5	29.5	32	32	30	30	26	15.5	20	18	30	30	40	6.5	10	14	11	11
25	24.5	31.5	29	29	29	29	13	41	31	31	29	29	23.5	32	30	30	30	30
26	24.5	37	36	36	33	33	12	45	35.5	34	33	33	26	33.0	31	31	31	31
27	17	28	26	26	25	25	9	19.5	9	9	25	25	16	25.5	22	22	23	23
28	15.5	22.5	20	20	20	20	3	26	11	8	20	20	11.0	24	18	18	20	20
29	33	39	39	39	39	39	7.5	39	29	28	39	39	41	30	32	32	32	32
30	8	13	11	11	11	11	4.5	23	12.5	10	11	11	43.5	34	35	34	34	34
31	46	46	46	46	46	46	17	38	34	33	46	46	46	36	46	46	45	45
32	36.5	27	28	28	28	28	35	33	38	37	28	28	15	23	19	19	19	19
33	4	6	4	4	4	4	6	8.5	6	5	4	4	13.5	16	14	12	15	15
34	18	21	22	21	23	23	28.5	31	32	32	23	23	19	22	20	21	18	18
35	21	20	23	23	22	22	40	42	45	45	22	22	26	21	24	23	21	21
36	24.5	18.5	21	22	21	21	41	30	39	40	21	21	17	17	17	16	16	16
37	1	4.5	3	3	3	3	1	4	2	2	3	3	3	3	2	2	2	2
38	45	43	45	45	45	45	19.5	43	40	39	45	45	28.5	27	29	28	27	27
39	2	2	2	2	2	2	2	2	1	1	2	2	2	4	3	3	3	3
40	11	15	14	13	12	12	15	35	30	30	12	12	6	2	4	4	4	4

Table (4) Continued: Ranks of sugarcane problems in the three villages according to the results of different assessment methods

Problem	Alashraaf village						Alhefnawiah village						Kagog village					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
41	7	8	7	7	7	7	30	11	17	17	7	7	1	1	1	1	1	1
42	5	7	5	5	5	5	24.5	8.5	8	11	5	5	5	5	5	5	5	5
43	35	34.5	37	37	36	36	36	21	25	27	36	36	19	8	8	8	7	7
44	40.5	29.5	31	31	32	32	38	19.5	24	24	32	32	23.5	11	11	10	10	10
45	3	1	1	1	1	1	10	3	4	3	1	1	19	10	9	9	9	9
46	6	10	9	8	8	8	28.5	10	14	15	8	8	28.5	12	12	13	12	12

Source: Determined from data in Appendix (2).

*1 = Ranking according to mean importance, 2 = " '' '' " achievement,

3 = " '' '' (Importance x Achievement), 4 = " '' '' Borich value,

5 = " '' '' Delta N value, 6 = " '' '' The Modified Delta N value

Conclusion:

Based on the above results, it can be concluded that sugarcane growers in Qena and Asswan governorates face various problems. Marketing problems are the most perceived problems. Spatial differences exist among farmers in these two governorates in their perception and evaluation of sugarcane production and marketing problems. The extension system should be aware of such differences and plan its extension programmes and activities on the basis of problems identification and assessment in each area using effective and precise assessment methods and techniques.

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Appendix (1): Suggested Error Weights for Computing Delta N

	Importance*				
Achievement*	1	2	3	4	5
1	0.7071	0.5303	0.3536	0.1768	0.000
2	0.7289	0.5590	0.3953	0.2500	0.1768
3	0.7906	0.6374	0.500	0.3953	0.3536
4	0.8839	0.7500	0.6374	0.5590	0.5303
5	1.000	0.8839	0.7906	0.7289	0.7071

Source: Misancuk, 1984: 30.

- Both importance and achievement are measured on a five point Likert-type scale ranging from 1 (very low) to 5 (very high). Values in the body of the Table show the error weigh. If all respondents fall in the cell (1,5) where their level of competence is very low and the degree of importance of the item is very high, the error will equal zero, and if all respondents fall in the cell (5,1) where their level of competence is very high and the degree of importance is very low, the error will equal one. The error weights increase as one moves through any direction from cell (1,5) t0 cell (5,1)

Appendix (2): Results of the application of different assessment methods on sugarcane production and marketing problems*

Problem	Alashraaf village						Alhefnawiah village						Kagog village					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1	4.07	2.14	1.93	7.86	5007.	7199.	4.04	2.79	1.25	5.07	.5007	7199.	4.53	2.76	1.77	8.02	4057.	6666.
2	4.13	1.99	2.14	8.84	5415.	7428.	4.08	2.57	1.51	6.14	5415.	7428.	4.37	2.48	1.89	8.26	4474.	6900.
3	3.90	2.91	99.	3.86	2756.	5936.	3.57	2.79	78.	2.79	2756.	5936.	4.23	3.66	57.	2.41	1009.	4956.
4	3.86	3.13	73.	2.82	2078.	5556.	3.32	2.97	35.	1.17	2078.	5556.	4.01	3.07	94.	3.77	2075.	.5554
5	3.94	1.51	2.43	9.57	5742.	7611.	3.20	2.11	1.09	3.48	5742.	7611.	3.90	2.19	1.71	6.67	4175.	6732.
6	4.00	2.65	1.35	5.40	3547.	6380.	3.68	2.67	1.01	3.72	3547.	6380.	4.08	2.47	1.61	6.57	3870.	6561.
7	3.92	2.54	1.38	5.41	3595.	6407.	3.73	2.46	1.26	4.71	3595.	6407.	4.11	2.56	1.55	6.37	3783.	6512.
8	3.94	2.94	1.00	3.94	2724.	5918.	3.51	3.32	19.	65.	2724.	5918.	4.15	3.90	25.	1.04	0030.	4407.
9	3.96	2.38	1.58	6.26	4050.	6662.	3.30	3.05	24.	80.	4050.	6662.	3.96	3.78	18.	71.	0066.	4427.
10	3.99	1.17	2.82	11.25	6520.	8048.	3.47	1.18	2.30	7.98	6520.	8048.	4.35	2.00	2.35	10.22	5561.	7510.
11	3.97	2.63	1.34	5.32	3585.	6401.	3.55	3.40	15.	55.	3585.	6401.	4.24	3.69	55.	2.33	0766..	4820.
12	4.01	1.86	2.15	8.62	5421.	7431.	3.80	3.63	18.	67.	5421.	7431.	4.19	4.19	00.	00.	-.068	4008.
13	4.13	1.42	2.71	11.19	6462.	8015.	3.81	3.52	30.	1.13	6462.	8015.	4.01	3.74	27.	1.08	0412.	4621.
14	4.15	1.20	2.95	12.24	6984.	8308.	3.75	1.95	1.80	6.75	6984.	8308.	3.43	2.46	97.	3.33	2276.	5667.
15	3.83	3.04	79.	3.03	2230.	5641.	3.79	4.07	-.27	-1.0	2230.	5641.	4.09	3.68	41.	1.68	0651.	4755.
16	3.96	2.17	1.79	7.09	4633.	6989.	3.57	2.75	82.	2.94	4633.	6989.	4.09	3.92	17.	70.	0171.	4486.
17	4.10	1.92	2.18	8.94	5624.	7545.	3.90	2.59	1.31	5.10	5624.	7545.	4.37	2.82	1.55	6.77	3590.	6404.
18	4.10	1.85	2.25	9.22	5806.	7647.	3.98	2.66	1.32	5.25	5806.	7647.	4.39	2.82	1.57	6.89	3570	6393.
19	3.85	3.13	72.	2.77	2096.	5566.	3.38	3.45	-.07	-.22	2096.	5566.	4.01	3.88	13.	52.	-.011	4330.
20	3.90	2.70	1.20	4.68	3355.	6272.	3.81	3.25	.56	2.14	.3355	.6272	4.11	3.33	78.	3.21	1695.	5341.

Appendix (2) Continued: Results of the application of different assessment methods on sugarcane production and marketing problems

Problem	Alashraaf village						Alhefnawiah village						Kagog village					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
21	3.97	1.76	2.21	8.77	5387.	7412.	3.35	2.23	1.12	3.76	5387.	7412.	4.39	1.90	2.49	10.93	6037.	7777.
22	4.00	1.55	2.45	9.80	5886.	7692.	3.55	2.62	93.	3.32	5886.	7692.	4.06	2.16	1.90	7.71	4816.	7092.
23	3.96	2.03	1.93	7.64	4840.	7105.	3.74	2.99	75.	2.79	4840.	7105.	4.34	2.33	2.01	8.72	4813.	7090.
24	3.85	2.34	1.51	5.81	4078.	6678.	3.69	2.62	1.08	3.98	4078.	6678.	4.00	1.90	2.10	8.40	5057.	7227.
25	3.99	2.38	1.61	6.42	4308.	6807.	3.95	3.55	40.	1.56	4308.	6807.	4.20	2.83	1.37	5.75	3405.	6300.
26	3.99	2.66	1.33	5.31	3645.	6435.	3.97	3.67	30.	1.18	3645.	6435.	4.19	2.87	1.32	5.53	3232.	6203.
27	4.06	2.23	1.83	7.43	4872.	7123.	4.02	2.68	1.34	5.39	4872.	7123.	4.30	2.56	1.74	7.48	4111.	6696.
28	4.07	1.99	2.08	8.47	5362.	7398.	4.12	2.80	1.32	5.43	5362.	7398.	4.36	2.55	1.81	7.89	4296.	6800.
29	3.94	2.85	1.09	4.29	3123.	6142.	4.04	3.49	55.	2.22	3123.	6142.	3.99	2.82	1.17	4.67	2952.	6046.
30	4.18	1.68	2.50	10.45	6057.	7788.	4.08	2.77	1.31	5.33	6057.	7788.	3.90	3.00	90.	3.51	2128.	5584.
31	3.69	3.27	42.	1.55	1504.	5234.	3.81	3.47	34.	1.30	1504.	5234.	3.01	3.30	-.29	-.87	-.050	4110.
32	3.92	2.21	1.71	6.70	4428.	6874.	3.53	3.30	.23	.81	.4428	.6874	4.33	2.52	1.81	7.84	4364.	6838.
33	4.35	1.23	3.12	13.57	7553.	.8627	4.07	2.31	1.76	7.15	7553.	8627.	4.34	2.40	1.94	8.42	4692.	7022.
34	4.03	1.97	2.06	8.30	5155.	.7282	3.63	3.24	38.	1.39	5155.	7282.	4.28	2.50	1.78	7.62	4367.	6840.
35	4.00	1.94	2.06	8.24	5180.	.7296	3.46	3.57	-.11	-.38	5180.	7296.	4.19	2.49	1.70	7.12	4273.	6787.
36	3.99	1.92	2.07	8.26	5248.	.7334	3.40	3.18	22.	75.	5248.	7334.	4.29	2.42	1.87	8.02	4645.	6996.
37	4.55	1.20	3.35	15.24	7927.	.8837	4.18	1.86	2.32	9.68	7927.	8837.	4.56	1.67	2.89	13.18	7080.	8362.
38	3.70	3.06	64.	2.37	2070.	.5551	3.80	3.60	20.	75.	2070.	5551.	4.16	2.62	1.54	6.41	3754.	6496.
39	4.54	1.14	3.40	15.44	8255.	.9021	4.14	1.54	2.60	10.79	8255.	9021.	4.58	1.71	2.87	13.14	7043.	8341.
40	4.13	1.77	2.36	9.75	5893.	.7696	3.86	3.34	52.	1.99	5893.	7696.	4.41	1.65	2.76	12.17	6717.	8158.

Appendix (2) Continued: Results of the application of different assessment methods on sugarcane production and marketing problems

Problem	Alashraaf village						Alhefnawiah village						Kagog village					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
41	4.24	1.41	2.83	12.00	6904.	.8265	3.59	2.40	1.20	4.30	6904.	8263.	4.60	1.42	3.18	14.63	7809.	8771.
42	4.34	1.37	2.97	12.89	7207.	.8433	3.73	2.31	1.42	5.28	7207.	8433.	4.44	1.78	2.66	11.81	6492.	8032.
43	3.93	2.63	1.30	5.11	3561.	.6388	3.52	2.73	79.	2.78	3561.	6388.	4.28	1.99	2.29	9.80	5670.	7571.
44	3.86	2.34	1.52	5.87	4012.	.6641	3.48	2.68	80.	2.79	4012.	6641.	4.20	2.11	2.09	8.78	5191.	7302
45	4.52	1.10	3.42	15.46	8258.	.9023	3.99	1.75	2.24	8.94	8258.	9023.	4.28	2.05	2.23	9.54	5515.	7484.
46	4.28	1.50	2.78	11.90	6863.	.8240	3.63	2.32	1.31	4.74	6863.	8240.	4.16	2.14	2.02	8.40	5030.	7212.

Source: Calculated from data collected by questionnaires

*1 = Mean importance,

2 = " achievement,

3 = Importance x Achievement

4 = Borich value,

5 = Delta N value,

6 = The Modified Delta N value

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Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs

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Abstract: The aim of this work was to investigate the possibility of employing semi-covalent molecularly imprinted polymers (MIPs) as a controlled release device for ibuprofen and naproxen in biological fluids, especially gastrointestinal ones, compared to non imprinted polymers (NIPs). The carboxyl groups of ibuprofen and naproxen were converted to vinyl ester group by reacting ibuprofen and vinyl acetate as an acylating agent in the presence of catalyst. The semi-covalent molecularly imprinted polymers (MIPs) were synthesized by free radical polymerization of vinyl esters derivatives of ibuprofen and naproxen in the presence of methacrylic acid and ethyleneglycol dimethacrylate (EGDMA) as the functional monomer and cross-linker, respectively. The composition of the cross-linked three-dimensional polymers was determined by FTIR spectroscopy. The hydrolysis of drug polymer conjugates was carried out in cellophane membrane dialysis bags and the in vitro release profiles were established separately in enzyme-free simulated gastric and intestinal fluids (SGF, pH 1 and SIF, pH 7.4). Detection of hydrolysis solution by UV spectroscopy at selected intervals showed that the drug can be released by hydrolysis of the ester bond between the drug and polymer backbone in low rate.
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Key words: molecularly imprinted polymer, pH-sensitive, anti-inflammatory drugs, sustained release.

1. Introduction

Molecular imprinting is an efficient technique for the introduction of regions with highly specific molecular arrangements into polymeric matrices [1, 2]. The first example of a molecularly imprinted polymer (MIP) was reported half a century ago, however it is only in the last decade that the use of molecular imprinting as a practical tool became established [3, 4]. MIPs were used as chromatographic stationary phases [5], for enantiomeric separation [6] and for Solid-Phase Extraction (SPE) [7] and also as receptors [8], antibody [9] and enzyme mimic [10]. In addition, in the last years, MIPs have been reported to be suitable as drug delivery systems (DDS) [11-15], as base excipients for controlled release devices of drugs with a narrow therapeutic index.

There are three different approaches to prepare MIPs: covalent (pre-organized approach), non-covalent (self-assembly approach) and semi-covalent approach. The covalent or pre-organized approach, involves the formation of reversible covalent bonds between the template and monomers before polymerization. Then the template is removed by cleavage of the covalent bonds, which will be re-formed upon rebinding of the target molecule. The semi-covalent approach is an intermediate option, where the template is covalently bound to a functional monomer, but the rebinding is based on non-covalent interactions. The non-covalent or self-assembly approach is based on the formation of relatively weak non-covalent interactions (e.g. hydrogen bonding, electrostatic interaction, hydrophobic interaction, Vander Waals forces and dipole-dipole bonds) between the template molecule and functional monomers before polymerization. The association and disassociation of the imprint occurs by plain diffusion in and out of the sites [16].

The ibuprofen and naproxen is non-steroid anti-inflammatory drug (NSAIDs) and are widely used for the treatment of rheumatoid arthritis. But, the use of NSAIDs is also limited by their irritant side effects on the gastro-enteric mucous and by their frequent poor water solubility. These problems can be solved by the preparation of polymeric prodrug backbones *via* hydrolyzable bonds. Polymer-drug conjugates of NSAIDs have been developed in order to minimize delivery problems and reduce gastrointestinal side effects by controlling the rate, duration, and site of release. These polymeric prodrugs have been designed for localized and prolonged duration of drug action by parental administration, or as dermal prodrugs [17].

In this work a new potential polymeric device, based on semi-covalent MIPs, for the sustained release of anti-inflammatory drugs are described. The vinyl ester type derivative of ibuprofen (VIP) and naproxen (VIN) was first synthesized by reacting ibuprofen and naproxen with vinyl acetate in the presence of mercuric acetate. The semi-covalent molecularly imprinted polymers (MIPs) were synthesized by free radical polymerization of VIP and VIN in the presence of methacrylic acid and ethyleneglycol dimethacrylate (EGDMA) as the functional monomer and cross-linker, respectively. The release of drugs from the obtained drug delivery vehicles was carried out in vitro by hydrolysis in buffered solutions at various pH values

and the quantity of the released drug detected by UV spectroscopy. Considerable differences in the capacity of the polymers to recognize and to bind the template selectively between imprinted and non imprinted polymers (NIPs) have been observed.

2. Experimental

2.1 Materials

The vinyl ester derivative of ibuprofen (VIP) and naproxen (VIN) were prepared by the method described in the literature [18]. Ibuprofen, naproxen and ethyleneglycol dimethacrylate (EGDMA) were purchased from Aldrich chemical company. Mercuric acetate, vinyl acetate, sodium acetate and MMA were obtained from Merck chemical company and were purified by distillation under vacuum. Azoisobutyronitrile (AIBN) was obtained from Fluka chemical company and recrystallized from methanol. Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the US Pharmacopeia [19].

2.2 Instrumental measurements

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker 400 AC spectrometer in $CDCl_3$. The IR spectra were recorded on a Shimadzu FT IR-408 spectrophotometer. The amount of released ibuprofen and naproxen was determined by a Philips PU 8620 UV spectrophotometer at the maximum adsorption of the free drug in aqueous buffered solutions using a 1-cm quartz cell.

2.3 Preparation of vinyl ester derivative of ibuprofen (VIP) and naproxen (VIN)

The amount of (12.6 mmol) of drugs and 0.3 g of mercuric acetate were dissolved in 30 ml of vinyl acetate and stirred for 30 min at room temperature. Then, 0.2 ml of concentrated sulfuric acid was added into the solution and refluxed for about 3 h. After this time, the solution was cooled to room temperature and 1.0 g of sodium acetate was added to quench the catalyst. The solution was filtered, concentrated and the crude product was then purified by silica gel column chromatography by eluting with petroleum ether/ethyl acetate (30:1, v/v) to give about (85%) of VIP and VIN as a colorless liquid (Figure 1).

For VIP:

FT-IR (KBr, cm^{-1}) 3050 (C-H aromatic and vinylic), 2890 (C-H aliphatic), 1740 (C=O ester), 1600, 1480 (C=C).

^{1}H NMR ($CDCl_3$, ppm) 0.8 (d, 6H, $-CH(CH_3)_2$), 1.55 (d, 3H, $-ArCHCH_3$), 1.9 (m, 1H, $-CHMe_2$), 2.5 (d, 2H, Ar-CH_2-), 3.8 (q,1H, Ar-CH-), 4.5 (dd, 1H, CH_2=C), 4.9 (dd, 1H, CH_2=C), 7.0-7.27 (q, 4H, aryl-H), 7.3-7.34 (q, 1H, CH_2=CH).

^{13}C NMR ($CDCl_3$, ppm) 20 (1C, $-CH-CH_3$), 21 (2C, $-CH(CH_3)_2$), 22 (1C, $-CHMe_2$), 30 (1C, Ar-CH_2-), 45 (1C, $-CH-CH_3$), 125 (1C, CH_2=CH-), 155 (1C, CH_2=CH-), 126, 129, 138, 140 (6C, aromatic carbons), 172 (1C, C=O).

For VIN:

FT-IR (KBr, cm^{-1}) 3050 (C-H aromatic and vinylic), 2890 (C-H aliphatic), 1750 (C=O ester), 1644, 1480 (C=C).

^{1}H NMR ($CDCl_3$, ppm) 1.64 (3H, d, CH_3), 2.11 (1H, m, C_6H_4CH), 3.94 (3H, t, CH_3O), 4.58 (1H, dd, CH_2=C), 4.88 (1H, dd, CH_2=C), 7.29 (1H, dd, CH_2=CH), 7.38–7.15 (6H, m, ArH).

^{13}C NMR ($CDCl_3$, ppm) 18.8, 45.6, 55.7 (aliphatic carbons), 126.5 (1C, CH_2=CH-), 158.1 (1C, CH_2=CH-), 98.3, 106, 119.5, 127.7, 129.3, 129.7, 134.3, 135.2, 141.8 (aromatic carbons), 172.1 (1C, C=O).

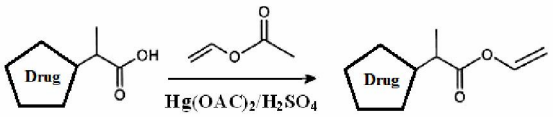

Figure 1. The synthesis route of vinyl ester type derivatives of ibuprofen and naproxen.

2.4 Synthesis of ibuprofen and naproxen molecularly imprinted polymer: (MIPs 1-4)

Methacrylic acid was used as the functional monomer to prepare the MIP by the semi-covalent imprinting method. Briefly, templates, methacrylic acid, EGDMA and AIBN with a variable feed ratio as shown in Table 1 were dissolved in acetonitrile (20 mL) in a thick-walled glass tube. The obtained solution was purged with nitrogen and sonicated for 10 min. The mixture was then incubated under a nitrogen atmosphere at 70 °C for 48 h. The resultant bulk rigid polymer systems were crushed, grounded into powder and sieved through a 63 µm stainless steel sieve. Reference NIPs matrices (acting as a control) were prepared under the same conditions without using the template. FTIR (KBr): 3380-2500 (broadened, -COOH group), 1730, 1705, 1245, 1225 cm^{-1}.

Table 1. Composition of molecular imprinted polymers and percentage of particles adhered onto rat intestine

MIPs	Molar composition of monomers in the feed				Percentage adherence
	VIP	NIP	MAA	CA	
MIP-1	1	--	6	30	61
MIP-2	1	--	10	30	71
MIP-3	--	1	6	30	64
MIP-4	--	1	10	30	69

2.5 Extraction of the templates from the polymer matrix: (MIP′s 1-4)

The resultant MIP materials were extracted with 100 mL mixtures of CH_3OH: NaOH (1:3, 1N) solution at 70 °C for at least 20 day and the solution were changed every
24 hours in order to remove any template. The washed MIPs (MIP′s) materials were checked to be free of drugs and any other compound by HPLC analysis. The extracted MIP′ materials were dried overnight in an oven at 60 °C.

2.6 Binding experiments

The binding experiments were performed, at room temperature in aqueous media (water solution pH 7). The sieved MIP′ and NIP particles (50 mg) were placed in 10 mL of ibuprofen or naproxen (1 mg.ml-1) for 240 min. Then the mixture was centrifuged for 10 min and the drug concentration in the liquid phase was measured by UV–VIS spectroscopy. The difference between the amount of drug initially employed and the drug content in the liquid phase is taken as an indication of the amount of drug entrapped. The amount of drugs bound to the polymer matrix was obtained by gentle washing of MIP′ and NIP for remove of any surface absorbance drug and comparing the drug concentration in the MIP′ samples and in the NIP ones. Experiments were repeated five times. The amounts of binding experiments are given in Table 2.

Table 2. Percentage of bound drugs by the imprinted and non-imprinted polymers after 240 min, in aqueous media (pH 7)

MIPs′	SA	DB	NIPs	SA	DB
MIP′-1	21	79	NIP-1	39	61
MIP′-2	16	84	NIP-2	66	34
MIP′-3	13	87	NIP-3	45.5	54.5
MIP′-4	30	70	NIP-4	40	60

Surface absorbance: SA (%)
Drugs bound: DB (%)

2.7 Drug Loading by the Soaking Procedure

The MIP′ and NIP particles (2.0 g) was immersed in a drug solution in water (20 mL, 5.5 mM) and soaked for 1 day at room temperature. During this time, the mixture was continuously stirred and then the solvent was removed by filtration. Finally the powder was dried under vacuum overnight at 40°C.

2.8 In vitro release studies

The MIP, MIP′ and NIP (50 mg) were poured into 3 mL of aqueous buffer solution (SGF: pH 1 or SIF: pH 7.4). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 20 mL of the same solution maintained at 37° C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The volume removed was replaced with SGF or SIF. Triplicate samples were used. The sample of hydrolyzate was analyzed by UV spectrophotometer (ibuprofen: (λmax=264 nm) and naproxen: (λmax=315 nm)), and the quantity of drugs was determined using a standard calibration curve obtained under the same conditions.

2.9 In situ Bioadhesivity Studies

Bioadhesivity testing was done by a novel in situ method as described by Ranga Rao and Buri [20]. A freshly cut 5-6 cm long piece of small intestine of rat was obtained and cleaned by washing with isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of MIPs were added evenly on the mucosal surface. The intestinal piece was maintained at 80% relative humidity for 30 mts in a desiccator. The piece was taken out and phosphate buffer pH 6 was allowed to flow over the intestinal piece for about 2 mts at a rate of 20 ml/min. The perfusate was collected and dried to get the particles not adhered. The percent of bioadhesion was estimated by the ratio of amount applied to adhere MIPs. The values are given in Table 1.

3. Results and discussion

Yang et al. [21] and Cai et al. [22] have already reported a method for conversion of carboxylic acids to the related vinyl ester by using vinyl acetate as an acylating agent. In this present work, ibuprofen and naproxen reacted with vinyl acetate in the presence of mercuric acetate as a catalyst, and the related vinyl esters were collected in high yield after purification by column chromatography. The resultant FT-IR and ^{1}HNMR spectra confirmed the structure of vinyl esters and its purity.

Analysis of the MIPs by IR spectra shows that with increase of pH from 2 to 8, the composite passes into the anion form, the band at 1705 cm^{-1} (the stretching vibrations of the carboxylic group) disappears, and in its place new absorption bands appear at 1560 and 1420 cm^{-1}, which are assigned to the stretching vibrations of the carboxylate anion COO^-.

4. Drug release in vitro

It has been widely demonstrated that the side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the drug polymer chemical bonds, the structure of the polymer and the surrounding condition. The hydrolysis of a linkage is also dependent on its distance from the polymer backbone, but the length and hydrophilicity of the spacer unit between the drug and the polymer chain can

also affect the release rate.

Release studies were carried out for semi-covalent MIPs (MIP 1-4) and drug loading matrices (MIP′ 1-4). For drug loading matrices were supposed to have a better ability in controlling drug release in comparison to NIP. The data obtained from the experiments clearly show that drug release from NIP was remarkably faster than that observed when MIP was used. In particular, it is possible to note that while in the first case the drug is completely released within five hours, for MIP samples even after 8 hours the release is not yet complete. Under these conditions the non-imprinted polymers do not have specific binding cavities in which the drug is bound with semi-covalent interactions, whereas MIP, due to its specific network structure, still retains a significant percentage of drugs. Such behavior is in accordance with results obtained from the binding experiments (Table 2). This observation supports a model of retention mechanism which assumes that the selective sites have stronger interaction with the drug than the non-selective sites [23].

It appears that the degree of hydrolysis of network polymers depends on the amount of the MAA units in copolymer and reticulated degree of cross-linking. In MIP systems as drug delivery vehicles, because high reticulated degree of the polymer, diffusion of the hydrolyzing agents in the network's polymer is reduced and the hydrolysis rate in the basis is slower.

As shown in this Figures 2 and 3, the drug release proceeds more efficiently at a higher pH (SIF). As the content of MAA in the feed monomers increased, hydrolysis rate decreased at pH 1 but increased at pH 7.4. This was because a higher MAA content in the polymer networks led to higher carboxylate anion concentration at high pH. In other words, the existence of hydrogen-bonding interactions between –COOH groups in the polymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum hydrolyzing of the gel in a medium of pH 1. However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of –COOH groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged $–COO^-$ groups. These two factors ultimately result in a greater increase in the water uptake. In pH 7.4 with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing agents on polymer is increased and the hydrolysis rate increased [24].

Therefore, in alkaline pH value, the polymers are easily degraded to release of drug.

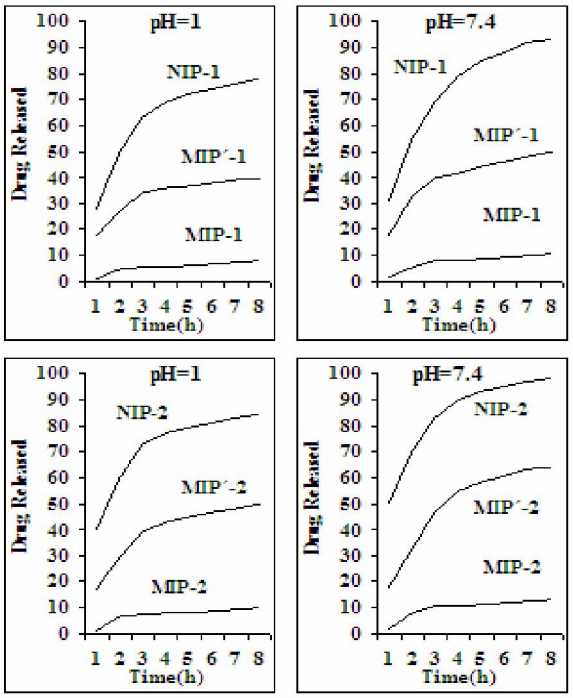

Figure 2. Release of ibuprofen from MIP, MIP′ and NIP as a function of time at 37 °C.

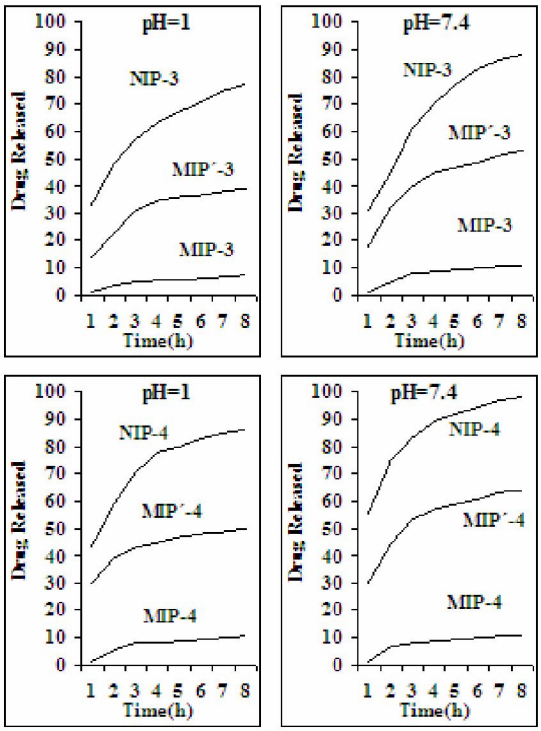

Figure 3. Release of naproxen from MIP, MIP′ and NIP as a function of time at 37 °C.

5. Conclusions

The starting point of this work was the preparation of a specific delivery system for ibuprofen and naproxen based on semi-covalent molecularly imprinted polymers synthesized using MAA as a functional monomer and EGDMA as a crossilnker in the presence of vinyl esters derivatives of ibuprofen and naproxen as templates. The particles are able to selectively re-bind the bioactive agent in aqueous media, under acidic conditions as well as under neutral conditions. The percentages of drug bound by the imprinted matrices were significantly higher than those obtained when the non imprinted ones were used. Despite, the imprinted polymers bound much more drugs than the corresponding non-imprinted ones and showed a controlled/sustained drug release, with MIPs release rate being indeed much more sustained than that obtained from NIPs.

The results obtained from the in vitro release studies indicated that these polymeric matrices are also suitable for a controlled/sustained delivery of the tested anti-inflammatory agent in biological fluids, both in gastrointestinal and in intestinal fluids. The release using the imprinted polymers cannot be easily classified according to the usual mechanisms of delivery because every matrix is highly specific for the drug used as a template; in fact, in order to obtain a matrix suitable for another drug it is necessary to synthesize a different imprinted polymer. Finally, because of their selective binding properties, the new polymeric networks reported in this paper represent a promising device for the preparation of novel controlled release dosage forms.

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4/21/2011

Chemical Reaction in Tomato Plants in Response to A biotic Elicitors Treatments

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Abstract: Early blight resistant cultivar "Tezier" and susceptible cv. "Castle rock" were tested to identification their response to *A. solani* infection on tomato seedlings pre-treated with chemical inducers: Salicylic acid (SA), Isonicotinic acid (INA), and Thiamine (vit. B_1), under greenhouse conditions. Resistant cv. "Tezier" exhibited rapid reaction represented in higher significant endogenous SA levels compared to the susceptible cv. "Castle rock" for all chemical treatments. "Tezier" endogenous SA levels surpassed "Castle Rock", 5 folds in exogenous SA, 2 fold in INA, and about 5 folds for vit. B_1 application. "Tezier" also had higher quantities in PRs accumulation (β-1, 3-glucanase, chitinase and peroxidase) in time course intervals 3, 24, 48, 72, and 96 hrs after pathogen inoculation, through increase of PRs activity which was started one day after inoculation in all the induced plants and reached maximum level after three to four days compared to "Castle rock" for all chemical inducers. Total protein content and polyphenol oxidase activity were also observed, their levels were highly significant in "Tezier".
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Key Wards; Early blight disease; Chemical inducers: Salicylic acid (SA), Isonicotinic acid (INA), and Thiamine (vit. B_1).

1. Introduction:

Infection of plants with a necrotizing pathogen can enhance resistance to subsequent infections by various fungal, bacterial and viral pathogens. This induced resistance, known as systemic acquired resistance (SAR), extends to plant tissue distant from the infection site and can persist for weeks after the initial infection. Salicylic acid (SA) plays an important role in signal transduction in plants and is believed to initiate SAR (Malamy *et al.*, 1990). Peng *et al.*, (2004) indicate that the SA pathway is involved in a wide range of plant defense responses, and SA is a key regulator of pathogen-induced systemic acquired resistance. SA has also been found to activate the expression of genes that encode pathogenesis-related proteins (Yalpani *et al.*, 1991). Exogenous application of SA to roots of hydroponically grown tomato can increase resistance against *A. solani*, the causal agent of early blight (Spletzer and Enyedi, 1999).

Pathogenesis-related proteins (PRs), identified as inducible proteins that have been implicated in active defense and could play a role in restricting pathogen development and spread in the plant against various pathogens (Chen and Zhu 2004; Eulgem, 2005). The recognized PRs have been extensively reviewed and currently comprise 17 families of induced proteins (van Loon *et al.* 2006). Chitinases and β-1,3-glucanases catalyze the hydrolysis of chitin and β-1,3-glucan, respectively, both polymers major components of the fungi cell walls. Typically they are expressed constitutively at low levels in plant cell and accumulate in response to fungal, bacterial, viral attack, or other inducers of acquired resistance (Gunter *et al.*, 2008; Cota *et al.* 2006). Time-course accumulation of chitinase and β -1,3-glucanase in induced plants was significantly higher than the control. Maximum activities of these PR-proteins were recorded after three days of inoculation in all induced plants. Thereafter, the activity decreased progressively (Saikia *et al.* 2005). Polyphenoloxidase (PPO) the oxidative enzyme converts phenolic compounds of plants into polyphenols and quinones, the toxic substances for theextracellular enzymes produced by the pathogens (Raju *et al.*, 2008). Peroxidase (POD) is a key enzyme in thebiosynthesis of lignin, where lignification leading to disease resistance (Bruce and West, 1989). The late and generalized necrosis in the susceptible cultivars seems to be related to the intervention of the PPO, contrary to the resistant cultivars in which the fast and localized induction of necrosis was associated to the POD (Diani *et al.* 2009). Specific chemicals such as salicylic acid (SA), benzo[1,2,3]thiadiazole-7-carbothionic acid-S-methyl ester (BTH, also named acibenzolar-Smethyl), and dl-3-amino-n-butyric acid (BABA) have been reported to induce SAR in a variety of plants against

a wide range of microbial pathogens without possessing direct antimicrobial activity (Barilli *et al.* 2010).

The objective of this study was to determine differences between resistant and susceptible cultivars in rate of induction of SA expression and monitoring accumulation of PRs during time intervals due to the induction by certain chemical activators.

2. Materials and methods

Tomato (*Lycopersicon esculentum* Mill) cultivars Tezier (resistant) and Castel rock (susceptible) were grown under greenhouse conditions (24-26 °C) in 14 cm pots containing : mixture of 1:2:1 sand: clay: peatmoos planted at the rate of 5 seedlings per pot. For seedlings inoculation, conidial obtained from (*Alternaria solani*, virulent isolate, obtained from Mycological center Assiut University).

Ellis and Martin) culture grown on potato dextrose agar for 10 days according to the method described by El-Samra *et al.* (2009). Eight tomato plants (5-week-old) of each cultivar were sprayed with Salicylic acid (SA) 500 ppm, Isonicotinic acid (INA) 750 µl/L, and Thiamine (vit. B_1) 100 mM on the upper and lower leaf surface which were solubilizing in water or ethyl alcohol. As a control treatment, H_2O was used instead of chemical inducer solution in each case. The same tomato seedlings pretreated by chemical inducers were inoculated by spraying with a suspension of 1×10^4 conidia ml^{-1}. Inoculated plants were incubated at 100% RH by covering bots with plastic bags for 24 h. Individual leaves: at each sampling time; 3, 24, 48, 72, 96 hrs after fungal inoculation to account for variation in SA content and PR protein levels were collected and pooled in liquid nitrogen for further studies.

Chemicals

Salicylic acid (SA) Sigma-Aldrich Chemie-France**,** Isonicotinic acid (INA), Vitamin B_1 hydrochloride (vit.B_1), were purchased from Sigma-Aldrich chemicals company (Cairo). Other chemicals and solvents used in this work were analytical or chromatographic grade. A standard HPLC calibration solution of salicylic acid, concentrations; 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 µg were prepared by accurate step-by-step dilutions of stock solution 10 µg by weighing 1 g SA and dissolving in 100 ml methanol.

Quantification of free salicylic acid (SA):

Quantification of endogenous SA was carried out twice, the first after 7 days from chemical application and the second after 7 days from pathogen inoculation on the resistant and susceptible tomato cultivars. Salicylic acid levels were measured in leaf tissue by high performance liquid chromatography (HPLC).

Extraction of free SA

Free SA was extracted from tomato plants according to the method of Malamay and Klessig (1992) with some start time after 3 hours of fungal inoculation.

Determination of SA:

Leaf material was grounded without sand particles, Supernatant evaporation was carried out by heating at 40 °C for 24 hours, were analyzed by HPLC-electrospray ionisation using an Agilent 1100 HPLC coupled to an Applied Biosystems Q-TRAP 2000 (Applied Biosystems, California, USA). Chromatographic separation was carried out on a Phenomenex Luna 3 µm C18 (2) 100 mm × 2.0 mm column, at 35°C. Determination of endogenous SA levels was performed according to the method of Forcat *et al.*, (2008).

Preparation of leaf homogenates

For determining enzyme activities of peroxidase (PO), and polyphenoloxidase (PPO), entire leaves, collected at different time intervals (3, 24, 48, 72, 96 hrs) following inoculation, immersed in liquid N2 and homogenized with 0.1 M Na-acetate buffer (pH 5.2) (1 g plant material in 10 ml). The homogenated leaves were centrifuged at 15000 rpm for 30 min at 4 ˚C, and the enzyme activities were determined in the supernatants. For determining pathogenesis related (PR) proteins (chitinase, β -1,3-glucanase), detached leaves were immersed in liquid N_2 then homogenized in 2 ml 0.1 M Na-acetate buffer of pH 5.2 consisting of 1% (v/v) PVPP (polyvinylpolypyrolidone), 5% (v/v) glycerol, 0.1 M phenylmethansulfanylfluorid, and 0.1 M DTT (dithiothreitol). Homogenates were centrifuged at 15000 rpm for 30 min at 4 ˚C. Supernatants were used to determine enzymatic activities.

Determination of protein

Protein concentration was determined for all experiments using the method described by Bradford (1976) spectrophotometrically at 595 nm using bovine serum albumin (BSA) (0-5.0 mg/ml) as standard.

Determination of enzyme activities Peroxidase (PO) activity

Peroxidase activity was determined spectrophotometrically using guaiacol as a common substrate for peroxidases as described by Abdal Razik *et al.* (2008). Peroxidase activity = OD436nm / mg protein.

Polyphenoloxidase (PPO) activity

Peroxidase activity was determined using the method of Batra and Kuhn (1975). PPO units = OD410nm / mg protein.

Chitinase activity

For chitinase assay, the substrate colloidal chitin was prepared from chitin powder according to

the method described by Ried and Ogrud-Ziak (1981). Reducing sugars were determined in 1 ml of the supernatant by dinitrosalicylic acid (Monreal and Reese, 1969) using 1 ml of 1% colloidal chitin in 0.05 M citrate phosphate buffer (pH 6.6) in test tubes, 1 ml of enzyme extract was added and mixed by shaking. Tubes were kept in water bath at 37˚C for 60 minutes, then cooled and centrifuged before measuring O.D. at 540 nm. Chitinase activity was defined as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes.

β-1,3-glucanase activity

Total β-1,3-glucanase activity was colorimetrically assayed by the laminaria-dinitrosalicyclate method described by Saikia *et al.* (2005). One gram of tomato leaves was extracted with 5 ml sodium acetate buffer (SAB; 0.05 M), pH 5. The extract was then centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was then used as crude enzyme extract. The extract (62.5 µl) was added to laminarin (4%, 62.5 µl) and incubated at 40°C for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic acid reagent and heated for 5 min in boiling water bath. The resulting coloured solution was diluted with 4.5 ml water, vortexed and absorbance at 500 nm was determined. The blank was the crude enzyme preparation mixed with laminarin with zero time incubation. The enzyme activity was expressed as µmol equivalent glucose release min^{-1} g^{-1} fresh tissue.

Statistical analysis

Salicylic acid data were compared using Scheffe's test ($P \leq 0.05$). Antifungal activity as well as chitinase, β-1,3-glucanase, polyphenoloxidase and peroxidase activity were statistically analyzed by Fisher's LSD Test ($P \leq 0.05$) (Gomez and Gomez, 1984). The package used for analysis was NCSS and PASS software version 2000.

3. Results:

SA expression in 'Resistant' and 'Susceptible tomato cvs.

A. Seven days after chemical treatment:

Data in Table (1) and Fig. (1) Showed no salicylic acid was detected in untreated control of resistant and susceptible cultivars. Moreover, in general SA content in resistant cultivar was in general SA content in resistant cultivar was significantly higher than those of susceptible ones. The highest SA contents 3.36 µg/$^{g-1}$ fresh weight, was obtained in Tezier cv., treated with thiamine, compared with that of the same treatment in Castle Rock cv. , 0.15 µg/g^{-1} FW SA contents in resistant cv., treated with SA or INA were recorded as 0.19 and 0.66 µg/g^{-1} FW, respectively. Unlike the resistant cv. treatments, the highest SA content in susceptible cultivar, 0.21 µg/g^{-1} FW, was obtained in seedlings, treated with INA, followed by those of SA and thiamine treatments as 0.03 and 0.15 µg/g^{-1} FW respectively

B. Seven days after inoculation with *A.solani*:

Similar trend was also observed concerning the susceptible cultivar Castle Rock.

Salicylic acid content in non-inoculated untreated control of resistant cv. (0.27 µg/g^{-1} FW) was higher than that of susceptible cultivar (0.04 µg/g^{-1} FW). Moreover, SA levels in all inoculated resistant treatments were, generally, significantly higher than those of the susceptible ones (Table 1 and Fig. 1). The highest SA content (0.34 µg/g^{-1} fresh weight) was obtained in inoculated Tezier cv., treated with thiamine, compared with that of the same treatment in Castle Rock cv. (0.07 µg/g^{-1} FW)**.** SA contents in inoculated resistant cv., treated with SA or INA (0.05 and 0.12 µg/g^{-}1 FW, respectively) were less than that of thiamine treatment (0.34µg/g^{-1} FW). Table (1). SA concentration (µg/g^{-1}) Contents of SA were estimated after 7 days both of chemical or fungal treatments days after inoculation with *A.solani*, under greenhouse conditions . Data in Table (1) and Fig. (1).

Pathogenesis related proteins (PRs)

Total protein determination

Protein content was determined by the method of Bradford (1976), with bovine serum albumin as the standard.

Chitinase activity

A standard curve was established for subsequent determination of induced chitinase in the tested tomato plants by resulted N-acetylglucosamine (mM per one gram fresh weight). Generally, untreated control inoculated with *A.solani* (C2) showed significant increase in chitinase activity in both resistant Tezier and susceptible Castle Rock cvs., compared with that of untreated non-inoculated control (C1). All the tested chemical inducers of SAR against *A.solani* significantly induced chitinases activity both in resistant and susceptible cvs., compared with both of untreated inoculated and non-inoculated control (C1 &C2). However, increasing rates significantly differed according to the applied chemical inducer, host resistance and time elapsed after inoculation with *A.solani*. Resistant Tezier cultivar, activity of chitinases, induced by SA increased gradually with time elapsed after inoculation, attaining maximum rates at the end of the experiment (14.68 and 12.98 and 6.96 times over that of C2 control, respectively). Meanwhile, INA and vit.B_1, increased chitinases activity gradually up to 48 hrs then decreased.

As for susceptible Castle Rock cv., treatment with vit.B_1 resulted in the highest increase in chitinases activity after three hours of treatment

(11.62-fold Moreover, the highest induction rate was realized by vit. B1 (1.77-fold), compared with SA and INA (1.15 and 1.19- fold of untreated inoculated control, respectively). Generally, vit. And SA were more efficient in inducing enzyme activity than INA. Pronounced decrease in enzyme activity was realized at the end of the experiment (0.29% of that of C2), start time was after 3 hours of fungal inoculation.

Treatment with vit.B_1 resulted in the highest increase in chitinases activity after three hours of inoculation (11.62-fold of C_2, compared with SA and INA (5.11 and 1.55-fold, respectively). The highest induction rate was realized by vit.B_1 (14.137 mM/g^{-1} FW), compared with SA and INA (9.189 and 9.521 mM/g^{-1} FW, respectively, after 48 hours of inoculation). Generally, vit.B_1 and SA were more efficient in inducing enzyme activity than INA. Pronounced decrease in enzyme activity was realized at the end of the experiment (0.29% of that of C_2 in INA treatment).

β-1, 3-Glucanase activity

Standard curve was established for subsequent determination of induced β -1,3-Glucanase in the tested tomato plants by resulted glucose (µmol per one gram fresh weight).

According to the results shown in Table 3 and illustrated in figs, 4 and 5, all the tested chemical inducers of SAR against *A.solani* significantly induced β -1,3-Glucanase activity both in resistant and susceptible cvs., compared with that of untreated non-inoculated control C1 and most of untreated inoculated control C2. However, increasing rates significantly differed according to the applied chemical inducer, host resistance and time elapsed after inoculation with *A.solani*.

In resistant Tezier cultivar, activity of β -1,3-Glucanase, induced by SA, INA and vit.B_1 increased gradually with time elapsed after inoculation, attaining maximum rates 48 hours after inoculation (6.009, 5.962 and 5.747 µmol /g^{-1} FW, respectively), then gradually decreased until the end of experiment. The highest enzyme activity values 96 hrs. After inoculation was detected in vit.B_1 treatment. Although enzyme activities induced by the tested inducers were higher than those induced by inoculated untreated control, however, differences were mostly limited (Table 3 and Fig.4).

In susceptible Castle Rock cv., treatment with SA, INA and vit.B_1 resulted in significant increase in β -1,3-Glucanase directly after inoculation with *A.solani* (54.68, 54.54 and 55.11%, respectively more than that of C1). Enzyme activity in treated inoculated plants increase with time elapsed after inoculation, attaining relatively highest levels after 48 hours, and then showed slight gradual decrease until the end of the experiment. Increases in enzyme activities, observed in inoculated untreated control C2 along the experiment were in close similarity to those induced by all the tested inducers at all the tested time intervals after inoculation, however, activities in C2 surpassed those induced by SA and vit.B_1. Generally, differences in enzyme activities among inducer treatment and those of C2 were not pronounced in the susceptible cultivar, compared with those of the resistant cv. (table 3 and fig. 5).

Data in table 3, illustrated in figs. 4 and 5 showed pronounced increase in â-1,3-Glucanase between resistant Tezier cv. and susceptible Castle Rock cv.. in particular 48 hours after inoculation of tomato plants, pretreated with SA, INA and vit.B_1 with *A.solani* (12, 12.07 and 7.64%, respectively, more than those of susceptible cv.). At the fourth day after inoculation,, treatment of resistant cv. with vit.B_1 exhibited 5,66% increase in enzyme activity, compared with that of susceptible cv.

Peroxidase (PO) activity

In resistant Tezier tomato cv., it was detected that inoculation of untreated plants with *A.solani* resulted in significant induction of peroxidase activity, compared with that of C1 control. Moreover, enzyme activity increased with time elapsed after inoculation, attaining highest values after 48 hours (4-fold over that of C1 control). Gradual decrease was the occurred until the end of the experiment, where enzyme activity in C2 control was 2.65-fold over that of C1 control.

According to results of (Table 4, Fig. 6), treatment with any of the tested SAR chemical inducers resulted in significant increase in enzyme activity, compared with that of the untreated inoculated C2 control. However increasing rates differed according the tested inducers. Three hours after inoculation significant increase in enzyme activity, in particular those pretreated with SA and INA (2.54 and 2.15-fold, respectively over that of C2 control). The highest levels of peroxidase activity occurred 48 hours after inoculation in plants pretreated with any of the tested inducers, however INA was more active in this respect (2-fold of C2 control), followed by vit. B_1 (1.3-fold of C2 control).

At the end of the experiment, increasing rates in enzyme activity were, generally lower than the other tested intervals, however INA and SA treatments realized, relatively higher rates (about 1.6-fold of that of C2). M β

Generally, INA was the most effective in inducing peroxidase activities after the second day of inoculation until the end of the experiment, followed by SA

In susceptible Castle Rock cv., it was evident that inoculation of untreated susceptible cv.

with *A.solani* resulted in significant increase in PO activity directly after three hours and 24 hours (3.68 and 3.8-fold over that of untreated non-inoculated C1 control), attaining maximum values at the end of the experiment 96 hours after inoculation, where PO activity was 6.18-fold over that of C1 control.

Results in table 4 and Fig. 7 showed that pretreatment of susceptible plants with the tested SAR chemical inducers, i.e. SA, INA and vit.B_1 before inoculation with *A.solani* significantly increased PO activity mostly from the first day of inoculation and till the end of the experiment. However, increasing rates, compared with PO activity in C2 control significantly differed according to the tested inducers and time elapsed after inoculation.

Three hours after inoculation, significant increase in PO activity was detected in both SA and vit.B_1 treatments (9% and 24.73%, respectively more than that of C2 control). After 48 hours of inoculation, INA exhibited the highest values of PO activity (2.36-fold over that of C2 control), whereas SA showed the highest PO activity after 72 hours (2.22-fold of C2 control). PO activity was then decreased until the end of the experiment.

Generally, INA and SA increased more PO activity particularly after the second and third days of inoculation.

Polyphenoloxidase (PPO) activity

In resistant Tezier cv., inoculation of untreated plants (Table 5) with *A.solani* significantly increased PPO activity throughout the duration of the experiment, attaining maximum activity (0.352 units/mg protein) at the end of the experiment (1.68-fold over that of C1). Treatment resistant tomato plants with the tested chemical inducers significantly increased PPO activity, compared with C2, however, significant decrease in enzyme activity at the end of the experiment, compared with that of the untreated inoculated control (C2).

Increasing rates differed according to the tested inducer and time after inoculation with *A.solani* (Table 5). A pronounced increase in PPO activity was detected (Table 5) in plants pretreated with vit.B_1 directly after inoculation (73.22% more than C2 control), attaining highest PPO activities after 48 hours (0.802 units/mg protein), compared with those induced by SA and INA inducers (0.572 and 0.514 units/mg protein, respectively). Gradual decrease in PPO activity was observed at the end of the experiment, where PPO activity values were less than that of C2 control. Generally, vit.B_1 was more efficient in inducing PPO activities in resistant cv. inoculated with *A.solani* than the other tested inducers, followed by SA (Fig. 8).

In susceptible Castle Rock cv., inoculation of untreated plants with *A.solani* resulted in an increase in PPO activity over that of C1 control, attaining maximum value 48 hours after inoculation (0.165 units/mg protein) then gradually decreased to reach the minimum enzyme activity at the end of the experiment (0.087 units/mg protein) (Table 5). Pretreatment of the susceptible Castle Rock cv. with the chemical SAR inducers INA and vit.B_1 significantly induced an increase of PPO activity, attaining maximum values two days after inoculation (0.241 and 0.211 units/mg protein, i.e. 31.54% and 21.80%, respectively than that of C2 control) (Table 5). Although treatment with SA resulted in the highest PPO activities (45.20% more than C2 control), three hours after inoculation, however, it decreased, attaining values less than that of C2 control at both the second and the third days of inoculation. Generally, treatment of the susceptible cv. with INA realized the highest induction of PPO, at the second day of inoculation with *A.solani*, followed by vit.B_1 (Fig. 9).

4. Discussion

Plant pathogen interactions are rapid and dynamic, with both host and pathogen constantly wrestling to modify signaling networks and reconfigure metabolism in favor of defense or disease (Truman *et al.* 2010). SA accumulates in leaf tissue following infection by an avirulent pathogen, but SA levels have not been reported in susceptible cultivars and compared to resistant ones. Though SA accumulation in susceptible cultivars has not been measured, a susceptible cultivar of tomato has the ability to take up exogenous SA (Spletzer and Enyedi, 1999), INA, and vit. B_1 and express resistance to *A.solani*. The link between a SA response and a hypersensitive reaction (Conrath *et al.*, 1995) suggests that resistant cultivars can accumulate SA more quickly than susceptible cultivars. In a test of this hypothesis, early blight resistant cv. 'Tezier' showed a more rapid accumulation of SA than 'Castle Rock'. This supports the hypothesis that faster accumulation of SA upon pathogen recognition occurs in resistant cultivars than in susceptible cultivars. In these experiments, we demonstrated that SA can activate a form of systemic resistance against *A. solani* in tomato plants grown under greenhouse conditions. This was accomplished by providing 500 ppm SA, 750ml/L INA, and 100 mM vit. B_1, directly foliar application.

Table (1). Contents of SA (µg/g^{-1}) in resistant (Tezier) and susceptible (Castle Rock) tomato cultivars pretreated with abiotic inducers under greenhouse conditions.

cultivar	treatment	SA content µg/g^{-1} FW	
		7 days after inducer application	7 days after pathogen inoculation
Tezier	Control (H_2O)	0.00^{a}	0.27^{a}
	SA	0.19^{c}	0.05^{b}
	INA	0.66^{d}	0.12^{c}
	vit. B_1	3.36^{e}	0.34^{d}
Castle Rock	Control (H_2O)	0.00^{a}	$0.04b^{e}$
	SA	0.03^{a}	0.01^{e}
	INA	0.21^{c}	0.06^{b}
	vit. B_1	0.15^{b}	0.07^{b}

Values followed by the same letter(s) in each column don't differ significantly according to Scheffe's test ($P \leq 0.05$)

Table (2). Chitinase activities in early blight resistant and susceptible tomato cvs. pretreated with the tested chemical SAR inducers and inoculated with *A. solani* at different periods following inoculation.

Enzyme activity (mM/g^{-1} FW)							
		Time after inoculation (hrs)					*FLSD*
Cultivars	Treatments	3	24	48	72	96	(0.05)
Tezier	C1	0.623^{a*}	1.205^{a*}	1.839^{a}	1.890^{a}	3.056^{a*}	0.159
(resistant)	C2	1.164^{b}	2.39^{b*}	12.205^{b*}	10.638^{b*}	1.101^{b}	0.180
	SA	9.106^{c*}	11.975^{c*}	14.158^{c*}	15.01^{c*}	16.174^{c*}	0.824
	INA	2.723^{d*}	3.201^{d*}	17.588^{d*}	11.808^{d*}	6.759^{d*}	0.208
	Vit.B_1	8.170^{e}	8.503^{e}	14.49^{e*}	12.91^{e}	13.097^{e}	0.826
Castle Rock	C1	0.528^{f}	0.645^{f}	1.210^{f*}	0.989^{f*}	0.542^{f}	0.160
(susceptible)	C2	0.696^{g*}	1.538^{g*}	7.995^{g*}	5.667^{g*}	7.563^{g*}	0.179
	SA	3.555^{h*}	9.272^{h}	9.189^{h}	10.893^{h*}	13.617^{h*}	0.181
	INA	1.081^{i*}	2.245^{i}	9.521^{i*}	9.771^{i*}	2.162^{i}	0.162
	Vit.B_1	8.087^{k*}	8.648^{k*}	14.137^{k*}	11.101^{k*}	12.203^{k*}	0.139
FLSD (0.05)		0.050	0.069	0.020	0.074	0.030	

Values followed by the same letter(s) in each column don't differ significantly according to Fisher's LSD Test ($P \leq 0.05$).
Values with superscript star (*) differs significantly than other values in each row according to Fisher's LSD Test ($P \leq 0.05$).
C1 = untreated non-inoculated control, C2 = untreated inoculated control.

Table (3). β-1,3-Glucanase activities in early blight resistant and susceptible tomato cvs. Pre-treated with the tested chemical SAR inducers and inoculated with *A.solani* at different periods following inoculation.

Enzyme activity (µmol/g^{-1} FW)							
		Time after inoculation (hrs)					*FLSD*
Cultivars	Treatments	3	24	48	72	96	(0.05)
	C1	1.682^{a*}	2.663^{a}	2.616^{a}	1.915^{a*}	2.757^{a*}	0.080
Tezier	C2	4.878^{b}	4.785^{b}	4.588^{b}	4.785^{bd}	4.616^{cd}	0.113
(resistant)	SA	4.523^{cg}	5.242^{bc*}	6.009^{c*}	5.130^{dg*}	4.523^{c}	0.083
	INA	4.869^{b}	5.009^{bd}	5.962^{c*}	4.373^{b}	4.766^{df}	0.815
	Vit.B_1	4.747^{be}	5.439^{cd}	5.747^{c}	5.000^{cde}	5.121^{g}	0.915
Castle Rock	C1	2.766^{d*}	1.897^{e*}	2.383^{a}	2.467^{a}	2.252^{b}	0.199
(susceptible)	C2	4.626^{ce}	4.551^{b}	5.411^{c}	5.355^{efgh}	5.177^{g}	0.214
	SA	4.467^{cf}	4.850^{bc}	5.280^{bc}	5.074^{cdf}	4.738^{de}	0.827
	INA	4.728^{be}	4.672^{b}	5.242^{bc}	5.785^{h*}	5.289^{g}	0.198
	Vit.B_1	4.383^{fg}	4.878^{bc}	5.308^{bc*}	4.504^{bc}	4.831^{ef}	0.200
FLSD (0.05)		0.160	0.488	0.780	0.582	0.178	

Values followed by the same letter(s) in each column don't differ significantly according to Fisher's LSD Test ($P \leq 0.05$).
Values with superscript star (*) differs significantly than other values in each row according to Fisher's LSD Test ($P \leq 0.05$).
C1 = untreated non-inoculated control, C2 = untreated inoculated control.

Table (4). Peroxidase (PO) activities in early blight resistant and susceptible tomato cvs. pre-treated with the tested chemical SAR inducers and inoculated with *A.solani* at different periods following inoculation.

Enzyme activity (units/mg protein)							
Cultivars		Time after inoculation (hrs)					*FLSD*
	Treatments	3	24	48	72	96	(0.05)
Tezier (resistant)	C1	0.001^{a}	0.022^{a}	0.075^{a}	0.098^{a}	0.070^{a}	0.037
	C2	0.054^{b}	0.082^{b}	0.302^{b}	0.206^{b}	0.186^{b}	0.113
	SA	0.137^{c}	0.285^{c}	0.333^{c}	0.306^{c}	0.295^{c}	0.181
	INA	0.116^{d}	0.221^{d}	0.601^{d*}	0.338^{d}	0.298^{d}	0.117
	Vit.B_1	0.054^{b}	0.097^{b}	0.390^{e}	0.221^{e}	0.217^{e}	0.199
Castle Rock (susceptible)	C1	0.019^{e}	0.021^{a}	0.072^{f*}	0.032^{f}	0.017^{f}	0.077
	C2	0.070^{f}	0.080^{e}	0.167^{g}	0.121^{g}	0.105^{g}	0.140
	SA	0.077^{g}	0.199^{f}	0.289^{h}	0.269^{h}	0.201^{h}	0.293
	INA	0.055^{b}	0.096^{f}	0.394^{i}	0.199^{i}	0.136^{i}	0.269
	Vit.B_1	0.093^{h}	0.101^{h}	0.153^{k}	0.145^{k}	0.128^{k}	0.162
FLSD (0.05)		0.001	0.001	0.002	0.005	0.002	

Values followed by the same letter(s) in each column don't differ significantly according to Fisher's LSD Test ($P \leq 0.05$).
Values with superscript star (*) differs significantly than other values in each row according to Fisher's LSD Test ($P \leq 0.05$).
C1 = untreated non-inoculated control.
C2 = untreated inoculated control.

Table (5). Polyphenoloxidase (PPO) activities in early blight resistant and susceptible tomato cvs. Pre-treated with the tested chemical SAR inducers and inoculated with *A.solani* at different periods following inoculation.

Enzyme activity (units/mg protein)							
		Time after inoculation (hrs)					*FLSD*
Cultivars	Treatments	3	24	48	72	96	(0.05)
Tezier (resistant)	C1	0.131^{a}	0.180^{a}	0.201^{a}	0.198^{a}	0.210^{a}	0.269
	C2	0.169^{b}	0.259^{b}	0.281^{b}	0.209^{b}	0.352^{b}	0.304
	SA	0.211^{c}	0.498^{c}	0.572^{c}	0.293^{c}	0.231^{c}	0.128
	INA	0.437^{d}	0.366^{d}	0.514^{d}	0.339^{d}	0.350^{ad}	0.115
	Vit.B_1	0.631^{e}	0.662^{e}	0.802^{e}	0.395^{e}	0.283^{e}	0.140
Castle Rock (susceptible)	C1	0.070^{f}	0.099^{f}	0.101^{f}	0.122^{f}	0.052^{d}	0.121
	C2	0.097^{g}	0.146^{g}	0.165^{g}	0.150^{g}	0.087^{f}	0.116
	SA	0.177^{h}	0.195^{h}	0.138^{h}	0.136^{h}	0.118^{g}	0.081
	INA	0.121^{i}	0.176^{a}	0.241^{i}	0.218^{i}	0.150^{h}	0.082
	Vit.B_1	0.160^{k}	0.218^{k}	0.211^{a}	0.154^{g}	0.138^{i}	0.182
FLSD (0.05)		0.006	0.012	0.022	0.003	0.007	

Values followed by the same letter(s) in each column don't differ significantly according to Fisher's LSD Test ($P \leq 0.05$), C1 = untreated non-inoculated control, C2 = untreated inoculated control.

In each experiment performed, there was a significant decrease in when the pathogen inoculation was preceded by SA, INA, and vit. B_1 treatments. These chemicals are known to be a potent inducers of systemic resistance in many plants including; tobacco, cucumber, potato, and Arabidopsis (White 1979; Ward *et al.* 1991; Coquoz *et al.*, 1995; Dann and Deverall 1995; Lund *et al.*, 1998; Dong and Beer, 2000; and Ahn *et al.* 2005) consequently, it is not surprising that these chemicals exerts a similar effect in tomato. In tomato, SAR can be induced using several different biotic elicitors; earlier work has shown that inoculation with *P. infestans* (Enkerli *et al.*, 1993 and Heller and Gessler 1986), tobacco necrosis virus (Anfoka and Buchenauer, 1997) and the host-incompatible *Meloidogyne incognita* nematode (Ogallo and McClure 1996) all induce SAR in tomato.

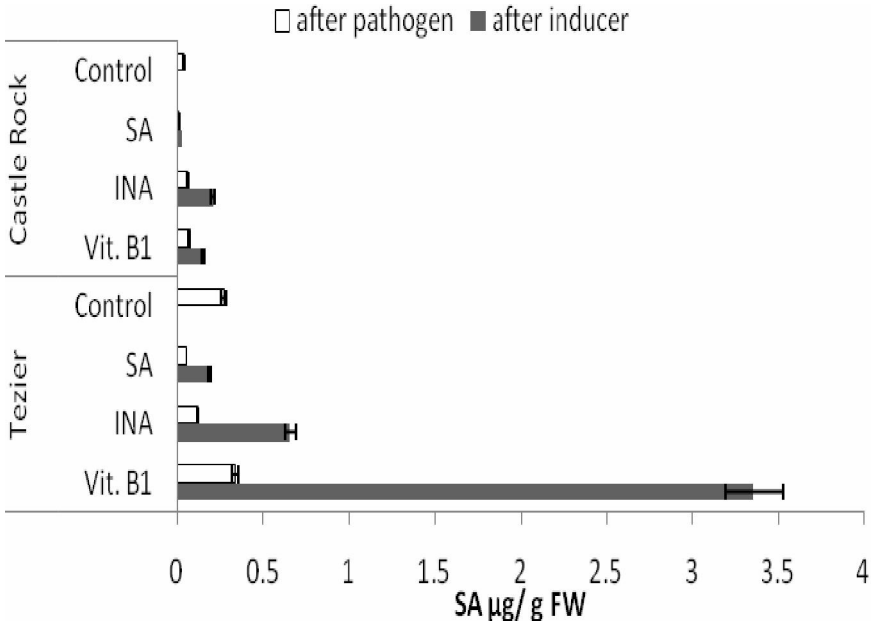

Fig.1. Endogenous levels of free salicylic acid (SA) in resistant cv. Tezier and susceptible cv. Castle Rock after inducer application (shaded bars) and after pathogen (*A. solani*) inoculation (open bars) on tomato plants. Leaves were harvested 7 and 14 days respectively, after treatment with H_2O (control) or 500 ppm SA, 750 ml/L INA, and 100 mM vit. B_1. Data bars are the means (± standard error) of three replicates.

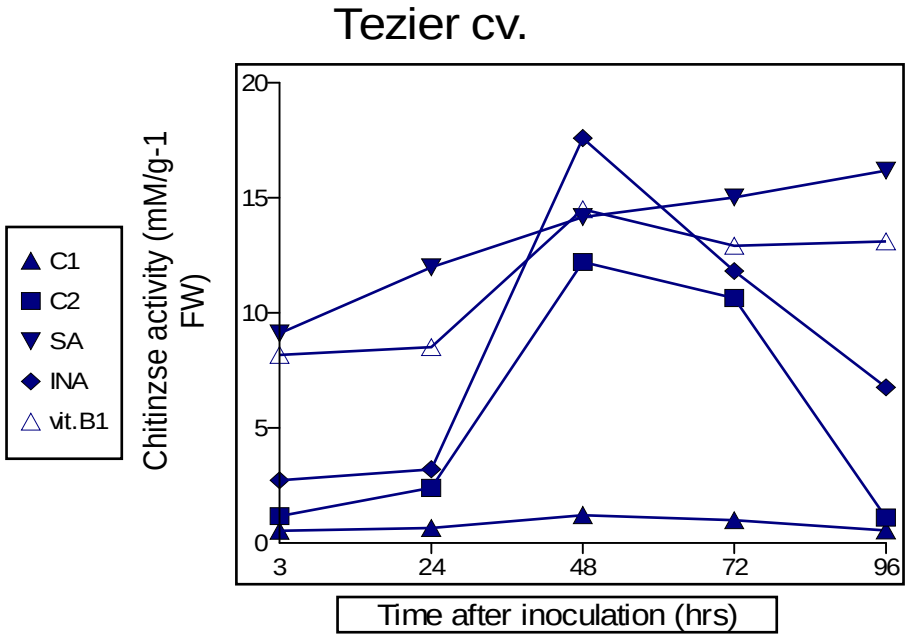

Fig. (2). Time course of chitinase activities in early blight resistant tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with *A.solani*, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

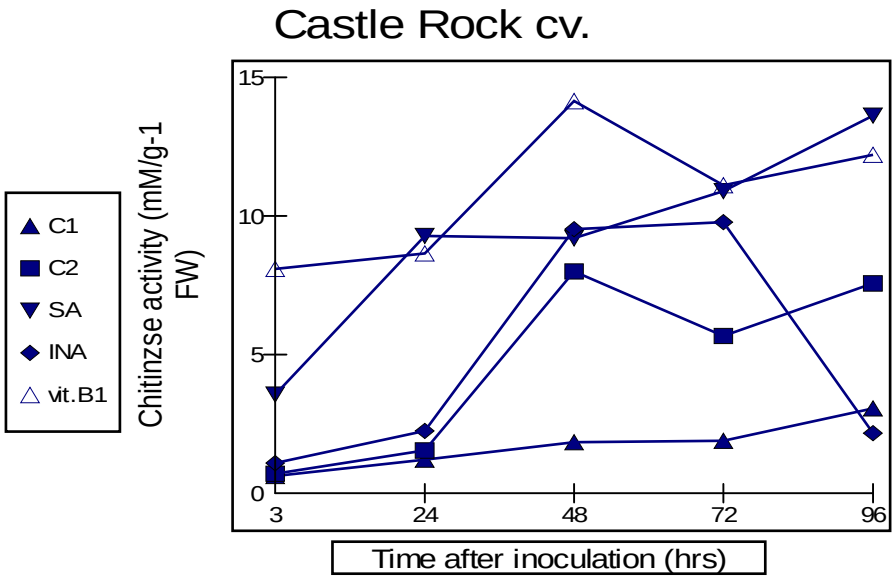

Fig. (3). Time course of chitinase activities in early blight susceptible tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with *A.solani*, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

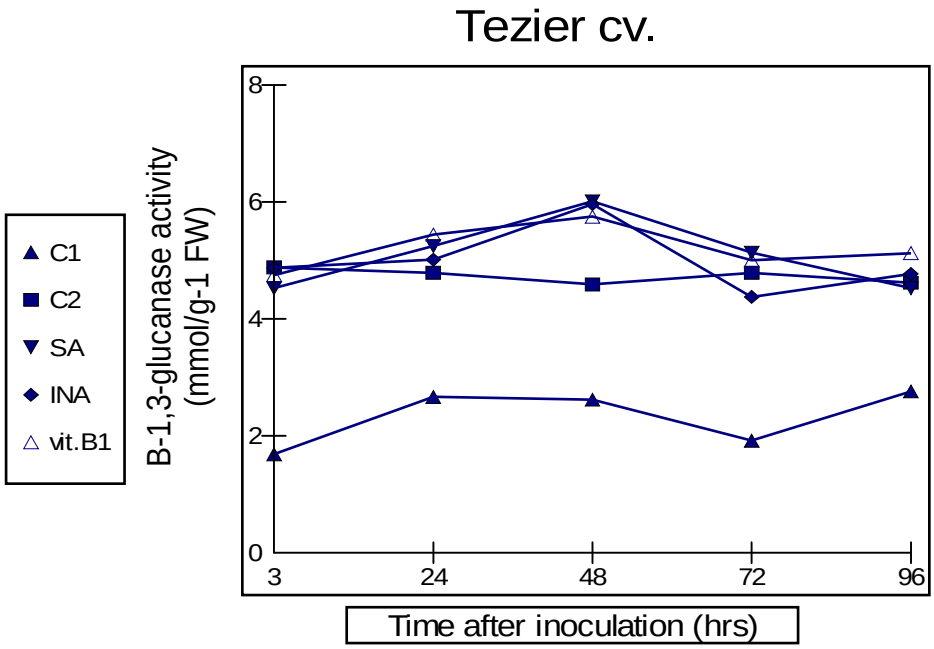

Fig. (4). Time course of β-1,3-glucanase activities in early blight resistant tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with *A.solani*, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

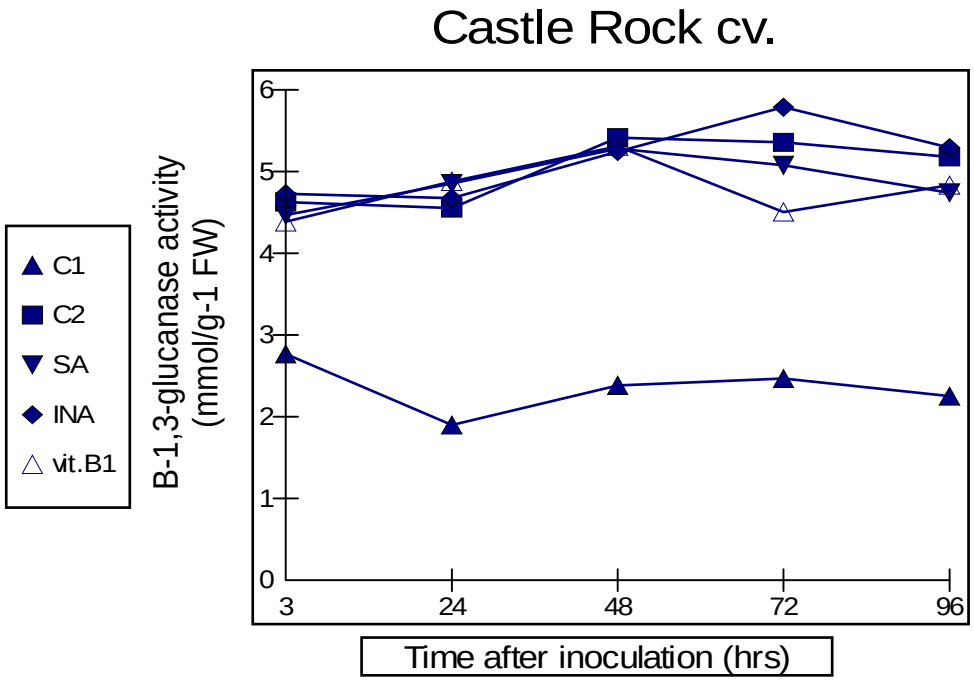

Fig. (5). Time course of β-1,3-glucanase activities in early blight susceptible tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with *A.solani*, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

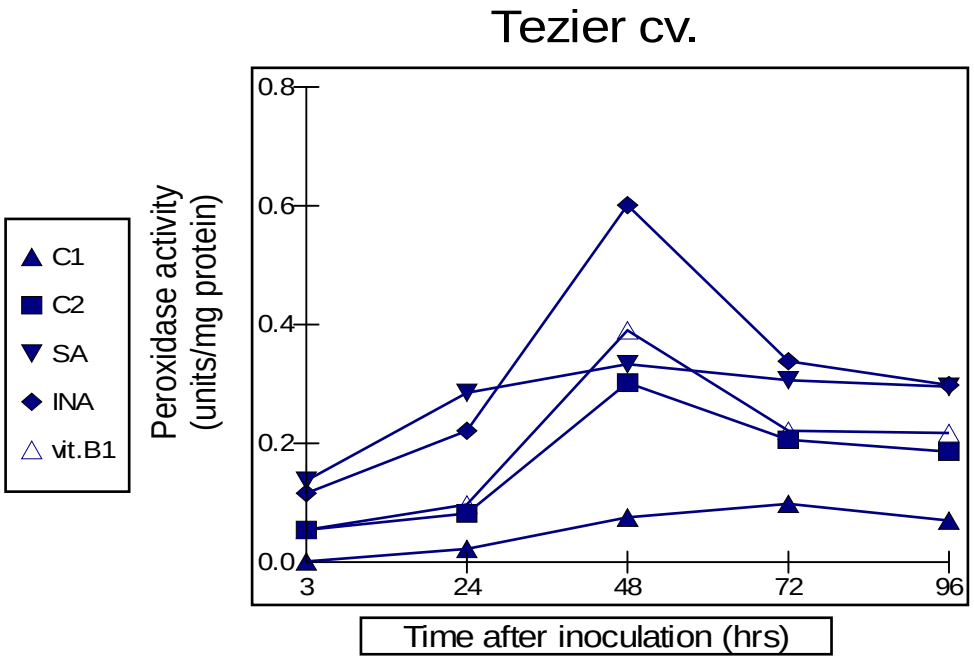

Fig. (6). Time course of peroxidase activities in early blight resistant tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with A.solani, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

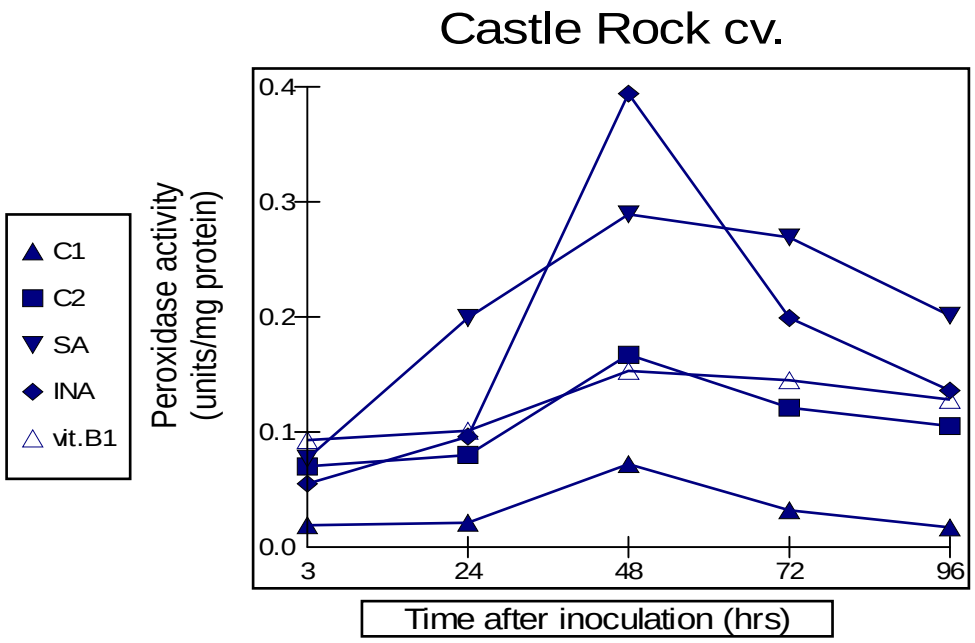

Fig. (7). Time course of peroxidase activities in early blight susceptible tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with A.solani, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

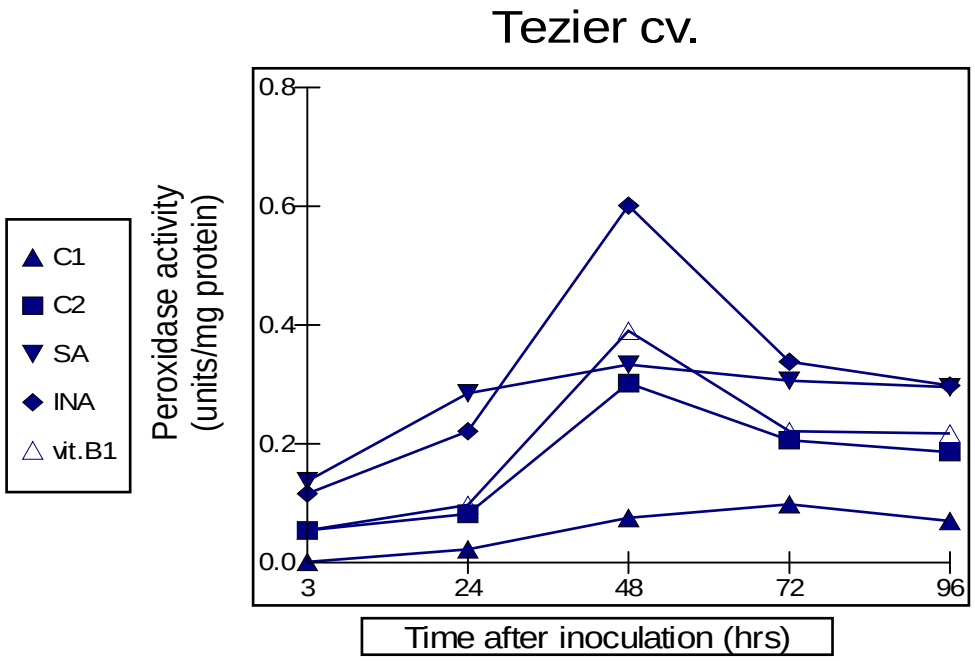

Fig. (6). Time course of peroxidase activities in early blight resistant tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with A.solani, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

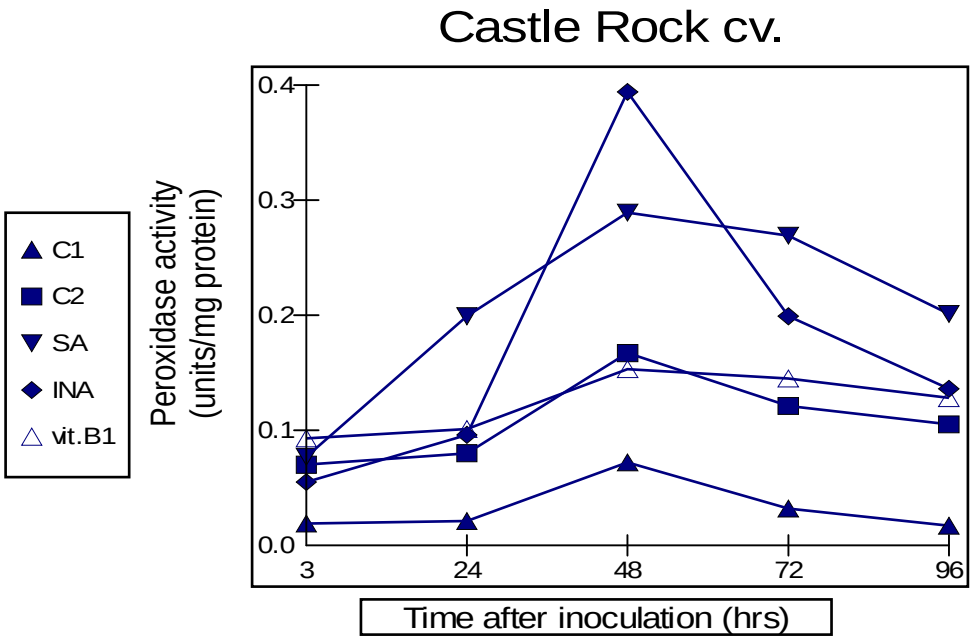

Fig. (7). Time course of peroxidase activities in early blight susceptible tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with A.solani, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

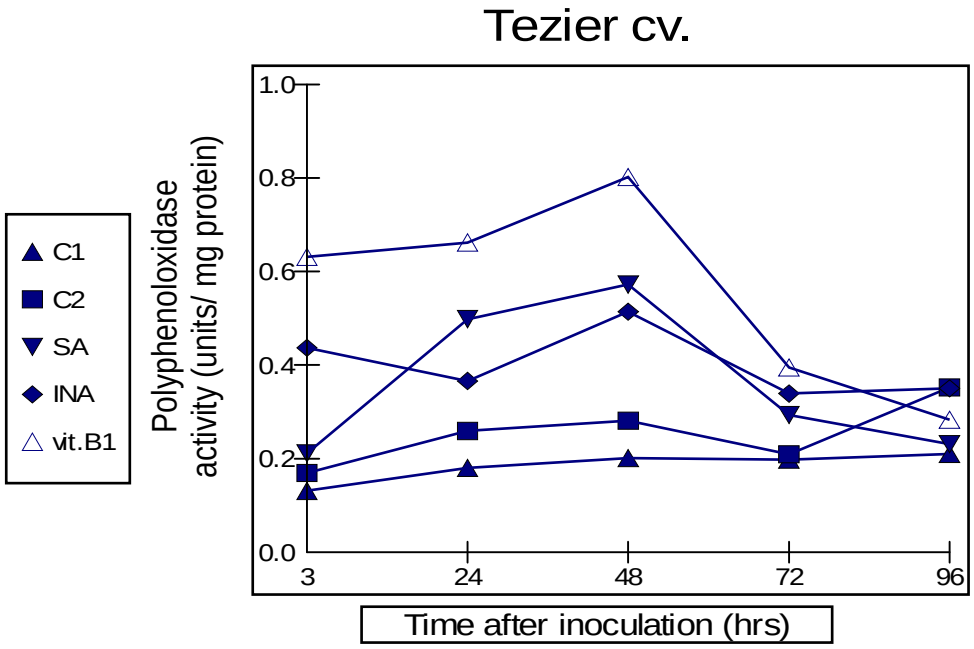

Fig. (8). Time course of polypenoloxidase activities in early blight resistant tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with *A.solani*, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

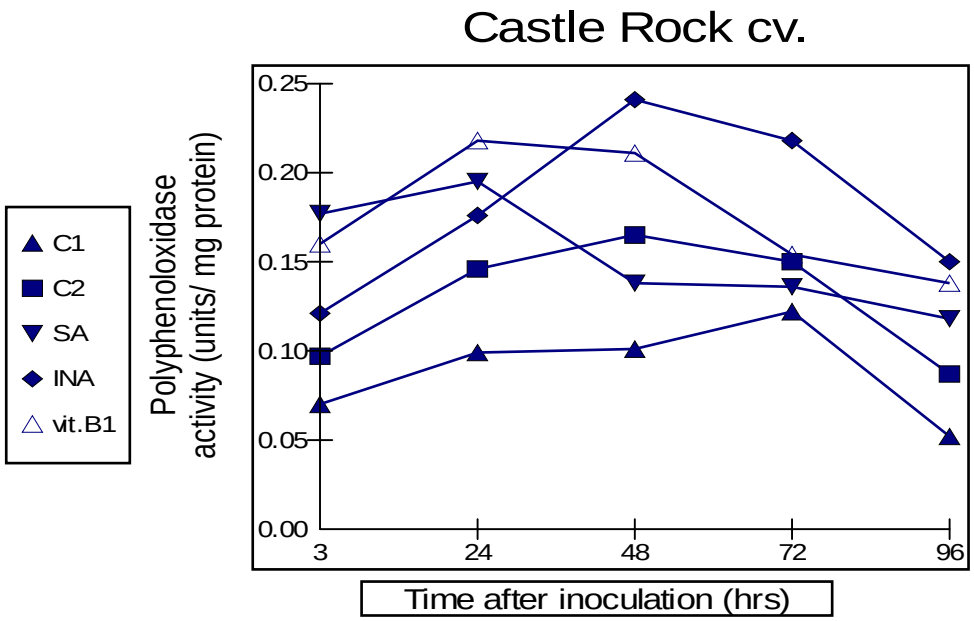

Fig. (9). Time course of polypenoloxidase activities in early blight susceptible tomato cv., pre-treated with the tested chemical SAR inducers and inoculated with *A.solani*, at different periods following inoculation. C1 = untreated non-inoculated control, C2 = untreated inoculated control.

Because SA is an important signal molecule (Enkerli *et al.* 1998 and Enyedi *et al.* 1992), its level may increase endogenously prior to the activation of SAR in each of the host-pathogen interactions described above (Malamy *et al.* 1990; Metraux *et al.* 1990). In the current study, the acquisition of systemic resistance to *A. solani* in tomato tightly correlated with elevated endogenous SA levels and the expression of the PRs genes. The minimum level of SA that is required to activate SAR in tobacco is approximately 0.33 µg g^{-1} FW (Yalpani *et al.* 1991) this conflict with our observation of SAR induction in tomato at foliar levels of 0.01 µg g^{-1} FW. and were unable to induce the expression of SAR. Following pathogen infection, increased levels of SA are known to occur (Enyedi, *et al.* 1992; Malamay *et al.* 1990 and Metraux *et al.* 1990). In our findings there was a fluctuation in endogenous SA levels of pretreated plants with chemical inducers then inoculated with pathogen *A. solani*) between the various treatments. Spletzer and Enyedi (1999) wondered that it was unclear why SA levels should fluctuate following plant pathogen infection from initial elevated SA levels. Plants that did not receive the chemical inducers exhibited endogenous SA levels following infection by *A. solani* and surpassed some inducers treatment levels in many cases either in resistant or susceptible cvs. It might be presumably, during early and later stages, pathogen serve as SAR inducer.

Regardless of the chemical inducers concentration used in PDA, there was no significant decrease in the radial growth of *A.solani* mycelium (not published). This indicates that SA does not have a direct antifungal effect on *A. solani*, but rather serves to trigger the signal transduction pathway that ultimately gives rise to SAR (Hunt and Ryals, 1996 and El-Mougy, 2002). Chemical-induced resistance to *A. solani* is likely due to the elicitation of a set plant defense responses. PR proteins (Vernooij *et al.* 1995) are considered to constitute one important portion of the induced defense responses employed by SAR (Enyedi *et al.* 1992; Hunt and Ryals, 1996; Van Kan *et al.* 1995). In spite of many reports approved that INA activates components of the SAR signaling pathway downstream of SA accumulation (Vernooij *et al.* 1995; Dong and Beer, 2000), our results demonstrated that there is considerable levels of endogenous SA accumulation. Although these levels were higher against other treatments but it continued greater significantly in resistant cv. (Tezier) than in susceptible (Castle Rock).

The development of SAR is associated with the induction of pathogenesis related (*PR*) gene expression. Increases in the endogenous SA levels in the pathogen-inoculated plants coincide with the increased levels of the *PR* gene expression and enhanced disease resistance (Sandhu *et al.* 2009) and plants are able to coordinate the expression of specific

PR genes in response to attack by relevant pathogens at the molecular level (Zhang et al. 2010). Several mechanisms that mediate the disease protection induced by certain chemicals have been described, including the direct inhibition of pathogen growth, blocking of the disease cycle (Fabritius *et al.*, 1997; Thompson *et al.*, 2000; Vicentini *et al.*, 2002), and the induction of plant resistance to pathogen infection (Dong and Beer, 2000; Nakashita *et al.*, 2003). Given the disease-progress-inhibiting activities of thiamine (vit. B_1) and riboflavin (vit. B_2) against fungal, bacterial, and viral pathogens, it would be unusual if these compounds acted as specific antibiotics. Media containing thiamine (vit.B_1, SA, and INA, did not inhibit the growth of *A. solani* on plates (data was done by authors in separate work). These results imply that these chemicals induce resistance in plants to infection by various pathogens. Broad-spectrum effects and the absence of direct effects on the pathogen are distinctive characteristics of other plant defense activators, including DCINA (Delaney, 1997), probenazole (Midoh and Iwata, 1996), probenazole derivatives (Yoshioka *et al.*, 2001), and brassinolide (Nakashita *et al.*, 2003).

Thiamine confers disease resistance through the priming of several plant defense responses, leading to a restriction of pathogen growth in planta and suppressed propagation of the inoculums. The maintenance of the resistance mimic status for a long period indicates that thiamine is a good candidate as a plant defense activation agent (Ahn, *et al.*, 2005). In addition, thiamine did not result in phytotoxicity at any of the tested concentrations. These results show that thiamine satisfies the requisites for an activator of plant SAR (Friedrich *et al.*, 1996), as previously suggested. Our results demonstrated that the higher accumulation levels of PRs due to exogenous application of thiamin (vit. B_1) against *A.solani* began at early stage after pathogen inoculation about 1-2 days, that's agreed with some suggestions of (Ahn, *et al.*, 2005) who found that the transcripts of all of the tested defense-related genes accumulated within 24 h after thiamine treatment, but the high transcript levels did not persist. However, following pathogen infection, SAR-related proteins were rapidly and strongly expressed in thiamine-treated plants, mirroring the expression patterns that occur during the interaction between resistant and susceptible host plants and pathogens.

Formulations of INA have been shown to be effective in decreasing susceptibility to pathogens and inducing PR proteins in several other plant species (Metraux *et al.*, 1991; Hijwegen and Verhaar, 1994; Kogel *et al.*, 1994; Nielsen *et al.*, 1994; Dann and Deverall, 1995; Vernooij *et al.*, 1995). According to colorimetric analysis in the present study, INA exhibited significantly higher levels in accumulation of *β*-1, 3-glucanase and chitinase in "Tezier" at 2nd day, peroxidase in resistant and susceptible to *A. solani* in the 2nd day and polyphenol oxidase in "Tezier" at the 2nd day and in "Castle rock" against *A. solani* in the 2nd - 4th days, that's confirmed the capability of this compound in induction of quantitative amounts of defensive proteins limits the progress of pathogen, this similar to Van Kan *et al.* (1995) whom they found that the spray of tomato leaves with INA formulation material alone apparently induced mRNAs for two PR proteins (osmotin and PR-4). INA is interpreted to act by moving rapidly into and systemically through plants, as shown by Metraux *et al.* (1991) using a radioactive form. The subsequent increases in the PR protein, chitinase, and in levels of resistance in parts of the plant remote from the point of application were concluded to be consequences of the accumulation of INA in these parts. This conclusion is supported here by the action of INA alone in raising activities of *β*-1, 3-glucanase, chitinase and peroxidase and resistance to early blight, in tomato seedlings, when contrasted with the effects of a control (H_2O) and untreated plants (pathogen only).

Salicylic acid (SA) played an important role in plant defense by the development of a systemic acquired resistance against pathogens (Ryals *et al.*, 1994) and by increasing antioxidant enzymes (Janda *et al.*, 1999). Exogenous applications of SA, either by direct injection or by spraying, have been reported to cause a multitude of effects on the morphology and physiology of plants (Pancheva *et al.*, 1996; Peng *et al.* 2004). In this series of experiments, we demonstrated that SA can activate a form of systemic resistance through accumulate a significant quantities of PR proteins against *A. solani* in greenhouse grown tomato plants. This was accomplished by providing 500 ppm SA directly to the shoot system of the plant, although Van Kan *et al.* (1995) reported that foliar application of SA to tomato has been attempted, but is an ineffective method for the introduction of SA to the leaf and did not result in accumulation of PR proteins. When we analyzed the PRs activity calorimetrically following SA treatment, we found the higher expressed levels of both, β-1, 3-glucanase and peroxidase in resistant "Tezier" and susceptible "Castle rock" at 48-h time point, but for polyphenol oxidase was at both 24- and 48-h time points, while for chitinase levels the increase was maintained throughout over the period of study up to 4th day. Induction of PR gene expression following SA application has also been demonstrated in tobacco, Arabidopsis, and cucumber plants (Metraux *et al.* 1990; Ward *et al.* 1991). Van Kan *et al.* (1995) report a similar finding after 24 h for an extracellular PR-1 transcript (P6) following SA feeding of an excised tomato leaf; however, this particular study made no

distinction between SA, and other inducers (vit.B_1, and INA) in PRs induction.

5. Conclusion

We have found that the accumulation patterns of chitinase, β-1,3-glucanase, peroxidase and polyphenol oxidase varies between early blight resistant cultivar (Tezier) and the highly susceptible variety, Castle rock. The activities of these defense enzymes were more in the compatible than the incompatible interaction of tomato with *A. solani*, suggesting that induction of these enzymes may be significant in symptom development in tomato shoots. That's supported by the higher endogenous levels of SA in resistant cv. compared to susceptible one, which the strong marker of systemic acquired resistance. In further investigations we plan to evaluate the effectiveness of chemical inducers in the field on some plant pathogens.

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Toxigenic Potential of Co-occurring Aflatoxin and Ochratoxin A Detected in Poultry feed on *Clarias gariepinus* Larvae

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Abstract: The worldwide contamination of poultry feeds with aflatoxins and ochratoxin A (OTA) independently and in co-occurrence has been reported in several countries. However, there is paucity of information on the co-occurrence of aflatoxins and OTA and their detection by immunoassay in Nigerian poultry feed. Fourty-seven locally formulated poultry samples collected from 13 locations within Southwestern Nigeria were analyzed for total aflatoxins (TA) and ochratoxin A (OTA) using the Immunoassay method. The potential toxicities of the samples were tested by the *Clarias gariepinus* day-old larvae bioassay. Approximately 98.2% samples were positive for TA and OTA with concentrations above the limits of quantitation. The ranges of TA and OTA in the samples were <4.0 µg kg^{-1} to 575 µg kg^{-1} and <2.0 µg kg^{-1} to 14.2 µg kg^{-1} respectively. Toxicity to *C. gariepinus* larvae was concentration dependent and 17, 21 and 7 samples containing the co-occurring toxins showed high, moderate and low toxicities respectively. On the average, 88.9% and 65.5% of the total samples had concentrations above the EU permissible limits for TA in immature and mature poultry feed respectively. The overall contamination risk for TA and OTA in samples in a significant (P = 0.01) decreasing order was: chick mash, broiler finisher, layers mash, broiler starter and growers mash.
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1. Introduction

The worldwide contamination of poultry feeds with aflatoxins and ochratoxin A (OTA) independently and in co-occurrence has been reported in Nigeria (Aletor, 1990), Morrocco (Kichou and Walser, 1993), United States (Schweitzer *et al*., 2001) and Turkey (Nizamlyoglu and Oguz, 2003), and in Argentina (Dalcero *et al*., 1998), India (Thirumala-Devi *et al*., 2002) and Greece (Vlachou *et al*., 2004), respectively. Also is the contamination of independent feeding stuffs involved in poultry feed formulation with aflatoxins and OTA (Aletor, 1990; Atawodi *et al*., 1994; Yazdanpanah *et al*., 2001; Egal *et al*., 2005; Zinedine *et al*., 2007). However, we could not lay hands on any data for the co-occurrence of aflatoxins and OTA and their detection by immunoassay in Nigerian poultry feed.

IARC in 2002 reaffirmed naturally occurring aflatoxins as members of the Group 1 carcinogens while OTA, a frequent natural contaminant of many foodstuffs including poultry feed, eggs and milk (Weidenborner, 2001), was classified as 2B by IARC evaluation in 1993. OTA induces nephrotoxicity, teratogenicity, carcinogenicity and immune-suppression in many animal species including poultry (Huff *et al*., 1974, 1975; Stoev, 1998; IARC, 1993). No animal species is resistant to acute aflatoxicoses. However, animal species respond differently in their susceptibility to chronic and acute aflatoxicoses. Smith and Hamilton (1970) reported that aflatoxin is a very potent hepatotoxin to young broiler chickens whereas Huff and Doer (1981) showed that the targeted effect of the combination of aflatoxins and OTA is on the kidney. The LD50 value of aflatoxins for most species ranges from 0.5 to 10 mg kg^{-1} body weight. Toxicity is influenced by exposure level and duration and environmental factors beside age, health and nutritional status (FAO, 2000).

Due to the above mentioned detrimental effects of these toxins, the European Union (EU) set the maximum tolerable levels for total aflatoxins (TA) in poultry feed and feeding stuffs meant for immature and mature poultry as 10 and 20 µg kg^{-1} respectively: the strictest standards for TA in the world (EC, 2003). Considering the need for appropriate regulation of mycotoxins in the Nigerian feed industry and the serious damaging effects caused by the dietary toxin combinations in poultry this research aimed at

surveying some states in Southwestern Nigeria for locally formulated poultry feed, detecting and quantifying TA (AFB_1 + AFB_2 + AFG_1 + AFG_2) and OTA contamination in the feeds. The potential toxicity of the contaminated samples was evaluated using *Clarias gariepinus* larvae bioassay.

2. Materials and Methods

2.1 Sample Collection

Five categories of poultry feed were collected between April and September, 2009 and examined in this study. The feed categories were chick mash, growers mash, layers mash, broiler starter and broiler finisher. The locations where the local poultry feed rations were formulated were surveyed and samples collected according to the categories above. The bags containing the raw materials (maize kernels, soybean meal, wheat offals and groundnut cake) from which the samples were formulated were stacked on each other on the very dusty cemented floors of the warehouses. The bags had been in store for at least 4 months prior to collection time. These conditions were enough to serve as avenue for entry of toxigenic fungi, their growth and consequent toxin liberation. For each sampled bag of feed (50 kg), three identical sampling sizes (1 kg each) from three different points of the bulk feed (top, middle and bottom) were taken at same time and mixed thoroughly to form the sample lot (3 kg per sample lot) for analysis. From the sample lot, 1 kg representative sample was taken and ground into fine powder with a Waring blender. Ground samples were stored at -4 ^oC until subsequent extraction and analysis.

Samples were collected from 13 locations in four states of Southwestern Nigeria after a preliminary survey. The states and representative locations were Lagos (Ikorodu, Iyana-Ipaja and Okoko), Ogun (Ijebu-Ode, Shagamu, Abeokuta and Ikenne), Oyo (Ibadan, Oyo, Moniya and Apata) and Osun (Ife and Oshogbo). Representative samples were labeled prior to grinding and analysis of samples for TA and OTA (October 2009 – May 2010).

2.2 Chemicals and Kits

ACS-grade methanol was purchased from Sigma-Aldrich (Germany). Validated Immunoassay [direct competitive Enzyme-linked Immunosorbent Assay (ELISA)] kits for Total Aflatoxins (B_1, B_2, G_1, G_2) and Ochratoxin A quantitation were purchased from Romer Labs® (Singapore).

2.3 Toxin Extraction and Quantitation by ELISA

A 20 g analytical sample was taken from each comminuted representative sample into a clean jar. To this jar 100 mL of 70% (v/v) methanol extraction solution was added and the jar sealed. The jar was shaken vigorously in a rotatory shaking incubator (150 rpm for 3 min) and then allowed to stand for 5 min. The top layer was filtered off into a clean glass bottle through 2-folds of Whatman #1 filter paper and the pH of the filtrate was tested. The extraction procedure was applied for both TA and OTA extraction from the feed matrix bearing in mind the need to pay full attention to sample preparation even if it is impossible to attain 100% certainty in determining mycotoxin concentration in a bulk lot (Whitaker, 2006).

Quantitation of TA (AFB_1 + AFB_2 + AFG_1 + AFG_2) and OTA in µg kg^{-1} were carried out following the procedures outlined in 96-well commercial AgraQuant® Total Aflatoxin Assay 4/40 kits and 96-well commercial AgraQuant® Ochratoxin Assay 2/20 kits respectively. The screened samples were read using the EL301 microwell reader (BIO-TEK® Instruments, Inc) at absorbance OD of 450 nm and 630 nm differential filters. The results were calculated using the Romer® Log/Logit spread sheet and interpreted with the Log/Logit regression model. The Limit of quantitation (LOQ) and Range of quantitation (ROQ) for TA were 4 µg kg^{-1} and 320 µg kg^{-1} respectively and 2 µg kg^{-1} and 40 µg kg^{-1} respectively for OTA. Repeated dilutions of 5 – 20 µg kg^{-1} were made on samples to extrapolate quantitation value above 320 µg kg^{-1} for TA.

2.4 Toxicity Assay

The Brine shrimp (*Artemia salina* L.) larvae bioassay for mycotoxin toxicity as described by Korpinen (1974) was modified slightly in the choice of the test organism and conditions. Day-old larvae of freshwater African catfish, *Clarias gariepinus*, otherwise called fingerlings were used in our study. The choice of *C. gariepinus* larvae was based on availability as well as similar morphology and physiology of *Clarias* to *A. salina*. Moreover, Gbore *et al*. (2010) reported the application of *Clarias* in mycotoxicity studies using Fumonisin B_1 as target toxin.

Actively motile day-old larvae were maintained under constant purified oxygen in clean oxygen bags filled with 150 mL tap water containing 0.002% (w/v) NaCl. Treatments were set up in a completely randomized design such that each oxygen bag contained 10 larvae. This was replicated in four sets per treatment (feed sample) to give a total of 200 oxygen bags and 2000 larvae. The fingerlings in each bag labeled against each test feed sample were exposed to 100 mg of the test feed sample. Positive control bags (P_1, P_2, P_3) contained larvae that were exposed to 5 µg L^{-1} pure aflatoxin, P_1; 5 µg L^{-1} pure ochratoxin, P_2; a mix of 2.5 µg L^{-1} each of pure aflatoxin and OTA, P_3. The concentration ratio of individual aflatoxin types in the 5 µg L^{-1} TA was 5:1:3:1 for AFB_1, AFB_2, AFG_1 and AFG_2 respectively. Negative control bags (N_1 and

N_2) contained larvae exposed separately to the two feed samples (L3 and F5) that contained < 4 µg kg^{-1} TA and < 2 µg kg^{-1} OTA respectively.

The exposed larvae were incubated at 25 ^oC for 24 h under oxygen. The number of dead larvae was recorded after the incubation period. Total loss of locomotive action of larvae was interpreted as death. The total number of fingerlings per bag was counted after freezing the bags to kill the survivors at -10 ^oC for 6 h. Results were recorded as High toxicity (H) when x < 75% mean mortality of larvae, Moderate toxicity (M) for 50 < x ≤ 75% mean mortality of larvae, Low toxicity (L) for 25 ≤ x ≤ 50% mean mortality of larvae and No toxicity (NT) when x = 0% mean mortality of larvae (Youssef, 2009).

2.5 Statistical Analysis

The results from individual analyses of samples are presented in µg kg^{-1} (ppb). The individual and combined mycotoxin concentrations were evaluated for feed categories for the comparison of the exposure risk of poultry to the toxic feed. The non-parametric Wilcoxon rank sum test (WRST) of Gad and Neil (1982) was used for this comparison at P = 0.01.

3. Results

A total of 47 samples were collected and analyzed for TA and OTA in this study. TA and OTA were detected at varying levels in all of the feed samples analyzed in this study except L3 (N_1) and F5 (N_2). These two samples (L3 and F5) had either TA or OTA concentration below their LOQs and they were from Ife and Apata respectively. All the feed types collected contained different quantities of the combinations of corn, groundnut cake, soybean meal, wheat offals, fish meal, sodium chloride and different kinds of additives including premixes, ferrous tabs, etc. Table 1 shows that the order of TA and OTA contamination in samples decreased in the order: chick mash, broiler finisher, layers mash, broiler starter and growers mash (P = 0.01). The summary of the analysis of poultry feed types for TA and OTA (Table 1) show that the growers mash had the lowest TA range of contamination (6.1 – 95.4 µg kg^{-1}) with 28.0 µg kg^{-1} as mean value while chick mash had the highest contamination range of 25.1–575 µg kg^{-1} and mean of 268.1 µg kg^{-1}. All feed categories had TA contamination of 100% except the layers mash which recorded 92.3%. The broiler finisher feed had the lowest range of OTA contamination (<2.0 – 14.2µg kg^{-1}) with mean value of 10.9 µg kg^{-1} while the range for chick mash samples was the highest (11.4 – 13.6 µg kg^{-1}). Only broiler finisher feed had a percentage contamination level lower than 100 (92.3%).

Table 1. Analysis of Total Aflatoxins and Ochratoxin A profiles in local poultry feed

Parameter	Poultry feed types				
	Chick mash *n = 8*	Growers mash *n = 9*	Layers mash *n = 10*	Broiler starter *n = 10*	Broiler finisher *n = 10*
Total Aflatoxin					
Range (µg kg^{-1})	25.1 – 575	4.7 – 95.4	<4 – 146.2	4.7 – 107.7	4.1 – 124.9
Mean value	268.1	28.0	49.5	33	63.9
% Positive	100	100	92.3	100	100
Ochratoxin A					
Range (µg kg^{-1})	11.4 – 13.6	6.1 – 14	3.2 – 13.9	6 – 13.7	<2 – 14.2
Mean value	12.5	11.0	11.2	10.5	10.9
% Positive	100	100	100	100	92.3

n = number of samples assayed

The independent analysis for TA and OTA contamination levels in the five categories of poultry feed, mean percentage mortality of the exposed larvae and the toxicity levels are presented in Tables 2 – 6. All the eight chick mash samples were contaminated with TA far above the EU permissible limits for immature poultry (Table 2) while the toxicity level studies expressed from the mean percentage mortality values showed that only 25% (2/8) of the samples that violated the EU limit caused moderate toxicities to *C. gariepinus* larvae. The other six samples where highly toxic. Positive control bags (P_1 and P_3) exhibited high toxicities whereas P_2 which contained 5 µg L^{-1} pure OTA showed low toxicity to the day-old larvae.

The data for the growers mash samples (Table 3) showed that only 44.4% of the samples had TA concentration levels above EU limits for mature poultry: an insignificant value (P = 0.01) as compared to the samples of other feed categories that violated the limits for mature birds. The toxicity levels of the violating samples range from moderate to high toxicity. From Table 4, 80% of the layers mash samples violated the EU regulation of TA levels in mature poultry feed. One particular sample coded as L3 (N_1) had TA level below the LOQ (4 µg kg^{-1}) for the method used. Toxicity levels of the violating samples ranged from moderate to high toxicities.

Table 2. Total Aflatoxins (TA) and Ochratoxin A (OTA) concentrations in chick mash samples and *C. gariepinus* toxicity

	Positive controls (n = 3) and Chick mash samples (n = 8)										
Analysis	P_1	P_2	P_3	C1	C2	C3	C4	C5	C6	C7	C8
$^+$TA	-	-	-	427.5*	230*	251.8*	470*	575*	25.1*	35*	130.3*
$^+$OTA	-	-	-	12.5	13.6	11.4	11.9	11.6	12	13.6	13.3
Mean % mortality	85	32.5	87.5	95	87.5	87.5	100	100	65	70	87.5
Toxicity	H	L	H	H	H	H	H	H	M	M	H

$^+$TA and OTA values are in (µg kg^{-1})
*samples above EU Permissible Limits for TA in immature poultry feed (% samples = 100%)
P_1: Positive control 1, P_2: Positive control 2, P_3: Positive control 3
H: High toxicity, M: Moderate toxicity, L: Low toxicity

Table 3. Total Aflatoxins (TA) and Ochratoxin A (OTA) concentrations in growers mash samples and *C. gariepinus* toxicity

	Growers mash samples (n = 9)								
Analysis	G1	G2	G3	G4	G5	G6	G7	G8	G9
$^+$TA	18.3	25.7*	4.7	6.1	18.4	18.1	25.6*	95.4*	40*
$^+$OTA	6.1	12.1	10	10	9.7	14	13	11.8	12.6
Mean % mortality	62.5	67.5	25	27.5	67.5	42.5	65	77.5	75
Toxicity	M	M	L	L	M	L	M	H	M

$^+$TA and OTA values are in (µg kg^{-1})
*samples above EU Permissible Limits for TA in mature poultry feed (% samples = 44.4%)
H: High toxicity, M: Moderate toxicity, L: Low toxicity

Table 4. Total Aflatoxins (TA) and Ochratoxin A (OTA) concentrations in layers mash samples and *C. gariepinus* toxicity

	Layers mash samples (n = 10)									
Analysis	L1	L2	N_1	L4	L5	L6	L7	L8	L9	L10
$^+$TA	20.8	20.5	<4	30.5	20	33	109.1	80	31.1	146.2
$^+$OTA	6.2	13.9	3.2	11.5	13.1	12.3	13.1	12.9	13.5	12.2
Mean % mortality	65	62.5	0	70	65	70	80	77.5	62.5	87.5
Toxicity	M*	M*	NT	M*	M	M*	H*	H*	M*	H*

$^+$TA and OTA values are in (µg kg^{-1})
*samples above EU Permissible Limits for TA in mature poultry feed (% samples = 80.0%)
N_1: Negative control 1 (L3)
H: High toxicity, M: Moderate toxicity, NT: No toxicity

Table 5. Total Aflatoxin (TA) and Ochratoxin A (OTA) concentrations in broiler starter samples and *C. gariepinus* toxicity

	Broiler starter samples (n = 10)									
Analysis	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
$^+$TA	76*	4.7	20.1*	6	20*	17.8*	22.2*	45*	107.7*	10.2*
$^+$OTA	13.2	2.8	13.1	6.5	13.3	13.7	12.8	11.4	13.1	5.5
Mean % mortality	75	25	60	27.5	60	60	60	72.5	77.5	32.5
Toxicity	M	L	M	L	M	M	M	M	H	L

$^+$TA and OTA values are in (µg kg^{-1})
*samples above EU Permissible Limits for TA in immature poultry feed (% = 80%)
H: High toxicity, M: Moderate toxicity, L: Low toxicity

Table 5 shows that 80% of the 10 broiler starter samples had concentrations above EU limits for immature poultry. The samples with levels above EU limits also showed moderate to high toxicities. Only 70% of the broiler finisher samples violated TA regulation in mature poultry feed by EU (Table 6). One sample known as F5 (N_2) had

OTA concentration below the LOQ (2 µg kg^{-1}) and TA concentration of 4.1 µg kg^{-1} thereby producing no toxicity to the larvae. Toxicity of the feed samples to *C. gariepinus* larvae was concentration dependent.

Table 6. Total Aflatoxin (TA) and Ochratoxin A (OTA) concentrations in broiler finisher samples and *C. gariepinus* toxicity

Analysis	Broiler finisher samples (n = 10)									
	F1	F2	F3	F4	N_2	F6	F7	F8	F9	F10
$^{+}$TA	79*	80*	75*	5.7	4.1	120.7*	18.3	56*	124.9*	75.2*
$^{+}$OTA	12.7	12.1	11.7	4.8	<2	13.2	10.4	14	14.2	13.9
Mean % mortality	77.5	77.5	80	25	0	87.5	62.5	75	87.5	77.5
Toxicity	H	H	H	L	NT	H	M	M	H	H

$^{+}$TA and OTA values are in (µg kg^{-1})
*samples above EU Permissible Limits for TA in mature poultry feed (% = 70%)
N_2: Negative control 2 (F5)
H: High toxicity, M: Moderate toxicity, L: Low toxicity, NT: No toxicity

4. Discussion

Subsistence poultry farming has become the major source of livelihood in some parts of Nigeria with the Southwestern and Eastern regions having more of these farms. Reports from the local farmers indicated the use of low quality grains which do not meet human consumption standards in poultry feed formulation. These grains showed moldiness, discolorations and numerous cracking from insect infestation. Quality poultry feed is necessary for the maintenance of physiological functions and animal defense systems against diseases and parasites. Traditionally feed quality has been specified on basis of the nutritional value of every individual feed component (Fink-Gremmels, 2004). This may not be true microbiologically as recorded in this study since it is expected that quality feed should be highly nutritious and at the same time free of any form of hazardous contaminants such as microbes, toxins and/or heavy metals.

The worldwide co-occurrence of aflatoxins and OTA in poultry feed formula and feeding stuffs as documented earlier has been upgraded for Nigeria by our study although TA occurred in higher concentrations than OTA in every feed sample. The low quantities of OTA present in the samples may be due to a low occurrence of OTA-producing fungi, unfavorable conditions for expression of OTA biosynthetic genes if toxigenic fungi are present or other fungal interactions and environmental conditions (Schmidt-Heydt *et al*., 2007; Wagacha and Muthomi, 2008). OTA contamination on its own may not necessarily pose any serious harm since it occurred in low quantities for all samples. But the high values obtained for TA in the samples with levels above EU limits and the percentage violating sample per feed type is alarming. It is logical to suggest that the individual feed components may have originally been contaminated with somewhat moderate levels of these toxins bearing in mind that cereal- and oil-based ingredients are suitable substrates for fungal growth and mycotoxin liberation. This is in line with the suggestions of Egal *et al.* (2005) and Zinedine *et al.* (2007) that toxin contamination of feed may be due to the presence of contaminating toxigenic fungi in individual feeding stuffs (corn, groundnut cake and wheat) or in finished product, feed processing environments and/or storage environs and conditions. This high level of TA may therefore pose a great risk to the poultry industry in terms of fowl death, reduced income, low egg production and quality, and administrative tension.

It is on record that the co-occurrence of OTA with aflatoxins could lead to higher degree toxicity in poultry (Huff *et al*., 1983; Petzinger and Ziegler, 2000). The day-old larvae may have died following the proposed cascade of events by Eaton and Gallager (1994), Creppy (1995), Riley and Norred (1996) and Benford *et al.* (2001) for the toxicity of co-occurring mycotoxins. The events may have progressed from metabolic activation of the toxin through DNA modification or inhibition of protein synthesis or altered membrane permeability and calcium transport disruption to cell deregulation, and cell death which was expressed in our study by the total loss of locomotive action of the larvae.

The observation from Table 2 where the positive control with 5 µg L^{-1} pure OTA (P_2) showed low toxicity as compared to the high toxicity of the other positive controls corresponds with the findings of Sokol *et al*. (1988) and this confirms that OTA alone even at relatively high concentrations is less toxic to this species of *Clarias*. Sokol et al. reported that OTA is less toxic in independent administration but when in

combination with aflatoxins exhibits a very high toxicity level that is dose dependent. The moderate to high toxicities observed in test experiments having moderate combinations of TA and OTA could have resulted from synergistic and potentiated effects of both toxins. Huff and Doerr (1981) reported a synergistic effect in a study of the combined effects of AFB_1 and OTA in broiler chicken. In further studies carried out by Huff *et al.* (1983) and Huff *et al.* (1988) they noticed that the toxic effects of aflatoxins and OTA combinations when compared to the toxicity expressed by some other mycotoxin combinations in poultry and pigs was found to be the most toxic. Similarly Sedmikova et al. (2001) reported that the co-occurrence of OTA with AFB_1 in same substrate even at very low concentrations was capable of inducing a potentiated mutagenic activity of AFB_1 in organisms as assayed by the *Salmonella typhimurium* Ame's test. Therefore our data confirm previous reports on combined toxicity of OTA and aflatoxins thereby adding to the data.

From the toxicity studies also, we are suggesting the likelihood that the type of aflatoxin present and the specific quantities in which they occur in a sample goes a long way in influencing the toxicity level induced when combined with OTA. This is deduced from the case of sample G6 which had TA and OTA concentrations of 18.1 and 14 µg kg^{-1} respectively. Considering these values which are almost similar to those of G1 and G5 one would have expected a moderate toxicity effect. Since G6 caused a low toxicity effect it could be that this sample contained more of the less toxic aflatoxin types such that they could not synergize with OTA to induce a moderate form of toxicity as we know from literature the decreasing order of toxicity in aflatoxin types (AFB1, AFG1, AFB2, AFG2) (Wogan *et al.*, 1971). This therefore calls for further analysis of the samples to determine the specific aflatoxin types and ratios.

Conclusively our study has shown that aflatoxin contamination of locally formulated poultry feed is very high in Nigeria unlike ochratoxin A contamination. We have also exposed the impending danger of poultry toxicity upon dietary consumption of aflatoxin and OTA in combination and suggested that toxicity may be concentration dependent for this duo depending on the type of aflatoxin present and the proportion of occurrence. This is the first report of the co-occurrence of aflatoxins and ochratoxin A in poultry feed from Nigeria by the Immunoassay method. Further investigations are underway towards the analysis of the poultry feed samples for other contaminating toxins, isolation and characterization of the mycotoxigenic fungi present.

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Estimating external demand functions for Egyptian exports of grapes In light of the current global economic variables

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Abstract: The research aim is to estimate the functions of foreign demand for Egyptian exports of grapes to the most important foreign markets, imported. These markets are the markets "the United Kingdom, the Netherlands, and Italy", considering that these markets are the main importing markets for Egyptian grapes, which absorbed about 69% of the amount of exports of Egyptian grapes during the period (2005-2009). The results showed that, an increase in export price of Egyptian grapes to the UK market by about 1% leads to a decrease in demand of about 2.412% of any commodity to be flexible in this high demand market. The cross demand elasticity's noted that the increase in the Egyptian grapes price as the main rival to Egypt market to the United Kingdom are (Spain, Germany, and the United States) is estimated at 1% lead to increased demand for Egyptian grapes about 1.521%, 1.140%, and 0.175%, respectively. These refer to the replacement relationship between grapes exported from these countries and the grapes exported from Egypt. The spending elasticity indicated that the power to increase the true total spending import of the United Kingdom on grapes by about 1%, leading to increased spending on Egyptian grapes about 0.792%, which indicates that the Egyptian grapes is a commodity necessary within the UK market. With regard to the Netherlands market, the elasticity of demand price on Egyptian grapes showed that, the increase in the price of grapes, about 1% leads to a decrease in demand of about 1.594%, which means that a product with elastic demand in this market. The cross demand of elasticity's noted to increase in the price of exported grapes, from these states as the main rival to Egypt which are Spain, Germany, and Greece, about 1% leads to an increase of demand on Egyptian grapes about 0.481%, 0.659%, and 0.572%, respectively. These referred to the replacement relationship between Egyptian grapes from one hand and the exported grapes from those countries on the other hand. The spending elasticity power shown that to increase the real total spending import of Dutch grapes about 1%, leading to increased spending on Egyptian grapes about 0.851%, which indicates that the Egyptian grapes is a commodity necessary within the Dutch market. While, noting that the results of estimating model (ADIS) with respect to price elasticity of demand on Egyptian grapes in the Italian market, that the increase in prices by about 1% leads to a decrease in demand of about 0.469%, which means that a product with inelastic demand of the Italian market. Nevertheless, noting that cross demand's elasticity that an increase in the price of grapes, from the States as the main rival to Egypt-Italian market which are (Spain, Germany, the Netherlands, and Israel) about 1% lead to changes in demand for Egyptian grapes about 3.193%, 1.490, - 0.244%, and 1.738% respectively. These indicate the replacement relationship of exported grapes from those countries in the one hand, and the exported grapes from Egypt in the other hand (excluding the Netherlands). This relationship is complementary, in the case of high export prices of Spain, Germany, Israel, and Egypt, respectively. It is clearly seen from the spending power of elasticity that, to increase the real Italian total import spending of grapes about 1%, leading to the increased expenditure on the import Italian Egyptian grapes by 0.814%, which indicates that the Egyptian grapes is a necessary commodity in the Italian market. The study recommended that, there is a need for attention to specifications of the required quality and conformity to international standards, and develop systems to ensure quality control. As well as, working on creating a strong export institutions and export high-efficiency, to study foreign markets and their needs in terms of quantity, quality and time of export. In addition to the study of world markets, competition for Egypt in the important markets, with the need to open new markets for exports of Egyptian grapes, and not rely mainly on a single market or a limited number of markets, as it turned out coefficiency of geographic concentration of the quantity of exports of Egyptian grapes, that there is a heavy concentration in the amount of exports of Egyptian grapes, which may displays these exports to the violent tremors if was one of these markets.

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Key Words: Egyptian Grapes exports, Almost Ideal Demand System (AIDS), Competitiveness capability, demand-price elasticity, cross elasticity, indicated elasticity.

Introduction:

Exports sector is the key sectors to finance programs and economic development plans. The development of Egyptian exports comes to the leading of issues of the state's interest, as it is one of the most important challenges facing economic policy makers in agriculture. The development of Egyptian agricultural exports linked to the extent of their ability to achieve competitive position of goods, which can have access to the international markets. Especially in the light of global rapid changes and the formation of the World Trade Organization (WTO), which allowed the opening of markets to foreign products and reduce the constraints imposed on it, whether quantitative or tariffs with the abolition of all types and forms of protection, and the quality of the commodity and the cost are allowing the entry into these markets. This has led the trend towards globalization and liberalization of international trade and the technological revolution and information to the race of states to competitiveness, growth and increasing the volume of exports through the production of items of outstanding quality, high specifications and low prices, the ability of countries and institutions to cope with these challenges on the internal management, production methods, and available resources, as well as, the overall investment climate. So that, the gains made by the States, due to the economical globalization, is not balanced, and commensurate with the gains of the ability of each country to the compatibility and integration with the new world order. Recent years have seen a sophisticated and clear in the course of Egypt's agricultural development, which is demonstrated by the features in the evolution of production productivity and areas of most agricultural crops, with the entry of new types and varieties in Egyptian Agriculture. In addition to, the adoption of producers to the patterns and methods of modern technological, goals to the transition of strategy from replacing the imports to the strategy of production for export.

Egypt's foreign trade characterized by continuing increase in the value of agricultural imports compared to exports, as the value of agricultural exports about 1443 million, representing 8.7% of the total value of Egyptian exports. While, the value of agricultural imports about 3509 million, representing 13.5% of total Egyptian imports. Thus, the deficit in agricultural trade balance is about 2066 million dollars with coverage reached 41.1% of total agricultural exports to the agricultural imports during the average period (2005-2009). To cover part of this deficit in agricultural trade balance is required the maximizing the returns of the most important Egyptian agricultural exports, particularly horticultural crops, including the fresh grapes, which represents an important place among the most important items of Egypt's exports of fresh fruit. Where, the average total production is about 1488 thousand tons, representing 2.1% of the amount of global production of grapes. While, the average amount of exports of about 38.45 thousand tons, representing 2.58% of the total amount of the Egyptian production of grapes, and the value of exports of Egyptian grapes about 59.19 million dollars, representing approximately 32.21% of the value of fresh fruit exports, which amounted to 189.64 million dollars for the same period the previous average.[10]

Research problem:

Grapes occupy an important place among the varieties of Egypt's exports of fresh fruit. Despite the fact that recent years have seen significant improvement in the production and export situation of grapes in Egypt, but the exported quantities of it are still below the desired level. The amount of Egypt's exports of grapes represented 2.58% of the total production quantity to the average period (2005-2009). Egyptian exports of this crop, is facing stiff competition from many countries in most major markets imported Egyptian grapes. These may lose Egypt to its foreign markets, and the opportunity for countries competes to win these markets, which lead to lower revenues from the export of Egyptian grapes. Thus, increase the negative impact on the trade balance of Egyptian agricultural and economic development, especially in the light of the circumstances and the current international economic variables.

Research objective:

The research objective is to estimate the function of foreign demand for Egyptian exports of grapes to the most important foreign markets, particularly "the United Kingdom, the Netherlands, and Italy," considering that these markets are the main importing markets for Egyptian grapes. Through the study of the current reality of those exports and knowing of the most competitive countries the entry of the Egyptian exports grapes to these markets.

Research method and data sources:

In order to achieve the research goal, the method which was used is a descriptive and quantitative analysis to address the development of Egyptian exports of grapes during the period (1990-2009). The geographical distribution in global imported markets during the average period (2005-

2009) was used as the application form optimal Almost Ideal Demand System (AIDS) for Egyptian grapes, with the introduction of restrictions when estimating the model, demand functions on grapes imported into the Egyptian market study. This has been obtaining the necessary data for the area, productivity and total production from the Central Department of Agricultural Economics, Ministry of Agriculture and Land Reclamation, while the obtained data on the form of the United Nations site on the Internet http://comtrad, un.org/db GRAPES Fresh (0806100000)

As well as the site of the Food and Agriculture Organization "FAO" http://www.FAo.org

The World Bank Web site, on the Internet * http://www.albankadawli.org, the site of the Central Agency for Public Mobilization and Statistics on the Interne Http://www.Capmas.gove.eg, and the site and the Egyptian Ministry of Economic Development on the Internet http://www.mop.gov.eg As well as some data on the dates of export of Egyptian grapes, grape source of competitive countries, and that the Office of the European Commission in Cairo in 2010.

Description of the pattern analysis:

Application of optimal model is different from the other traditional models by estimating the demand that it takes into account the differences in the sources of goods. Also, it includes special restrictions on demand functions of the sources of goods, and explains the changes in demand and demonstrates the extent of competition between different sources, and provides the requirements of the economic policy of the estimates of the degree of response of demand prices and spending on imports. As it is to get rid of the problems of bias in the compilation of the sources of import and the expenditure function in the model reflect the behavior and the pattern of imports, which separates between import sources. It can be identified as the most important factors affecting it, and analyze the competitive relationship between the sources of import. The model is based on the value of expenditure on the item of any share of the total expenditure on the item instead of the quantity of each commodity separately.

This model has been provided by Deaton, Mulbauer "[2], [15]. The model is a flexible model is easy to use, as it is more applicable in economic studies. it is assumed when applied in economic studies two propositions: first, the assembly-level item It does not discriminate in this case between goods, according to imported sources, an assumption is possible if commodity prices change by the same percentage. But it seems a difficult assumption in the exports of agricultural commodities for reasons including the differing quality of products and tariffs, varying modes of formulas of contracts, the different services, conservation and transport of these products. The second assumption is the complete separation of goods, according to sources, import and this is may be contrary to logic. Due to the importance of differentiating between sources of imports in the analysis of demand for imports, some economic studies suggested the use of this form that is where the distinction between the sources of imported goods without restriction completes separation. Assuming that the expenditure function with utility U, which assumes a verifying between the goods according to different sources, can be derived form as follows:

$$\text{Ln } [E(P, U)] = (1-U) \text{ Ln } [a(P)] \text{ U Ln } [b(P)] \quad \ldots\ldots(1)$$

$$\text{Ln } [a(P)] = \alpha_0 + \Sigma \alpha_k \text{ Ln } P_k + \tfrac{1}{2} \Sigma_k \Sigma_j \gamma_{kj} \text{ Ln } P_k \text{ Ln } P_j \quad \ldots\ldots(2)$$

$$\text{Ln } [b(P)] = \text{Ln } [a(P)] + \beta_0 \prod_k P_k^{\beta_k} \quad \ldots\ldots(3)$$

Bringing equations (2.3) in equation (1) spending function can be formulated as follows:

$$\text{Ln } [E(P,U)] = \alpha_0 + \Sigma \alpha_k \text{ Ln } P_k + \tfrac{1}{2} \Sigma_k \Sigma_j \gamma_{kj} \text{ Ln } P_k \text{ Ln } P_j + \beta_0 \text{ U} \prod_k P_k^{\beta_k} \quad \ldots(4)$$

By differentiated Ln [E (P, U)] for the price of Ln P_i, it can get a share of the imported commodity spending W_i are as follows:

$$\frac{\partial \text{Ln } [E(P,U)]}{\partial \text{Ln } P} = \frac{P_i q_i}{E(P,U)} = W_i \quad \ldots\ldots(5)$$

therefore can be re-formulation of equation (4) as follows:

$$W_i = \alpha_i + \Sigma_j \gamma_{ij} \text{ Ln } P_j + \beta_i \text{ U } \beta_0 \prod_k P_k^{\beta_k} \quad \ldots\ldots(6)$$

Solving equation (4) for the benefit of (U) and substituted in the equation (6) can be obtained on the following:

$$W_i = \alpha_i + \Sigma_j \gamma_{ij} \text{ Ln } P_j + \beta_i \text{ Ln } \left(\frac{E}{P_{index}} \right) \quad \ldots\ldots(7)$$

Where:

$$\text{Ln } (P_{index}) = \alpha_i + \Sigma_k \alpha_k \text{ Ln } P_k + \tfrac{1}{2} \Sigma_k \Sigma_j \gamma_{kj} \text{ Ln } P_k \text{ Ln } P_j \quad \ldots(8)$$

The P_{index} is a non-linear and faced difficulty in the estimation had therefore replaced the index by the Engineering Stones Price Index is as follows:

$$\text{Ln } (P_{spi}) = \Sigma_i W_i \text{ Ln } P_i \quad \ldots\ldots(9)$$

Since the W_i refers to the percentage of expenditure, as it represents the dependent variable in the equations, the use of this record may cause some immediate problems in the model equations, so delays are used as follows:

$$\text{Ln } (P_{spi}) = \Sigma_i W'_i \text{ Ln } P_i \quad \ldots\ldots(10)$$

Where:

$$W'_i = \tfrac{1}{2} (W_{it} + W_{it-1}) \quad \ldots\ldots(11)$$

Note that it can be considered P_{index} an approximation for record number P_{spi} in case of duplication of the

pace of Multicolinearity high prices and, hence, the equation becomes (7) as follows:

$$W_i = \alpha_i + \Sigma_j \gamma_{ij} \text{Ln } P_j + \beta_i \text{ Ln } \left(\frac{E}{P_{spi}} \right) \text{…….....(12)}$$

That is under special conditions, to the demand of equation (12), which is represented in:

- Terms of Additively $\Sigma_i \alpha_i = 1, \Sigma_i \gamma_{ij} = 0, \Sigma_i \beta_i = 0$
- Terms of Homogeneity $\Sigma_j \gamma_{ij} = 0$
- Terms of symmetry $\gamma_{ij} = \gamma_{ji}$ for $i \neq j$

The importance of these conditions is that, it makes the model consistent with the theory of demand, which guarantees the conditions added a condition that the total spending = 1 ($\Sigma_i \quad W_i = 1$). While, the conditions of homogeneity guarantees the homogeneity of demand countries, and the conditions of parity check condition Slutsky Condition.

where: $\alpha,\beta,\gamma\iota$ indicates to the parameters of the function, P_i is the price of the commodity from source i, a (?), B (?) are functions in the parameters of the function and price, m number of sources of export item, W_i share of the imported commodity from spending, Pi, qi are price and quantity of the item from the source i, respectively, E is total expenditure on the item from all sources, P_{index} the standard price index, and Pspi the standard record of Stone.

Calculates the price, spending power and cross elasticity's of demand as follows:

- Price and cross elasticity, take the matrix (m × m) $\varepsilon_{Own,Cross} = -\delta_{ij} + (\gamma_{ij} / W_i) - \beta_i (W_j / W_i)$

Self price elasticity (diagonal matrix)

Cross price elasticity (outside diameter) ($\delta_{ij} = 1$, where $i = j$)

($\delta_{ij} = 0$, where $i \neq j$)

- Flexible spending power $\varepsilon_{expend} = 1 + (\beta_i / W_i)$

To validate the results, it is measurement by the relationship between the spending elasticity's of the imported commodity-weighted share of expenditure as follows:

$\Sigma_i W_i \varepsilon_{expend} = 1$

The autocorrelation test has been identified using "Breusch Godfrey". For the problem of non-homogeneity test using the error limit Engel test, and a problem is detected non-normal distribution reduce the error by using the Jarque-Bera test, in the absence of the significance problem, there is no standard formula. To estimate the model parameters of the equation no. (12) Use the method of Zellner to solve, Seemingly Unrelated Regression (SUR).

Results and discussion

1. The development of the most important variables affecting Egypt's exports of grapes during the period (1990-2009):

1.1. the development of fruitful area and productive feddan and total production of grapes:

It is clear from the overall time trend equations of numbers (1.2, 3) table (1), that there is a general trend of increasing statistically significant at the level of probability of 0.01 in the fruitful area, and productive fadden ($4200m^2$). The total production of Egyptian grapes was about 4.93 thousand fadden, 0.29 tons/ fadden, 60.89 thousand tons, with annual rates increase amounted to about 3.56%, 3.29%, and 5.06% of the average fruitful area, fadden production and total production of grapes, which recahed about 138.42 thousand per fadden, 8.82 tons/fadden, 1203.45 thousand tones, respectively. This is reflecting the adjusted coefficient of determination average value R^{-2}, that the changes reflected in the time element is responsible for 98%, 93%, and 97% of the changes in the fruitful area and productive fadden and total production of Egyptian grapes respectively during the period (1990-2009).

1.2. The evolution of the quantity and value and price of Egyptian exports of grapes:

Studying the evolution of the quantity, value and price of exports of Egyptian grapes during the mentioned earlier period, the time trend equations numbers (4.5, 6) table (1) indicated that, these variables have been taken an increasing and statistically significant general trend at 0.01 level of about 5.25 thousand tons, 7.15 million dollars, 38.93 dollars / ton, with increasing annual rates of about 30.67%, 36.11%, and 5.45% of the average quantity and price value of Egypt's exports of grapes, which amounted to about 17.12 thousand tons, 19.8 million U.S. $ and 714.30 U.S. $ / ton respectively. The adjusted coefficient of determination R^{-2} reflects the value of that the changes reflected in the time element is responsible for 66%, 52%, and 47% of the changes in these variables, respectively.

1.3. the development of the quantity and value of world imports and the price of grapes:

The time trend equations numbers (7.8, 9) table (1) indicated to a significant and confirmed statistically increase at the level of probability of 0.01 in the quantity, value and the price of world imports of grapes was about 134.62 thousand tons 289.46 million U.S. $ and 38.85 U.S. $ / ton, with rates of annual increase amounted to 4.87%, 7.87%, 2.79% of the average quantity, value and the price of the world imports of grapes, which are estimated at 2762.84

thousand tones, 3675.87 to $ 1391.16 $/ton, respectively. The adjusted coefficient of determination R^{-2} reflects the value of that changes reflected in the time element is responsible for 97%, 92%, and 86% of the changes in the quantity and value of world imports and the price of grapes, respectively, during the period (1990-2009).

Table (1): equations of general time trend of the evolution of the most important variables affecting Egypt's exports of grapes during the period (1990-2009)

The dependent variable 'Y'	Number equation	The unit of measure	The constant term $\propto$	Regression coefficient $\hat{\beta}$	average	**Adj. R-Sq**	**T $\hat{\beta}$**	**F Test**	Annual change rate%	The level of significance
Area grape fruit	1	Thousand fedden	12.09	4.93	138.42	0.98	26.13	682.78	3.56	0.01
Food productivity	2	Tons/ acre	6.38	0.29	8.82	0.93	22.57	509.41	3.29	0.01
Total production	3	Thousand tons	732.54	60.89	1203.45	0.97	21.86	477.87	5.06	0.01
The amount of exports	4	Thousand tons	- 23.45	5.25	17.12	0.66	9.55	91.22	30.67	0.01
The value of exports	5	Million dollars	- 27.18	7.15	19.80	0.52	4.12	16.98	36.11	0.01
Export price	6	Dollar/ton	336.60	38.93	714.30	0.47	3.17	10.07	5.45	0.01
The amount of world imports	7	1000 ton	1745.19	134.62	2762.84	0.97	19.48	379.49	4.87	0.01
The value of world imports	8	Million dollars	1476.32	289.46	3675.87	0.92	10.56	111.53	7.87	0.01
World import price	9	Dollar/ton	1019.46	38.85	1391.16	0.86	7.34	53.89	2.79	0.01

Where:

A diJ R^{-2} =Adjust coefficient of determination.

$\hat{\beta}$ T= T value of the estimated regression coefficient.

F test calculated for the model.

Source:

1 - Ministry of Agriculture and Land Reclamation, Economic Affairs Sector, Central Department of Agricultural Economics, Agricultural Economics Bulletin, Nos. (1990-2009).

2. http://www.comtrad.un.org/db.

3. http://www.FAO.org.

4. http:// www.Capmas.gov.eg.

2. Geographical distribution and market share for exports of Egyptian grapes in the most important foreign markets during the period (2005-2009).

The study of foreign markets, imported Egyptian grapes, and the geographical distribution of these exports is important; in order to identify priorities and policy directions for the export of this crop in the future and work to improve its competitiveness in foreign markets.

2.1. The geographical distribution of Egyptian grapes exports:

Reviewing the geographical distribution of grapes Egypt's exports during the average period (2005-2009), it was found that, it is distributed to different markets of the world, which can be divided in terms of the relative importance of the quantity exported about fifteen countries, in addition to a range of other countries less relative importance. The average annual quantity and value of imports of these countries (the fifteen) are about 36.99 thousand tons 57.56 million dollars, representing approximately 96.22%, 97.24% of the average quantity and value of total exports of Egyptian grapes. Moreover, about 38.45 thousand tons 59.19 dollars respectively to the average of the same period, as is clear from Table (2). This made the UK market in the first place among the most important importer of grapes Egyptian quantity and value of exports amounted to 13.95 thousand tons 23.57 Million dollars, respectively, representing approximately 36.30%, 39.82% of the average quantity and value of Egypt's total exports of grapes for the same period. While the markets occupied of "the Netherlands, Italy, Belgium, Russia, and Germany," the second to sixth places the exports

amounted to 7.41, 5.12, 2.83, 1.94, and 1.56 thousand tons, representing approximately 19.28%, 13.34%, 7.37%, 5.06%, and 4.06 % of the average amount of total exports of the Egyptian grapes. That mean that these markets next to the British market accounted for around 85.41% of the average amount of exports of Egyptian grapes for the same period. While the value of imports of these markets of Egyptian grapes about 10.04, 9.68, 5.37, 2.69, and 2.76 million USD representing approximately 16.96%, 16.36%, 9.08%, 4.56%, and 4.67% of the average value of total exports of the Egyptian grape, which means that these countries together beside the United Kingdom account for about 91.45% of the average value of Egypt's exports of grapes during the same period. As indicated data in the same table that, about 10.81%, 5.79% of the average quantity and value of total exports of Egyptian grapes arrangement is in the imports of the markets of "United Arab Emirates, Kuwait, Sudan, Saudi Arabia, South Africa, Ireland, Austria, Singapore, and The United States, and about 3.78%, 2.76% of the total amount and value of exports of Egyptian grapes is in the imports of other markets.

Calculating the coefficient of "Jenny Hirschman" to the geographical concentration of quantity and value of Egypt's grapes exports were approximately 46.35 and 50.88 respectively, which confirms the focus of Egypt's grapes exports in a limited number of countries, accounting for markets, "the United Kingdom, the Netherlands and Italy," about 69% of the amount of these exports, which requires the need to work on opening new markets in one hand, and try to increase the quantities of grapes exported to other countries with low market share, particularly Arab nations on the other hand.

2.2. market share for exports of Egyptian grapes:

Studying the market share of the Egyptian importing grapes exports to the foreign markets, it was necessary, in order to identify the extent to which the entry by force of these exports to these markets and the possibility of increased export efforts to increase the market share of the Egyptian grapes to these markets. It is clear from Table (2) that, there are countries characterized by low ratio of market share with high price and its import from grapes such as "United Kingdom, the Netherlands, Belgium, Russia, Germany and Ireland,". Where, the total market share of these countries, respectively are, 5.47, 2.79, 3.03, 0.57, 0.47 and 3.41 respectively, which means that there is a possibility to increase exports Egyptian grapes to these markets, taking into account the conditions of export to these markets, and consumer taste, and suitable dates for the export of systems, the quality control of exports. Also, there are countries characterized by high proportion of market share and prices of export with high and low import capacity markets, such as Italy, South Africa. Where, the total market shares of these markets, 18.59, and 29.35 respectively, during the average period (2005-2009), which means the need to preserve the exported quantities to such markets. While, the markets of Austria, Singapore and the United States of America, are characterized with small market share and high prices of Egyptian grapes imports, which means the need to increase the exported quantities of grapes to these markets to take advantage of higher prices, especially since these markets with a large import capacity, particularly the United States, to be combined with the marketing studies to identify the consumption patterns of these markets. As shown by the data in the same table that, the markets of all Arab countries characterized by a low price import of Egyptian grape, due to a decline in transport costs due to the proximity with Egypt. Where, the market share for grapes exports to United Arab Emirates, Kuwait, Sudan, and Saudi Arabia reached to 3.58, 9.94, 2.19, and 1.49 respectively during of the study period. In general, we should focus on the markets of Arab countries, where the advantage of increasing the capacity of these countries from grapes imports, as it does not require complex conditions for export compared to the European Union and the United States markets, in addition to the consumer acceptance of the Arab to the varieties of Egyptian grapes.

3. Estimate the function of foreign demand for Egyptian grapes in the most importing foreign markets:

The markets "the United Kingdom, the Netherlands, and Italy," are the most major overseas markets importing Egyptian grapes, as it absorbed those three markets around 26.49 thousand tons, representing 69% of the total amount of Egyptian grapes exports during the average period (2005-2009), amounting to 38.45 thousand tons, with a total value of about 43.29 million dollars, representing approximately 73.14% of the total value of Egyptian grapes exports in the same period average, amounting to about 59.19 million dollars. The following is a presentation to estimate the function of foreign demand for Egyptian exports of grapes for these markets using the demand form optimal (AIDS):

Table (2): geographical distribution and market share for the most important importer countries of Egyptian grapes during the period (2005-2009).

Country	exported Quantity (ton)	%	Exported value (thousand dollar)	%	Exported price (dollar/ton)	The total amount imports of the State	% world imports	Market share
UK	13956	36.30	23572	39.82	7.13	255181	7.13	5.47
The Netherlands	7414	19.28	10039	16.96	7.40	264921	7.40	2.79
Italy	5127	13.34	9685	16.36	0.77	27584	0.77	18.59
Belgium	2832	7.37	5373	9.08	2.61	93417	2.61	3.03
Russia	1947	5.06	2697	4.56	9.48	339429	9.48	0.57
Germany	1561	4.06	2765	4.67	9.26	331619	9.26	0.47
United Arab Emirates	982	2.56	539	0.91	0.77	27440	0.77	3.58
Kuwait	720	1.87	490	0.83	0.20	7245	0.20	9.94
Sudan	687	1.79	475	0.80	0.87	31276	0.87	2.19
KSA	521	1.36	255	0.43	0.97	34874	0.97	1.49
South Africa	490	1.27	549	0.93	0.05	1669	0.05	29.35
Ireland	340	0.88	470	0.79	0.28	9957	0.28	3.41
Austria	250	0.65	398	0.67	1.58	56522	1.58	0.44
Singapore	93	0.24	117	0.20	0.39	13871	0.39	0.67
USA	75	0.19	134	0.23	13.24	473927	13.24	0.01
The rest of the world	1452	3.78	1634	2.76	45.00	1611560	45.00	0.09
Total World	38447	100	59192	100	100	3580492	100	1.07
Gini coefficient *	46.35		50.88		--	--	--	--

Sources : 1. http://www.comtrade.un.org/db.GRAPES Fresh, SITC. Rev. 2 code (0806100000).
2. http: //www.FAO.org

* Gini-Hirchman Coefficient is used in the calculation of the degree of geographical concentration of the state exports which takes the following mathematical formula:

$$C_j X = 100\sqrt{\sum [x_{ij} / x_i]^2}$$

Where: X_{ij} indicates to exports or imports of the State (i) of Item (X) to state (j), xi total exports or imports of the State (i) of Item (x), this factor reach to the maximum value, by (100) in the case of exported to one country only. While this rate is less, whenever exports of the commodity distributed on a large number of countries. "Michaely" see that the coefficient of geographic concentration is considered high if it is larger than (40), which means that the occurrence of any severe price fluctuations in the value and quantity of the consequent negative effects on the economies of the exporting country foreign trade.

3.1. UK market:

3.1.1. The most competitive countries for the exports of Egyptian grapes within the UK market:

The UK occupies the fifth place among importers of grapes in the world, after the United States of America, Russia, Germany and the Netherlands. Where the volume of UK grapes imports about 255.18 thousand tons, representing 7.13% of the average amount of grapes global imports about 3580.49 thousand tons during the average period (2005-2009). While, the volume of Egyptian grapes exports to the UK market about 13.95 thousand tons, representing approximately 36.30% of the average amount of Egyptian exports of grapes, and about 5.47% of the average quantity of imports of the United Kingdom of grapes during the same average period. Spain, Germany, and the United States of America are considered as the major competitive countries for the entry by force of the Egyptian grapes exports to the UK market. Where the percentage of imports to the United Kingdom of grapes from these countries is about 13.12%, 7.09%, 6.45%, respectively, during the average period (2005-2009), and is competitive of grapes exports from these countries for the exports of Egyptian grapes in the UK market "competition," where more than the time period for grapes export from these countries about half of the season of Egyptian grapes export to the UK market, which continues for a period of (6) months starting from May and ends in October, the same time period for American grape exports to market. While the Spanish and German grapes exports to the UK market continue over almost the months of the year. It should be noted that there are many countries competing of Egyptian grapes in the markets of the United Kingdom, but "the particial competition," a competition that you are in a period of time less than about or equal to half of the season export of Egyptian grapes to the market, or about

three months. Those States includes "Morocco, Greece, Chile, Peru, Argentina, and India," where the exports of those countries of grapes to the UK market at the beginning of January until the mid-June, with the exception of exports of Indian grapes, that begins with the months of March to July. Nevertheless, the quantity is being very small during the month of June and July which does not affect the exports of Egyptian grapes in this market. Despite the presence of Israeli grapes in the United Kingdom during markers the season of exporting Egyptian grapes to the same market, but the supplied quantities are very small to affect the Egyptian grapes this market. On the other hand, despite the fact that about 23% of UK imports of grapes come from south Africa, but it is not considered one of the competitive countries to the exports of Egyptian grapes in this market, and to different export seasons where there are grapes from South Africa in the UK market at the beginning of November until April, while the Egyptian grapes as the above mentioned before, from May to October in the UK market.

3.1.2. Estimating demand functions on Egyptian grapes within the UK market:

Table (3) illustrated the demand for Egyptian grapes market function to the UK using the form "AIDS", showing the absence of standard problems that can affect the efficiency of the model, which is the autocorrelation, heterogeneity, and non-normal distribution. This has been confirmed non-significant of "Wald" Test, concerning the add limitations, consistency, uniformity, and non negativity. As can be seen from the same table that, the grapes prices in this market for all countries that are the main rival to Egypt, (Spain, Germany and the United States of America). In addition to the total true expenditure of imports grapes of the UK explains about 75% of the changes that occur in the proportion spending on Egyptian grapes market to UK. While the rest of the changes due to factors other than the measured function, and according to the adjusted value of the coefficient of determination Adj. R^{-2}.

Table (3) the results estimates of model "AIDS" to the demand for Egyptian grapes in the UK market during the period (1990-2009).

Country		α	LnP1	LnP2	Lnp3	LnP4	Ln(E/p) spi	Adj. R-sq	S.E of Reg	Auto	Hetro	Non-Norm
Egypt	Coffi	1.701	-0.140	0.082	0.107	-0.011	-0.054	0.749	0.011			
	T.Stat.	5.021	-6.120	3.970	7.55	-0.1325	-4.218			1.236	1.181	0.077
	Prob.	0.000	0.000	0.000	0.000	0.708	0.000			0.119	0.135	0.859
Spain	Coeffic.	0.975	0.071	0.145	-0.110	-0.114	-0.029	0.815	0.017			
	T.Stat.	2.513	2.194	3.508	-2.760	-3.909	-1.421			0.151	0.241	0.297
	Prob.	0.007	0.010	0.000	0.005	0.000	0.117			1.080	1.306	1.169
Germany	Coeffic.	1.136	-0.140	-0.027	0.072	0.145	-0.076	0.718	0.121			
	T.Stat.	3.111	-4.900	-1.113	2.451	2.724	-2.515			0.087	1.171	1.009
	Prob.	0.000	0.000	0.097	0.007	0.000	0.002			0.731	0.540	0.742
United states	Coeffic.	-2.101	0.370	-0.181	-0.116	0.020	0.147	0.681	0.124			
	T.Stat.	-5.317	6.490	-5.420	-5.111	0.748	7.489			0.191	1.163	1.093
	Prob.	0.000	0.000	0.000	0.000	0.462	0.000			1.454	0.661	1.137

Lnp1 - LnP4: the logarithm of export prices in dollars per ton of grapes, for "Egypt, Spain, Germany, the United States" respectively in the British market during the period (1990-2009).

Ln (E / P) spi: logarithm of total expenditure on the British import grapes, in thousands of dollars during the period (1990-2009).

Adj.R-Sq: adjusted coefficient of determination, S.E of Reg standard error of regression.

Auto: LaGrange multiplier for the self-correlation.

Hetro: LaGrange multiplier for instability of the variance.

Non-Norm: LaGrange multiplier for the non-normal distribution reduces of limit of the error.

Source: http: // www. Comtrade. Un.org/db. GRAPES Fresh, SITC. Rev. 2 Code (0806100000).

It is clear from Table (4) that the price elasticity of demand on Egyptian grapes within the UK market, indicating that the increase in prices by 1% which leads to a decrease in demand by 2.412%, which means that it is a product with elastic demand in this market. While recalling cross demand elasticity's that an increase in the grapes price, exported from the competitive countries to Egypt which are (Spain, Germany, and the United States) is estimated at 1% leads to increased demand for Egyptian grapes about 1.521%, 1.14%, and 0.175%, respectively, which refers to the replacement relationship between the exported grapes from these countries on the one hand, and between the Egyptian

grapes on the other hand. As can be seen that the spending power of elasticity to increases the total actual expenditure of the UK on grapes by about 1%, leading to increased spending on the Egyptian grapes by 0.792%, which may indicate that the Egyptian grapes is a necessary commodity in the UK market.

3.2. The Dutch market:

3.2.1. The most competitive countries for the exports of Egyptian grapes within the Dutch market:

The Netherlands is the fourth importer of grapes in the world, where the volume of import from grapes about 264.92 thousand tons, representing 7.40% of the average amount of global imports of grapes to the average period (2005-2009). The volume of Egyptian exports of grapes to the Dutch market is about 7.41 thousand tons, representing 19.28% of the average amount of Egypt's exported grapes, and about 2.79% of the average amount of imports grapes the Netherlands during the same average period.

Table (4): elasticity model of "AIDS" for the UK market on the grape.

Country	Price, cross elasticity				Expenditure elasticity
	Egypt	Spain	Germany	United States	
Egypt	-2.412	1.521	1.140	0.175	0.792
Spain	0.188	-0.857	-0.207	-0.279	0.907
Germany	-1.317	-0.109	-0.281	1.196	0.895
United States	0.751	-0.661	-0.490	-1.138	0.362

Source: table no.(3) in the current study.

Spain, Germany, and Greece are the major competitives for the entry of the Egyptian grapes exports to the Dutch market, where the percentage of grapes imports from these countries about 16.53%, 11.82%, and 8.71%, respectively, during the average period (2005-2009). The grapes exports from these competitive countries for the exports of Egyptian grapes within the Dutch market are "total competition" where the season continues, to export grapes from Spain and Germany to this market almost all year. However exports of Greek grapes to Dutch market from June until December while, the Egyptian grape in the beginning of May until September. While, the Chile, Morocco, Peru, Argentina, Mexico, and India, are competing to exports of Egyptian grapes within the Dutch market as "a partial competition ", where the grapes imported from those States to the Netherlands start from January until June. Also the American and Belgian grapes imported throughout the season of exporting the Egyptian grapes, but its quantity was very small to affect the exports of the Egyptian grapes, and it can not be replaced in full replacement in this market. As well as the Italian grapes are in the Dutch market but the majority of exports grapes Italian be Krmeson verity Red which preferred by Dutch consumer relatively. Despite the fact that 13.72% of the imports of the Dutch market for the grapes come from South Africa, but the grapes of South Africa is not to compete with the grapes of Egypt, where export grapes of South Africa to the Dutch market at the beginning of November to March, while exports of the Egyptian grapes are from the beginning of May to the end of September, and notes that all competitive countries to the exports of Egyptian grapes especially total competitive, are the countries in the European Union, which refers to the intensive competition faced by the exports of Egyptian grapes in the market Dutch.

3.2.2. Estimation of demand functions on Egyptian grapes within the Dutch market:

Table (5) illustrated the demand function of the Egyptian grapes within the Dutch market, using the model of "AIDS", showing that there is no standard problems can affect the efficiency of the model which is the autocorrelation, non-homogeneity, and non-normal distribution. This has been confirmed of non-significance test "Wald" which concerning with restrictions addendum, harmony, uniformity, and non-negativity. As can be seen from the same table that the prices of grapes in this market (for main competitive countries to Egypt, which are Spain, Germany, and Greece). In addition to the real total import Dutch expenditure of Grapes explains 85% of the changes that occur in the proportion of spending on the Egyptian grapes in Dutch market, while the rest of the changes due to factors other than the measured function, and according to the adjusted value of the coefficient of determination Adj. R^{-2}.

Table (5): Results of model estimates of "AIDS" to the demand for Egyptian grapes in the Dutch market during the period (1990-20009)

Country		α	LnP1	LnP2	Lnp3	LnP4	Ln(E/p) spi	Adj. R-sq	S.E of Reg	Auto	Hetro	Non-Norm
Egypt	Coeffi	1.671	0.019	0.104	0.072	0.017	-0.893	0.851	0.031			
	t.Stat.	7.114	0.651	0.137	1.293	0.579	-24.166			0.416	0.315	0.499
	Prob.	0.000	0.010	0.859	0.011	0.119	0.000			0.731	0.495	0.791
Spain	Coeffic.	-0.197	-0.107	0.121	0.175	-0.480	0.098	0.651	0.082			
	t.Stat.	-0.416	-0.351	1.119	0.018	-0.174	4.918			0.176	0.071	0. 695
	Prob.	0.172	0.050	0.001	0.000	0.001	0.000			0.714	0.796	0.841
Germany	Coeffic.	-0.445	0.078	-0.171	-0.041	0.019	0.130	0.721	0.015			
	t.Stat.	-0.381	0.691	-0.490	-0.710	0.785	0.184			0.690	0.489	0.312
	Prob.	0.899	0.909	0.907	0.801	0.040	0.471			0.655	0.740	0.410
Greece	Coeffic.	-0.795	0.127	-0.512	-0.017	-0.012	0.009	0.708	0.011			
	Tt.Stat.	-0.816	0.091	-0.189	-0.413	-1.514	1.193			0.871	0.067	0.291
	Prob.	0.093	0.009	0.077	0.050	0.002	0.009			0.319	0.702	0.012

Lnp1-Lnp4: the logarithm of export prices in dollars per ton of grapes, for each "Egypt, Spain, Germany, Greece", respectively, to the Dutch market during the period (1990-2009).
Ln (E / P) spi: logarithm of total expenditure on the Dutch import of grapes in thousand dollars during the period (1990-2009).
Adj.R-Sq: adjusted coefficient of determination, S. Eof Reg standard error of regression
Auto: LaGrange multiplier for the self-link.
Hetro: LaGrange multiplier for instability Altabanin
Non-Norm: LaGrange multiplier for the non-normal distribution reduce the limit of error.
Source: http://www. Comtrade. Un.org/db. GRAPES Fresh, SITC. Rev. 2 code (0806100000).

It is clear from Table (6) that the price elasticity of demand on the grape in the Dutch market, indicating that the increase in prices by about 1% leads to a decrease in demand by 1.594%, which means that this product with elastic demand in this market. While cross demand elasticity indicated that an increase in the price of grapes, imported from the main competitive States to Egypt (Spain, Germany, and Greece) by 1% lead to increased demand for Egyptian grapes by 0.481%, 0.659%, and 0.572%, respectively. Which are referring to a replacement relationship of the Egyptian grapes on one hand and grapes from those States on the other hand. It also describes the elasticity to increase the real total spending Dutch import grapes by 1%, leading to increased spending on the Egyptian grapes by 0.581%, which indicates that the Egyptian grapes is a necessary commodity in the Dutch market.

3.3. The Italian market:

3.3.1. The most important competitive countries of the exports of the Egyptian grapes in the Italian market

Despite the decrease in import capacity to Italy from grapes, amounting to 27.58 thousand tons representing 0.77% of the average total amount of world imports of grapes during the period (2005-2009), but the Egyptian exports of grapes to the market amounted to about 5.12 thousand tons, representing 13.34% of Egypt's total exports of grapes, and about 18.59% of the total imports of the Italian grapes during the same mentioned average period of the Italian grapes, which indicates the importance of exports of the Egyptian grapes in this market.

Table (6): Elasticities of model "AIDS" from the Dutch market on the grapes.

Country	Price, cross elasticity				Expenditure elasticity
	Egypt	Spain	Germany	Greece	
Egypt	-1.594	0.481	0.659	0.572	0.581
Spain	-1.737	-0.651	0.331	-0.548	1.170
Germany	0.417	-0.801	-0.903	0.862	1.336
Greece	0.508	-0.315	-0.761	-0.437	1.404

Source: Table No (5): in study.

Studying the most competitive markets for the exports of Egyptian grapes in the Italian market; it was found that the exports of grapes from "Spain, Germany, the Netherlands, and Israel" are

competitive countries to Egyptian grape "in a complete competition" within this market. Where, imports of the Italian market from these countries represent about 12.38%, 11.25%, 10.96%, and 8.05%, respectively during the period (2005-2009). With regard to the seasonal export of grapes from these markets to the Italian market, it became clear that the Spanish, German, and Dutch grapes hardly exist in this market throughout the year. While, the Israeli Grapes is exist in the same exported Egyptian grapes in the Italian market at the beginning of May until the first week of October. On the other hand, there are countries compete Egyptian Italian grapes market as a partial competition, include those countries include "Morocco, Chile, and India," as determined that the exports of grapes from these countries to the Italian market in the period from January to June. Despite that about 6% of imports of the Italian market of the grapes come from South Africa "but it is not considered a competitor to the exports of the Egyptian grapes, owing to different seasons of export, where the grape of South Africa in the Italian market is in the beginning of December and ends in April. The study noted that most competitive countries for the Egyptian grapes "total competition" within the Italian market of the European Union countries except Israel, which puts the Egyptian exports of grapes in a severe competition with the exports of those countries within the Italian market.

Table (7) the results of model estimates of "AIDS" to the demand for Egyptian grapes in the Italian market during the period (1990-2009).

Country		α	LnP1	LnP2	Lnp3	LnP4	LnP5	Ln(E/p) spi	Adj. R-sq	S.E of Reg	Auto	Hetro	Non-Norm
Egypt	Coeffi	3.714	-0.113	-0.185	-0.451	-0.317	0.971	-0.426	0.770	0.041			
	t.Stat.	2.521	-0.510	-2.457	-6.521	-7.489	9.418	-3.709			1.615	1.017	0.918
	Prob.	0.005	0.819	0.000	0.000	0.000	0.000	0.000			0.121	0.580	0.673
Spain	Coeffic.	5.730	-4.01	-0.725	1.109	4.931	0.777	-4.715	0.759	0.127			
	t.Stat.	4.731	-4.111	-0.070	0.089	5.423	0.655	-4.891			1.251	0.321	0.109
	Prob.	0.000	0.000	0.950	0.928	0.000	0.705	0.000			0.320	0.052	0.739
Germany	Coeffic.	-5.601	0.171	0.079	0.323	-0.139	-0.311	0.521	0.691	0.181			
	t.Stat.	-7.215	2.320	1.987	4.173	-3.601	-3.125	4.188			0.081	0.177	0.055
	Prob.	0.000	0.005	0.017	0.000	0.000	0.000	0.000			0.891	0.946	0.254
Nether Lands	Coeffic.	0.704	0.016	0.270	0.745	-0.171	-0.918	-0.034	0.912	0.127			
	t.Stat.	0.641	0.081	5.718	9.715	-1.181	-9.750	-0.398			0.491	0.512	0.271
	Prob.	0.500	0.742	0.000	0.000	0.209	0.000	0.723			0.449	0.170	0.214
Israel	Coeffic.	-3.707	0.250	-0.147	-0.591	0.181	0.319	0.248	0.231	0.045			
	t.Stat.	-3.651	3.459	-3.412	-8.801	2.940	3.045	2.791			0.536	0.218	1.450
	Prob.	0.000	0.000	0.000	0.000	0.000	0.000	0.000			0.027	0.619	0.781

Lnp1-Lnp5: the logarithm of export prices in dollars per ton of grapes, for each "Egypt, Spain, Germany, the Netherlands, and Israel," respectively to the Italian market during the period (1990-2009).

Ln (E / P) spi: logarithm of total expenditure on the import grapes in Italy, in thousands of dollars during the period (1990-2009)

Adj.R-Sq: adjusted coefficient of determination,

S.E of Reg standard error of regression.

Auto: LaGrange multiplier for the self-correlation.

Hetro: LaGrange multiplier for instability of variance

Non-Norm: LaGrange multiplier for the non-normal distribution reduce the limit of error

Source: http://www.Comtrade. Un.org/db. GRAPES Fresh, SITC. Rev. 2 code (0806100000).

Table (8): elasticity's of model "AIDS" for the Italian market on the grape

contry	Price, cross elasticity					Expenditure elasticity
	Egypt	Spain	Germany	Netherlands	Israel	
Egypt	-0.469	3.193	0.622	-0.244	1.738	0.814
Spain	-1.817	-0.415	-0.197	1.685	2.219	0.717
Germany	0.245	-1.599	1.110	-1.047	-5.407	0.572
Netherlands	0.247	-1.612	-5.460	-2.410	0.778	1.224
Israel	1.948	2.415	0.622	0.136	-2.458	0.409

Source table no. (7) in the current study

3.3.2. Estimation of demand functions on the Egyptian grapes in the Italian market:

Table (7) showed the demand function for Egyptian grapes in the Italian market, using the model of "AIDS" by showing that there is no standard problem, can affect the efficiency of the model are "self Linking, heterogeneity, and non-normal distribution". This has been confirmed not to be significance by the test "Wald" which concern with the adding restrictions, homogeneity, uniformity, and non-negativity. As can be seen from the same table that the prices of grapes in this market for the main competitive countries to Egypt, which are (Spain, Germany, the Netherlands, and Israel), in addition to the real total import expenditure on grapes in Italy, explains about 77% of the changes that occur in the proportion of spending on the Egyptian grapes to the Italian market. While the rest of the changes are due to factors other than the measured function, and according to the adjusted value of the coefficient of determination Adj. R^{-2}.

It is clear from Table (8) that the elasticity price of demand on the Egyptian grapes in the Italian market, indicating that the increase in its prices by 1% leads to a decrease in its demand by 0.469%, which means that a product with inelastic demand in this market. While, cross elasticity demand indicated that an increase in the price of grapes, from the main competitive countries to Egypt (Spain, Germany, the Netherlands, and Israel) by 1% lead to changes in demand for the Egyptian grapes by 3.193%, 1.490% - 0.244%, and 1.783% respectively. This refers to the replacement relationship of exported grapes from those countries on the one hand, and the grapes exported from Egypt on the other hand (excluding the Netherlands), where the relationship is complementary, in the case of high export prices of Spain, Germany, Israel and Egypt, respectively. As can be seen that the spending power of elasticity to increase the real total Italian spending import of grapes by 1% lead to increased expenditure on Egyptian grapes import by 0.814%, which may indicate that the Egyptian grapes is a necessary commodity in the Italian market.

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4/12/2011

Light Hydrocarbons in Niger Delta Oils: Geochemical Significance of Ring Preference.

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Abstract: The light hydrocarbon ring preference (RP) in crude oils from the Niger Delta has been investigated. The crude oil samples were analyzed using gas chromatographic fingerprints of ring preference light hydrocarbons. The ratios of P_2^3, P_3^3 (3RP) and N_2^5 (5RP) ranged from 9.73 to 13.27%, 4.04 to 7.90% and 8.75 to 14.71% with no compositional variation of ring preference for correlation and/or differentiation. The ratio of 6RP, N_1 ranged from 38.47 to 55.17% and revealed Niger Delta crude oils as exhibiting high 6RP. The ratio of parent P_1 separates the oils into two homologous sets. k_2 supports the grouping by P_1, compares well with RP ratio and classified EN-A4, EN-A9 (Eastern) and CE-B3, CE-C7 (Central) as marine source crude oils and WT-D5 (Western) as terrigenous source oil. Plots of ring preference further showed that Western Niger Delta oil remained distinct from the Central and Eastern oils. Gross differences observed on star plots of key ring preference parameters established that the Central and Eastern crude oils remained constrained and distinct from the Western. The ring preference appears to be reliable but must be interpreted within a complete understanding of the petroleum system under study
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Key words: Ring Preference, Light Hydrocarbons, Niger Delta, Geochemical, Star Plot.

1. Introduction

The petroleum fraction between C_1 – C_9 hydrocarbons are referred to as light hydrocarbons (LHs), and constitute about 50% of the carbon in petroleum (Mango, 1997). They are formed between 75-140^{o}C through catalysis of *n*-alkane parent into daughter iso- and cycloalkanes. This involves three-, five- and six-carbon ring closure and cleavage of C-C bond in the lipophilic domains of kerogen (Mango, 1992). The six carbon ring compounds N_1^6 originate via a six carbon ring cyclisation of the *n*-heptane parent, P_1. Second parent, P_2^3 forms N_2^5, by a five carbon ring closure and P_3^3 via a three carbon ring closure as seen in fig. 1 (Mango, 1990).

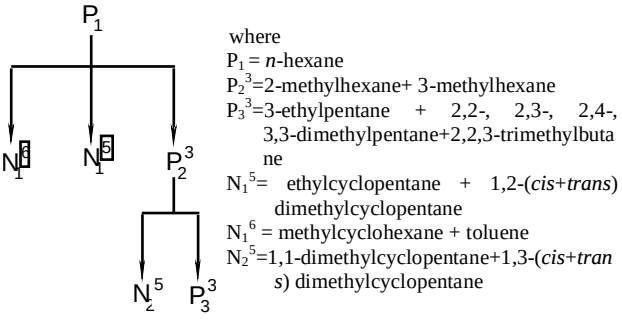


Figure 1: A schematic representation of the Formation of Light Hydrocarbons by Steady State Catalysis (Mango, 1990, 1994).

The LHs are commonly used in evaluating crude oils however, the most relevant geochemical criteria are those which reflect the genetic relationship between organic matter as well as provide information about migration of fluids (Osuji and Antia, 2005). Oils displaying a uniform overall geochemical composition show large compositional variations in LHs reflecting variations in three ring preference (3RP: $P_2^3$and P_3^3), five ring preference (5RP: N_2^5) and six ring preference (6RP: N_1^6) (Mango, 1994).

Mango (1994) divided C_7 LH ratios into two categories: invariance ratio of isoheptanes and dimethylcyclopentanes, k_2 and ring preference. The invariance ratio [$k_2 = P_3^3/ (P_2^3 + N_2^5)$] remains constant over the course of petroleum generation with homologous oil suites, but distinctly different from another suite of homologous oils (Ten Haven, 1996; Mango, 1990). However, it is the ring preference that gives the C_7 LHs high resolution in distinguishing genetically distinct oils (Mango, 1997). Star plots and ratios of ring preference LHs have proven effective in oil-oil and oil-source correlation (Halpern, 1995). Zhang et al. (2005) used differences observed in k_2 to characterize oils from the Tarim basin, north west China into marine and terrigenous.

Applying biological markers, Eneogwe and Ekundayo (2003) sorted crude oils from Western Niger Delta into three families. Manilla and Onyema (2008) used low molecular weight geochemical markers to characterize Niger Delta oils while Ekweozor and Udo

(1988) delineated Niger Delta oils with respect to their source on the basis of their oleanane content. Eneogwe (2003) in analysing Western Niger Delta oils based on variations in their LHs, observed methylcyclohexane and toluene as the most important discriminating variables.

The purpose of this study is to examine the potential of the ring preference of light hydrocarbons as biomarkers for crude oil correlation and speciation in Niger Delta crude oils.

2. Province Geology

The Niger Delta is situated on the West African continental margin at the Gulf of Guinea and is one of the world's largest tertiary delta systems (Doust, 1990). It formed during the continental breakup in the cretaceous era, with the delta developing from Paleocene. The Niger Delta basin covers an area of 75,000km^2 and consists of regressive clastic sequences (Sonibare et al., 2008). The lithostratigraphic sequence of the Niger Delta are distinguished on the basis of sand-shale ratios and is divided into three units namely Akata, Agbada and Benin formations (Short and Stauble, 1967). The Akata formation (Paleocene to Recent), at the base of the delta, consists of thick shale deposited under marine conditions. This formation transitions from the continental margin into the deep water portion of the basin. Deposition of the overlying Agbada formation (Eocene into the Recent) to the north consists of interbedded shale and sandstones about 4000km thick in the central part and thinning seawards towards the delta margins. The Agbada formation is overlain by the Benin formation (latest Eocene to Recent) is composed of coastal plain sands that are up to 2000km thick (Sonibare et al., 2008, Tuttle et al., 1999). The Niger Delta is an extremely prolific hydrocarbon province. The source rocks for crude oil in the Niger Delta are the marine shale facies of the upper Akata formation and the shale interbedded with paralic sandstone of the lower Agbada formation (Tuttle et al., 1999).

3. Materials and Methods

3.1 Samples

Five crude oil samples were collected from the Niger Delta region, Nigeria, and used for this study. The crude oil samples (one litre each) were obtained from producing well heads by field technicians, with the assistance of the Department of Petroleum Resources (DPR). Two crude oil samples each were collected from Akwa Ibom and Rivers States and the fifth from Delta State and were labeled as EN-A4, EN-A9 (Eastern) , CE-B3, CE-C7 (Central) and WT-D5 (Western) respectively.

3.2 Gas chromatographic analysis

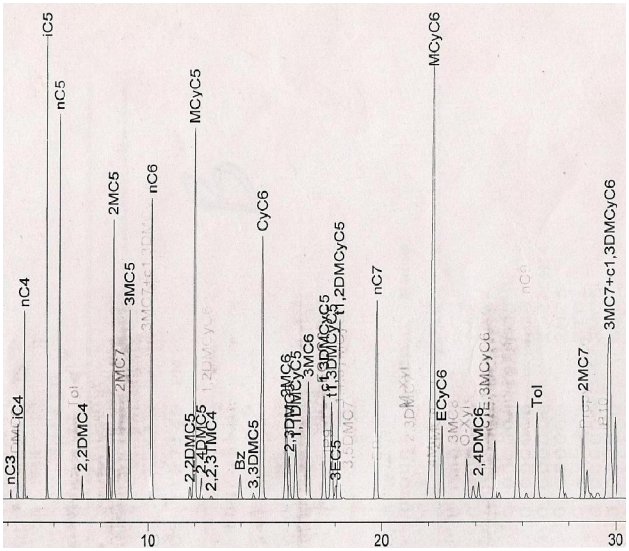
The light hydrocarbons were analyzed using the Hewlett Packard (HP) 6890 gas chromatography (GC) fitted to a fused silica capillary column (30m x 0.25µm) and equipped with a flame ionization detector (FID). Whole oil gas chromatography oven temperature was programmed with a 15min hold at 35^{o}C and ramped at 2^{o}C from 35^{o}C to 70^{o}C, 3^{o}C/min from 70 to 120^o. The final temperature was held for 20mins. Separated components were detected by FID Light hydrocarbon peak identification was based on data presented by Mango (1990 and 1994) and area integration of each peak was processed by the HP chemstation software.

3.3 Microscale correlation

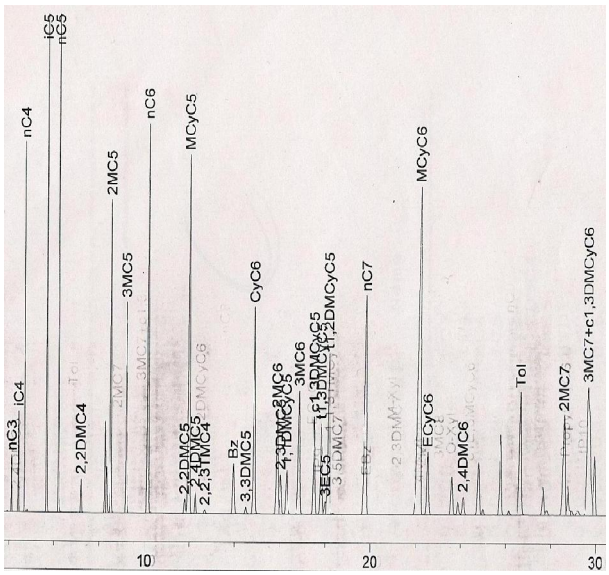
Microscale correlation technique using gas chromatographic analysis of LHs will be used for the purpose of correlation and/or differentiation between the Niger Delta oils under investigation by comparing ratios of compounds. The microscale correlation technique will be pictorially represented in a star diagram for easy comparison of the fluids.

4. Results and discussion

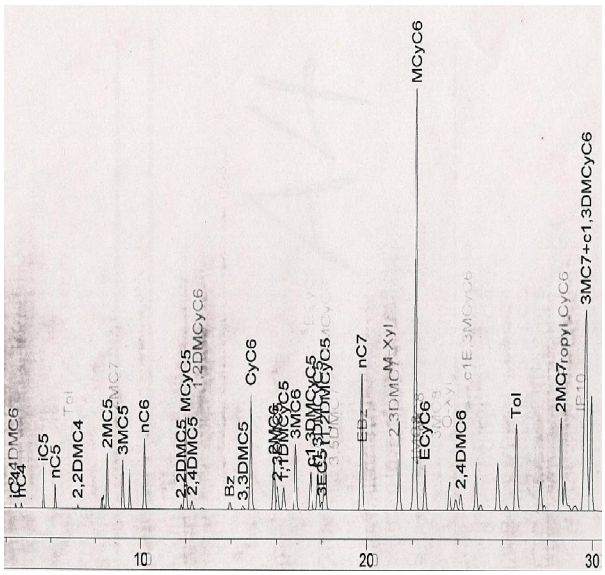
All the sample crude oils from the Niger Delta show characteristic light hydrocarbons which constitute the bulk of carbon in petroleum. GC fingerprints of all the Niger Delta oil samples are presented in Fig. 2.



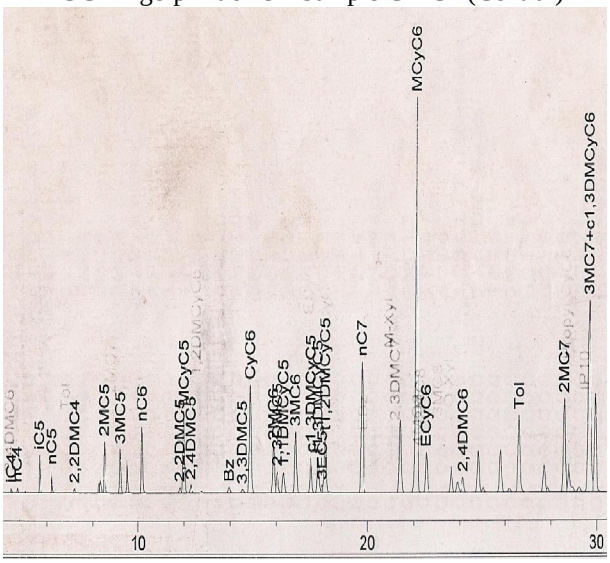
GC Fingerprint of Oil Sample CE-B3 (Central)



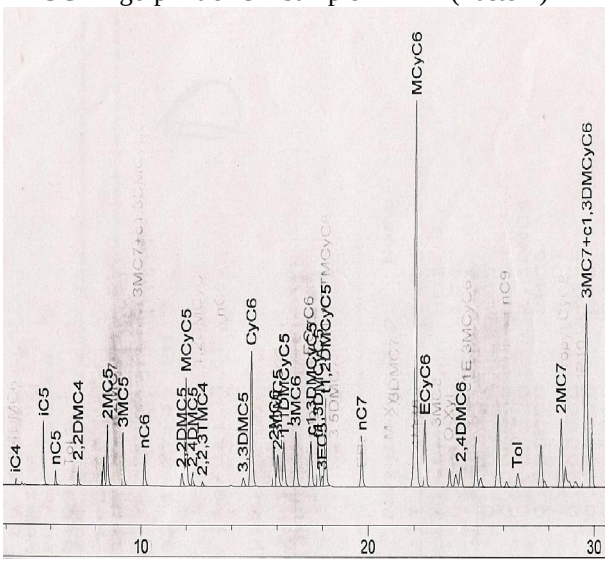
GC Fingerprint of Oil Sample CE-C7 (Central)



GC Fingerprint of Oil Sample EN-A4 (Eastern)



GC Fingerprint of Oil Sample EN-A9 (Eastern)



GC Fingerprint of Oil Sample WT-D5 (Western)

Figure 2: Gas Chromatographic Fingerprints of the studied Niger Delta Oil Samples showing characteristic Light Hydrocarbons.

4.1 Ring preference ratios

The results for the light hydrocarbon ring preference ratios of the parent (P_1), three ring preference (3RP: P_2^3 and P_3^3), five ring preference (5RP: N_2^5) and six ring preference (6RP: N_1^6) of the five crude oil samples are presented in Table 1. Of these, notably, P_1 discriminated between the samples. The data showed that the oil, WT-D5, from the western Niger Delta had low P_1 ratio of 5.29% and separated clearly from oils EN-A4 (12.99%), EN-A9 (13.39%) (Eastern) and CE-B3 (11.85%), CE-C7 (15.70%) (Central).

The ratios of P_2^3, P_3^3 and N_2^5 ranged from 9.73 to 13.27%, 4.04 to 7.90% and 8.57 to 14.71% with no compositional variation of ring preference for correlation and/or differentiation. The ratio of N_1^6 was observed to be high ranging from 38.47 to 55.17%. This result revealed that all the Niger Delta exhibited 6RP.

Light hydrocarbon parameter based on Mango's invariance ratio, k_2 involving isoheptanes and dimethylcyclopentanes, supports the grouping by P_1. k_2 is a reliable indicator of source organic matter. Zhang *et al* (2005) reported that marine oils are characterized by low k_2 values (average 0.23) and terrigenous oils by high k_2 values (average 0.35).

Table 2: Summary of Compositional Ring Preference Characteristics of Crude Oils from the Niger Delta.

	Eastern Niger Delta (Akwa Ibom State)		Central Niger Delta (Rivers State)		Western Niger Delta (Delta State)
Parameter	**EN-A4**	**EN-A9**	**CE-B3**	**CE-C7**	**WT-D5**
Total C_7	2374.89	2981.80	10087.06	13661.91	1194.35
P_1	308.52 (13.00%)	399.18 (13.39%)	1195.51 (11.85%)	2144.66 (15.70%)	63.22 (5.29%)
P_2^3	256.52 (10.80%)	323.89 (10.86%)	1209.04 (11.99%)	1812.59 (13.27%)	116.26 (9.73%)
P_3^3	103.83 (4.37%)	124.98 (4.19%)	496.03 (4.92%)	689.52 (5.05%)	94.39 (7.90%)
N_2^5	221.13 (9.31%)	271.89 (9.12%)	1484.21 (14.71%)	1899.12 (13.90%)	167.93 (14.06%)
N_1^6	1239.06 (52.17%)	1561.44 (52.37%)	4256.61 (42.20%)	5255.32 (38.47%)	549.60 (46.02%)
MCyC6	1025.63 (43.19%)	1309.08 (43.90%)	3706.01 (36.74%)	4075.86 (29.83%)	529.06 (44.30%)
Tol	213.43 (8.99%)	252.36 (8.46%)	550.60 (5.46%)	1179.46 (8.63%)	20.55 (1.72%)
k_2	0.22	0.21	0.18	0.19	0.33

MCyC6 = Methylcyclohexane Tol = Toluene

This data classifies WT-D5 crude oil (0.33) as terrigenous organic input to source and CE-B3 (0.18), CE-C7 (0.19), EN-A4 (0.22) and EN-A9 (0.21) as marine organic input to source.

A plot of isoheptanes and dimethylcyclopentanes is presented in fig. 3. The ring preference plot further supports k_2 in that CE-B3, CE-C7, EN-A4 and EN-A9 crude oils remained constrained and distinct from WT-D5 crude oil.

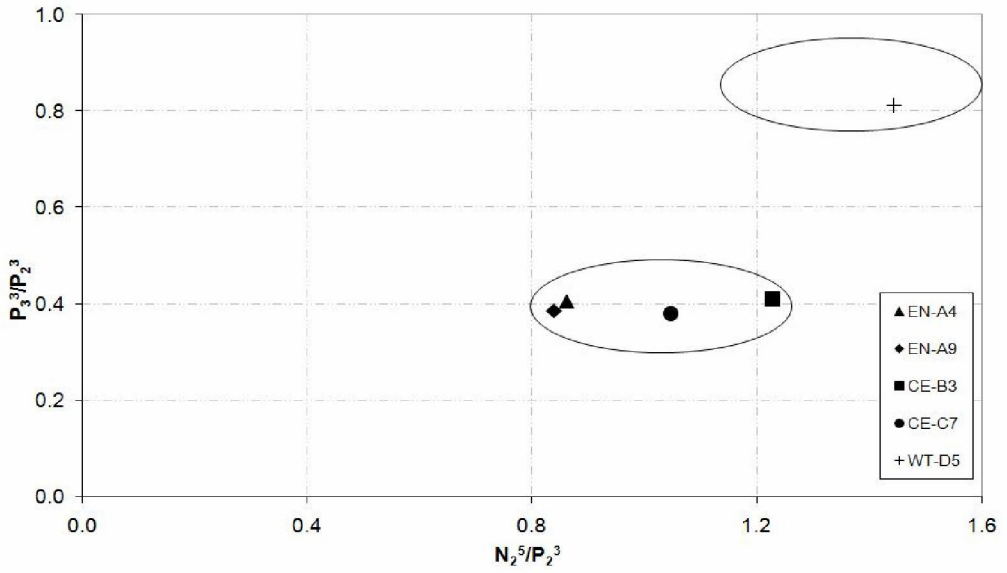


Figure 3: Plot of Isoheptanes and Dimethylcyclopentanes Establishing Homologous Sources for Crude Oils in the Niger Delta

4.2 Invariance of Ring Preference

An invariance plot of ring preference, fig. 4, establishes definitely that the Central (CE-B3, CE-C7) and Eastern (EN-A4, EN-A9) crude oils are similar and different from the Western (WT-D5 (Western) oils. This again remains consistent with two homologous sources (marine and terrigenous) for oils in the Niger Delta.

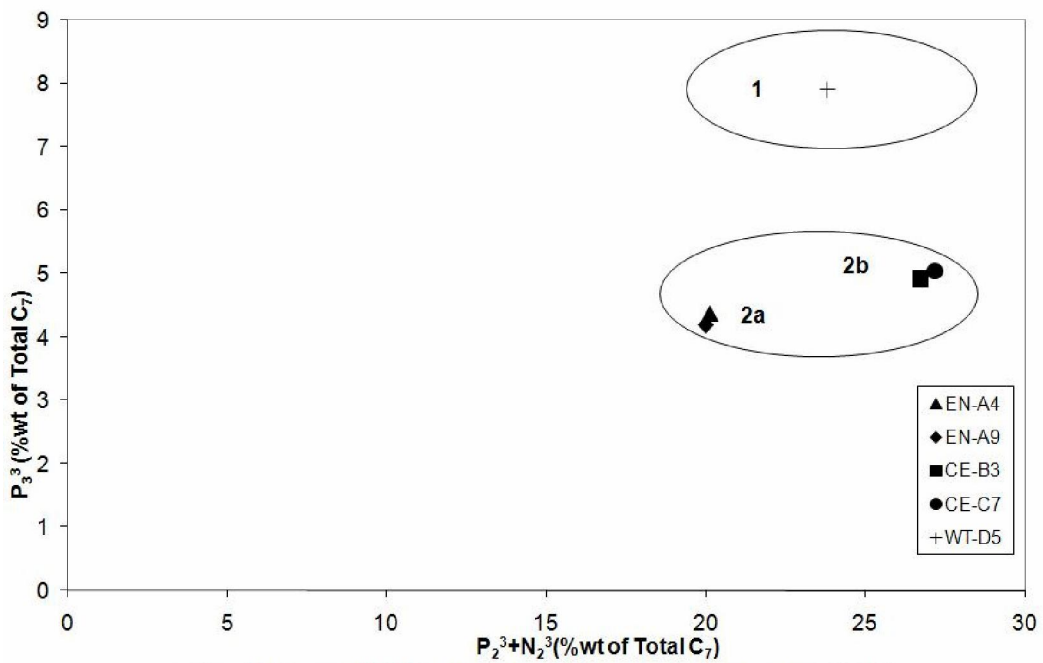


Figure 4: An Invariance Plot of Ring Preference of P_3^3(%C_7) and $P_2^3+N_2^5$ Establishing the two Homologous Oil Sets in the Niger Delta

This model not only discriminated between the two main genetic oils of the Niger Delta, but also classified the marine source oils into two sub-types. This may be indicative of two sub-petroleum system or that the Central crude oils have migrated distances from the east to their present accumulations.

4.3 Microscale correlation of ring preference

Comparisons of ratios of ring preference LH compounds are put in pictorial form of a star diagram to make correlation and/or differentiation of the Niger Delta oils easier. Star diagram have been used to represent chemical compositions of oils and water samples from reservoirs, as well as correlation and/or differentiation (Halpern, 1995; Ali et al., 2002). A star diagram, with five axes, of key ring preference parameters is presented in fig. 5 and will be referred to hereafter as ring preference star diagram (RPSD). Figure 5 depicts RPSD for all the studied oils.

The RPSD showed that the Eastern (EN-A4, EN-A9) and Central (CE-B3, CE-C7 (Central) crude oils followed patterns that were similar suggesting a close grouping among the oils and is reflective of oil generation from the same source rock (marine). Gross differences were observed in the RPSD of the Western oil (WT-D5) which followed patterns different from those of the eastern and southern oils. This differentiation in patterns followed by the oils is in line with differences in source rocks between the oils (Ali et al., 2002) further confirming different sources for the western Niger Delta oils.

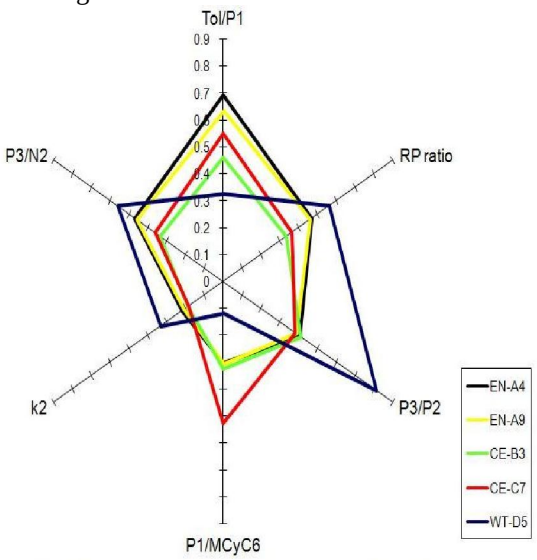


Figure 5: Star Plot of Key Ring Preference Parameters showing differences in pattern followed by the Western Oil from the Central and Eastern Oils.

5. Conclusions

Analyses of light hydrocarbon ring preference have revealed the Niger Delta oils as exhibiting high 6RP. Parameter P1 and invariance ratio of RP, k_2, grouped the Niger Delta oils into two: marine and terrigenuous sources. This was further confirmed by ring preference plots and star diagram which delineated the two genetic sources of oils in the Niger Delta. Thus the light hydrocarbon ring preference provides a technique for interpreting the Niger Delta oils.

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4/25/2011

Role of nitric acid or H_2O_2 in antioxidant defense system of *Pisum sativum* L. under drought stress

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Abstract: Water shortage is likely to be one of the major global environmental stresses of the 21st century. Drought is an important environmental constraint limiting the productivity of many crops worldwide. Experiments were conducted to investigate the effects of seed pretreatment by hydrogen peroxide at 70 mM or sodium nitroprusside (SNP; nitric oxide donor) at 10 µM on drought tolerance in pea seedlings. Osmotic stress was provoked by addition of polyethylene glycol to the nutrient solution at the flowering stage. H_2O_2 or SNP are active molecules involved in mediation of various biotic and abiotic stress induced physiological responses in plants. H_2O_2 or SNP pretreatment alleviate oxidative damage, accelerate proline accumulation and enhance total chlorophyll, carotenoid, photosynthetic activity ($^{14}CO_2$–fixation), and total yield/plant in leaves of pea seedlings subjected to osmotic stress. The results showed that osmotic stress induced decrease in the enzyme activities of ascorbate peroxidase, glutathione peroxidase, catalase and overproduction of $O_2^{\bullet-}$ in pea leaves, which in turn caused exacerbation of lipid peroxidation and depression of photosynthesis. Application of H_2O_2 or SNP significantly increased the enzyme activities and decrease $O_2^{\bullet-}$ production and hence inhibited lipid peroxidation. Level of H_2O_2, proline and Evan blue uptake in seedlings pretreated with H_2O_2 or SNP were markedly lower than under drought stress, indicating the operation of antioxidant system in them. Moreover, seedlings arising from H_2O_2 or SNP pretreatment enhanced the membrane stability, as revealed from greatly reduced malondialdehyde content. The present data suggest that pea seed pretreatment with H_2O_2 or SNP, a stress signal, could trigger the activation of antioxidants in seeds, which persists in the seedlings to alleviate the oxidative damage, leading to improvements in physiological attributes for the seedling growth under drought.

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Keywords: Antioxidative enzymes, Drought stress, Photosynthesis, Proline, Pea

1. Introduction

Drought stress is one of the main causes for crop yield reduction in the majority of agricultural regions of the world (Moussa, 2011). Reactive oxygen species (ROS) are enhanced during drought stress through the disruption of electron transport system and oxidizing metabolic activities occurring in chloroplast, mitochondria and microbodies (Sofo *et al.*, 2005). Plants eliminate ROS produced in non-stressful conditions through production of non-enzymatic and enzymatic antioxidants (Inze and Montagu, 2000), whereas during severe drought conditions the production of ROS exceeds the capacity of the antioxidative systems to remove them, causing oxidative stress (Sofo *et al.*, 2005). In these conditions cells could be protected either by the endogenous molecular systems or exogenously applied compounds that mitigate the stress (Ingram and Bartels, 1996). ROS are highly reactive and in the absence of effective protective mechanism, can seriously damage plants by lipid peroxidation, protein degradation, breakage of DNA and cell death (Beligni and Lamattina, 1999). Among various technique strategies, pre-sowing treatment and priming of plant seeds are easy, low cost, low risk and effective approaches to enhance plant tolerance to the stressful environments (Ashraf and Foolad, 2007). Hydrogen peroxide is a major kind of ROS in plant tissues. It acts as an important signal molecule involved in acclamatory signaling, triggering higher tolerance against various biotic and abiotic stresses at low concentrations, whereas at high concentrations, it is toxic to plant tissues and can trigger programmed cell death (Quan *et al.*, 2008). H_2O_2 pretreatment could improve salt stress (Fedina *et al.*, 2009), oxidative stress (Chen *et al.*, 2009), and multiple stresses (Uchida *et al.*, 2002). Many previous studies have reported presence of NO in the plant kingdom as an important endogenous plant signaling molecule and its involvement in growth, development and defense responses (Lamotte *et al.*, 2005). As a relatively stable free-radical molecule, and due to its highly lipophilic nature, NO diffuses through membranes and may act as a synchronizing chemical messenger that is involved in many physiological processes in plants (Franciele *et al.*, 2010). Nitric oxide is an important signal molecule involved in plant response to biotic and abiotic stresses (Jinfang

et al., 2008). However, there is very little information about the effect of exogenously applied H_2O_2 or SNP and drought stress in pea seedlings.

The aim was to provide experimental basis for understanding the mechanism of drought tolerance in pea induced by H_2O_2 or SNP pretreatment.

2. Materials and Methods

Plant material and growth conditions.

A homogenous lot of pea seeds (*Pisum sativum* L.), cv. Master; was obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt. The caryopsis was kept at 4°C. They were surface sterilized in 0.1 % (w/v) sodium dodecyl sulphate solution and then thoroughly rinsed with sterile deionized water. Clean-healthy dry seeds were soaked for 20 h in solutions of H_2O_2 (70 mM) or sodium nitroprusside (10 µM) and with deionized water as the control. The H_2O_2 and SNP–treated and water-soaked seeds were sown in black polyethylene pots (40 cm diameter, 45 cm height) filled with silicon sands. Ten seeds were sown per pot. After the seedlings reached the first true leaf stage, they were thinned to four plants per pot. The sand-filled pots irrigated with half strength Hoagland's solution (Rafi and Epstein, 1999) as well-watered conditions. Pots were kept in a controlled-growth chamber at photo flux density of 240 µmole $M^{-2}S^{-1}$ (12/12 h day/night period) at relative humidity of 55-60%, and 25±2 °C temperature. Cultural practices, such as weed control and irrigation, were performed as needed. Drought stress was achieved by adding polyethylene glycol (PEG-6000, –0.5 MPa) solution at the flowering stage (40 day after planting). The experiments conducted four times in complete randomization replicated design. Samples were collected 10 days after the water treatment was applied, between 9:30 and 10:30 a.m., and kept in liquid nitrogen until analyzed. At harvest, the effects of treatments on the total seed yield/plant were recorded.

Biochemical assays.

Free proline was determined according to the method described by Bates *et al.* (1973). The amount of total chlorophyll (*a*+*b*) and carotenoids were determined according to the method of Lichtenthaler and Wellburn (1983).

Determination of $O_2^{\bullet-}$ and H_2O_2 concentrations.

The superoxide radical ($O_2^{\bullet-}$) was determined by 2, 3-bis (2-methoxy-4-nitro-5-sulphenyl) (2H) tetrazolium-5-carboxanilide (XTT) assay (Frahry and Schopfer, 2001). To determine H_2O_2 concentration, the root extract was mixed with 0.1% titanium chloride in 20% (v/v) H_2SO_4. The mixture was then centrifuged at 6 000 *g* for 15 min. The absorbance was measured at 410 nm (Hsu and Kao, 2007).

Determination of lipid peroxidation and Evans blue uptake.

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content using the thiobarbituric acid reaction as described by Madhava Rao and Sresty (2000). The loss of plasma membrane integrity was detected by the non-permeable dye (Evans blue) uptake in the root cells, which has also been used as an indicator of cell death (Baker and Mock, 1994).

Determination of antioxidant enzyme activities.

The catalase (CAT, EC 1.11.1.6) activity was assayed from the rate of H_2O_2 decomposition following the method of Aebi (1983). The ascorbate peroxidase enzyme activity (APX, EC 1.11.1.1) was determined as the decrease in absorbance at 290 nm due to ascorbate oxidation (Nakano and Asada, 1981). The glutathione peroxidase (GPX, EC 1.11.1.9) activity was determined as the decrease in absorbance at 340 nm due to the oxidation of NADPH (Navrot *et al.*, 2007).

Photosynthetic activity ($^{14}CO_2$–fixation).

Photosynthetic activity was measured in the Atomic Energy Authority, Radioisotope Department, Cairo, Egypt, with the method of Moussa (2011). The seedlings from each treatment were placed under a Bell jar, which was used as a photosynthetic chamber. Radioactive $^{14}CO_2$ was generated inside the chamber by a reaction between 10% HCl and 50 µCi (1.87×10^6 Bq) $NaH^{14}CO_3$ + 100 mg Na_2CO_3 as a carrier. Then the samples were illuminated with a tungsten lamp. After 30 min exposure time, the leaves were quickly detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction by 80% hot ethanol. The ^{14}C was assayed from the ethanolic extracts in soluble compounds using a Bray Cocktail (Bray, 1960) and a liquid scintillation counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises, Edinburgh, UK).

Statistical analysis

All data were subjected to ANOVA and the means were compared using Duncan's multiple range tests (P<0.05).

3. Results:

Water deficit decreased significantly the total chlorophyll (*a*+*b*), carotenoid content, photosynthetic efficiency ($^{14}CO_2$–fixation) and total yield/plant (Table 1). However, pretreatment with H_2O_2 or SNP increased significantly the above parameters as compared to water deficit stressed plants (Table 1).

Table (1). Effects of H_2O_2 or SNP pretreatment on total chlorophyll *a+b* (mg/gFW), carotenoids (mg/gFW), photosynthetic efficiency (*KBq/mgFW) and total yield/plant (g) in pea seedlings under drought stress. Data presented are the means of four separate experiments.

Treatments	Total chlorophyll (*a+b*)	Carotenoids	Photosynthetic activity	Total yield/plant
Control	3.98^{c}	6.3^{b}	19829^{a}	58^{a}
Drought stress	2.57^{e}	3.9^{c}	15422^{d}	40^{e}
SNP	4.18^{d}	7.4^{d}	21498^{e}	63^{d}
Drought stress+ SNP	3.74^{b}	5.8^{a}	19032^{b}	55^{c}
H_2O_2	4.05^{d}	6.8^{e}	20602^{c}	60^{a}
Drought stress+ H_2O_2	3.51^{a}	5.5^{a}	18837^{t}	53^{b}

Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests (P<0.05). * KBq: kilobequrel.

H_2O_2 content increased significantly under drought stress as compared with the control (Table 2). Exogenous application of H_2O_2 or SNP in drought stressed plants decreased significantly H_2O_2 content under both normal and stress conditions (Table 2). Exposure to water stress increased malondialdehyde, proline concentration, Evans blue uptake and $O^{\bullet-}$ concentrations more significantly as compared with controls. Pretreatment with H_2O_2 or SNP alleviated Evans blue uptake, reduced the overproduction of $O^{\bullet-}$, proline and MDA accumulation as compared with water deficit plants (Table 2).

Table (2). Effects of H_2O_2 or SNP pretreatment on H_2O_2 (µM/gFW), MDA content (µM/ gFW), $O^{\bullet-}$ concentration (µM/gFW), Evan blue uptake (OD_{600nm}/gFW), and free proline level (µM/gFW), in pea seedlings under drought stress. Data presented are the means of four separate experiments.

Treatments	H_2O_2	MDA	$O^{\bullet-}$	Evans blue uptake	Free proline
Control	1.8^{a}	2.2^{b}	1.1^{a}	0.128^{a}	8.7^{a}
Drought stress	7.3^{e}	5.8^{e}	3.7^{d}	0.346^{e}	23.9^{e}
SNP	1.1^{b}	1.7^{a}	0.8^{c}	0.080^{b}	18.8^{b}
Drought stress+ SNP	2.6^{c}	3.0^{d}	1.7^{b}	0.204^{d}	14.3^{c}
H_2O_2	1.3^{b}	1.9^{a}	0.7^{c}	0.075^{b}	17.1^{b}
Drought stress+ H_2O_2	3.0^{d}	2.8^{c}	1.6^{b}	0.251^{c}	19.3^{d}

Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests (P<0.05).

Water deficit markedly increased proline content and nearly tripled. Drought stress decreased significantly the activity of CAT, APX and GPX as compared with the control (Table 3). However pretreatment H_2O_2 or SNP application to drought stressed plants enhanced the activity of theses enzymes (Table 3).

Table (3). Effects of H_2O_2 or SNP pretreatment on enzyme activities of APX (µM ascorbate/min.gFW), CAT (µMH_2O_2/min.gFW) and GPX (µMNADPH/min.gFW) in pea seedlings under drought stress. Data presented are the means of four separate experiments.

Treatments	APX	CAT	GPX
Control	2.6^{b}	1.8^{a}	5.1^{b}
Drought stress	1.7^{a}	0.8^{c}	3.5^{c}
SNP	2.4^{b}	2.3^{b}	8.4^{e}
Drought stress+ SNP	2.1^{c}	1.9^{a}	4.6^{a}
H_2O_2	2.8^{b}	2.5^{b}	7.3^{d}
Drought stress+ H_2O_2	2.2^{d}	1.7^{a}	4.6^{a}

Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests (P<0.05).

4. Discussion

Various environmental stresses such as drought can inhibit plant growth and development, leading to crop reduction. For food security in the world and agricultural production, more efforts are needed to develop multiple effective strategies to improve crop stress tolerance (Moussa, 2011). In this study, we investigated the effects of seed treatment with H_2O_2 or SNP on pea growth characteristics and physiological- biochemical response to drought stress. Overproduction of ROS, especially H_2O_2, is believed to be primarily responsible for drought triggered oxidative damage and cell death (Moussa, 2011). H_2O_2 acts as signal molecules in plant response to stress in low concentrations (Desikan *et al.*, 2004; Liheng *et al.*, 2009). H_2O_2 can effectively modulate the related gene expression and subsequently lead to the enhancement of plant tolerance to the stress (Hung *et al.*, 2005; Liheng *et al.*, 2009). Proline is such an antioxidant which accumulates in response to biotic and abiotic stresses, including water stress (Zhang *et al.*, 1995). The change in stability of biological membranes is a key indicator of cellular damage. Drought and other stresses always results in cellular membrane injures including the increase of membrane permeability and MDA content due to membrane lipid peroxidation (Farooq and Azam, 2006; Agrawal and Rathore, 2007). Like other physiological/biochemical processes affected by drought, the photosynthetic activity ($^{14}CO_2$-assimilation), total chlorophyll and carotenoid content is also adversely affected by drought stress (Akram *et al.*, 2007; Moussa, 2011). H_2O_2 treatment enhanced photosynthetic efficiency in wheat under water stress condition (Liheng *et al.*, 2009). Paralleling with the improvement of photosynthesis upon drought, the total yield/plant production largely increased. SNP promotes a significant increase in chlorophyll content and chloroplast membrane density in maize plants (Graziano *et al.*, 2002). Also, SNP application stimulating the photosynthetic efficiency and inhibiting the degradation of chlorophyll in wheat (Tue *et al.*, 2003). Opposite to this result, exposure to NO reduced photosynthesis in leaves of oats and alfalfa (Hill and Bennett, 1970). Also, Franciele *et al.*, 2010, reported that SNP treatment in soybean seedlings decreased H_2O_2 and Evans blue uptake concentration. Also, Tian and Lai (2006) investigated that pretreatment with SNP in wheat under drought stress increase the enzyme activities of CAT and GPX and decrease MDA content. Liheng *et al.* (2009) reported that H_2O_2 pretreatment enhanced membrane stability by greatly reduced MDA content and increasing the antioxidant enzyme activity of CAT and APX in drought stressed wheat seedlings (Liheng *et al.*, 2009).The increased stress tolerance is attributed partly to an enhanced level of antioxidant defense system induced by H_2O_2 or SNP pretreatment, which alleviates ROS accumulation and oxidative damage during the subsequent stress conditions (Hu *et al.*, 2005). As is now commonly accepted NO as a second messenger in plants, it is supposed that low concentration of NO might be a signal molecule to induce/stabilize the expression of many antioxidative enzymes including SOD, CAT (Frank *et al.*, 2000). The protective effect of NO may also be related to its ability to react with some ROS, such as $O_2^{\bullet -}$, making NO act as a chain breaker and show its proposed antioxidant properties (Conner and Grisham, 1996). Moreover it has been reported that NO can react with lipid alcoxyl ($LO^{\bullet}$) and peroxyl ($LOO^{\bullet}$) radicals, leading to the expectation that NO could stop the propagation of radical-mediated lipid oxidation in a direct fashion (Lamotte *et al.*, 2004), which is in well agreement with our result in the decrease of MDA content. Thus NO may help plants to survive stressful conditions through its action as signaling molecule to activate antioxidative enzymes and reaction with active oxygen and lipid radicals directly. Tolerance to drought is enhanced in wheat treated with SNP (García-Mata and Lamattina 2001, 2002). Incubation of soybean roots in solutions of SNP at very low concentrations (1–10 μM) promotes growth, whereas greater concentrations inhibit it (Hu *et al.*, 2005). Proline as a cytosolic osmoticum and a scavenger of $OH^{\bullet}$ radical can interact with cellular macromolecules such as DNA, protein and membranes and stabilize the structure and function of such macromolecules (Kavir-Kishor *et al.*, 2005). The data are of considerable value in understanding the mechanisms of plant stress tolerances and in developing effective methods for crop protection against environmental stresses. Thus, it may be a useful management tool in afforestation projects in arid and semiarid areas.

5. Conclusion

Applying H_2O_2 or SNP prior to water deficit stress could partially alleviate the detrimental effect of water stress on growth of pea through increasing assimilation of $^{14}CO_2$, and improving antioxidant system and reduce the oxidative damage of cellular membranes. It was concluded that H_2O_2 or SNP minimized the pea yield loss caused by water deficits.

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Protease Production and Growth Characteristics of *Aspergillus sydowii*

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Abstract: The present work was aimed to evaluate the optimization of medium composition for protease production by *Aspergillus sydowii*. The fungus was isolated from the garden soil of Banasthali University and was identified as *Aspergillus sydowii* on potato dextrose agar medium. It was maintained on potato dextrose agar medium for 7 days at 28^0C. Spore suspensions was prepared, inoculated in Czapek Dox broth medium supplemented with various substrates, incubated and the mycelium was separated from the culture medium by filtration and the filtrate was used to determine the protease activity. The biomass was determined after drying the mycelium at room temperature for 24 hours. Maximum growth rate of this fungus in casein as substrate was found after 8 days of incubation ($7.11 mgmL^{-1}$) at 35^0C. But maximum protease activity was obtained after 6 days ($11.95\ Uh^{-1}mg^{-1}$dry mycelium). The highest protease production was attained with casein, peptone and mung seedlings as nitrogen sources. The extracellular protease production and mycelial growth were influenced by the concentration of casein. Other protein sources (yeast extract) supported growth but did not induce such excellent protease synthesis and ammonia as end product repressed it, indicating catabolite repression in this microorganism. Optimal protease production was obtained at final pH 5.3.

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Key words: *Aspergillus sydowii*, Casein, Nitrogen sources, Protease.

1. Introduction

Proteases are a group of enzymes that have been found in several microorganisms like bacteria and fungi which are involved in breakdown of complex protein molecules into simple polypeptide chains (Absida, 1985). They are commercially employed in many industrial processes. In foods, proteases have two main applications: in the processing of traditional food products and in the processing of new protein-based ingredients called functional foods (Nagodawithana and Reed, 1993).

Microbial protease represents about 60% of all the industrial enzyme's sales in the world due to their applications in several industrial sectors (Gupta et al. 2002). Proteolytic activity has been known and studied since the 1950's. The genus *Aspergillus* is well known for their proteolytic activity (Heldstrom, 2002). Now these proteases from any *Aspergillus* species are used in leather treatment and protease from *A.oryzae* are utilized in the breakdown of wheat gluten and in the pharmaceutical industry (Chiplonkar et al. 1985). Acid proteases are very important in beverage production and in baking (Rao et al. 1998).

The induction of protease requires a substrate having peptide bonds including substrates like peptone, casein and other proteins. The ammonia, as final product of enzymatic reaction of substrate hydrolysis, represses enzyme synthesis by a well known mechanism of catabolite repression. This extracellular protease has also been commercially exploited to assist protein degradation in various industrial processes (Srinubabu et al. 2007).). The great advantages offered by fungal enzymes are low material costs coupled with high and faster productivity and the ease with which the enzymes can be modified (Sharma et al. 2007). At present, due to high cost of substrates and mediums used, the overall cost of enzyme production is very high and therefore, development of novel processes to increase the yield of proteases with respect to their industrial requirements coupled with lowering down the production cost is highly appreciable from the commercial point of view (Kammoun et al. 2008).

Extracellular protease has high commercial value and multiple application in various industrial sectors, such as detergent, food, pharmaceutical, leather, diagnostics, waste management and silver recovery industries (Godfrey and West, 1996). The factors like composition of the culture medium, initial concentration of the protein/ substrate, cultivation condition and the microorganism itself may control the mechanism of enzymatic regulation. Besides nitrogen source, carbon source plays a vital role in survival of the microorganism. Growth conditions like pH, temperature, incubation period, humidity, substrates and the products formed influence the growth of microorganisms. Different species of *Aspergillus* show different proteolytic activity at pH and temperature. Thus, the strain showing the appreciable activity with less pathogenicity is used in different industrial field.

The present study deals with the growth of *Aspergillus sydowii* under various parameters and protease production which may contribute to understand the mechanism of action of environmental factors (incubation period, temperature and pH in the culture medium) and substrates (effect of nitrogen sources) on the growth characteristic of this fungus.

2. Materials and Methods

(1) *Microorganism and Inoculum Preparation*

The fungus *Aspergillus sydowii* was isolated from the garden soil of Department of Bioscience and Biotechnology, Banasthali University, Rajathan and was identified on the basis of its colony morphology on potato dextrose agar medium and microscopic observations. The microorganism was grown and maintained on potato dextrose agar medium for 7 days at 28^0C. Spore suspensions for inoculation were prepared by adding 3 ml of sterilized distilled water to fungal slant and vigorously shaking the culture for 1 min. The number of spores was determined with a Neubauer counting chamber and the inoculum was adjusted to $1X10^7$ spores/ml for inoculum preparation.

(2) *Growth Medium and Cultivation*

The fungus was inoculated in Czapek Dox broth medium for growth. The Czapek Dox medium was employed with various protein substrates at a concentration 2.0% (w/v) as nitrogen source and with the solution of trace elements. The pH of the medium was adjusted to 6.6 with 1N HCl. The protease production was determined at several pH values ranging from 4.0 to 10.0, at several temperatures ranging from 20^0C to 50^0C and incubation period from 2 days to 12 days using the broth medium and the pH was adjusted with HCl and/or NaOH to various values in the 2..0 to 10.0 pH range.

The medium was inoculated with 0.5 ml of spore suspension and incubated for 3 days to 12 days at optimum pH and temperature. In the completion of respective incubation periods the mycelium was separated from the culture medium by filtration and the filtrate was used to determine the protease activity. The biomass was determined after drying the mycelium at room temperature for 24 hours. After getting the optimum growth conditions like pH, temperature and incubation period, *Aspergillus sydowii* was grown in increasing concentration of casein and its effect on biomass and protease production was analyzed.

(3) *Protease Assay*

Protease assay was assayed with slight modifications. Test tubes were arranged on a test tube strand. 1ml of the Enzyme extract was pipette in to the test tubes. 1ml of 1% soluble substrate in citrate-Phosphate buffer (pH 6.5) was added. The test tubes were incubated in a water bath at 40° C for 30 minutes. A blank was set of consisting of 2ml of the enzyme extract that has been boiled for 20 minutes (boiling inactivates the enzyme), added to the substrate solution and treated with the same reagent as the experimental tubes. The reaction was stopped by adding 10 ml acetone and 100 mg Ninhydrin at 1, 3, 5, 7, 9, 11, 13 & 15 minutes. Colour variations in the test tubes were spectrometrically analyzed. One unit of enzyme activity was defined as the amount of enzyme which hydrolyzes 1mg of casein/10 minutes under the above conditions. The specific activity was expressed as units per mg dry weight mycelium per hour.

(4) *Statistical Analysis*

Each experiment was carried out in triplicate. From this, arithmetic means, standard errors of mean and graph were plotted.

3. Results and Discussion

After inoculating soil suspension of dilution 10^{-4}, a mixed colony culture was obtained. *Aspergillus sydowii* was characterized and identified by its colony morphology and microscopic characteristic as describe in Microbiology; A laboratory manual sixth edition, by “cappuccino, Sherman”, 1st Indian, Reprint, 2004. After growing the individual species in modified Czapek Dox medium with 2% substrates & incubating the plate for 2 days, the hydrolytic zones were observed and measured using Nessler’s reagent. After incubation, the halo rounds the colonies was measured and presence of degraded zone was determined using Nessler’s reagent. (More diameter of the halo the more ability of the organism is to degrade the protein.). It has been found that *Aspergillus sydowii* was having maximum proteolytic activity followed by *A. kanagawensis* and *A. stellatus*. Minimum activity was seen in *A. niger*. Among the nitrogen sources screened for maximum biomass produced using the strain *Aspergillus sydowii*, the maximum biomass was produced on casein>peptone>mung seedlings>yeast extract>gelatin. At 2% casein concentration in the culture medium, fungal biomass was enhanced from 0.72mg mL^{-1} (no casein) to 6.93mg mL^{-1} (2% casein, w/v) (Table 1).

The fungus *Aspergillus sydowii* was grown under various pH and 2% casein source. As the organism was grown in pH 6.0, the pH of the broth medium was decreased gradually to 5.3 during protease production and the highest protease activity (9.91 U $h^{-1}mg^{-1}$dry mycelium) was found at the pH 5.3. The protease activity was also recorded in the 4.0 to 10.0 pH range (Table 2). Maximum biomass was

found at final pH 6.6 with a yield of 4.44mg mL^{-1} dry weight. For pH below 4.0 or above 8.0, the fungal biomass and enzyme production were reduced gradually in relation to the optimum.

The highest protease activity (6.58U $h^{-1}mg^{-1}$dry mycelium) was obtained at 35^0C. Maximun growth of mycelium (6.38 mg mL^{-1}) was also recorded at 35^0C. So 35^0C was found optimum temperature for the growth of fungus and the enzyme production. The enzyme synthesis was also recorded in temperature range 20^0C to 50^0C (Table 3). Temperature below 25^0C and above 35^0C, the enzyme production was not obtained.

When the fungus *Aspergillus sydowii* was grown at 2% casein concentration in various incubation days, protease activity was found to be highest on the 6th day of cultivation. It was 11.95 U $h^{-1}mg^{-1}$dry mycelium, decreasing thereafter (Table 4). The mycelia production was maximum on 8th day of cultivation as it was 7.11 mg mL^{-1} and the pH values decreased to 4.7 on the 4th day of inoculation. Although highest protease activities were found on the 2nd day of inoculation, but it is not taken in consideration as the fungus didn't grow with some other nitrogen sources (substrates) within 2 days of inoculation.

When *Aspergillus sydowii* was grown in an increasing concentration of casein (1%-5%) for 6 days, maximum biomass (8.96 mg mL^{-1}) was obtained at 2% casein concentration and the final pH was found to be 4.1 with a higher protease activity (13.66 U $h^{-1}mg^{-1}$dry mycelium) (Table 5). The level of enzyme activity was markedly dependent upon the concentration of casein in the broth medium. The biomass of fungus was suddenly decreased at a casein concentration higher than 3% and the final pH enhanced to 8.2 in the culture medium without casein.

The results showed that the protease activity of *Aspergillus sydowii* was regulated by protein supply. While the mycelial growth on 2% casein reached a maximum on 8th day of incubation, maximum protease activity was produced on 6th day of incubation. The decreased activity in the later phase of growth was probably due to the catabolite repression by ammonia as it is the final product during the protein breakdown by the fungus.

Overall data imply that protease from *Aspergillus sydowii* was induced by casein, peptone, yeast extract, mung seeds and repressed by ammonia as the end product of this enzymatic reaction. Enzyme synthesis was affected by various organic nitrogen sources and maximal activity was shown attained with casein. Fungal growth and protease production were found only within a narrow pH range (4.0-7.0).

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Table 1. Effect of nitrogen sources on growth of *Aspergillus sydowii*.

Serial No.	Nitrogen sources 2% (w/v)	Final pH	Mycelium dry weight (mg mL^{-1})
1	Control	6.2	0.72
2	Casein	4.4	6.93
3	Peptone	4.1	5.4
4	Mung seedlings	3.6	5.0
5	Yeast extract	5.5	4.12
6	Gelatin	4.2	1.0

Table 2. Effect of pH on the growth and protease production by *Aspergillus sydowii*.

Serial No.	Initial pH	Final pH	Mycelium dry weight (mg mL^{-1})	Protease synthesis ($Uh^{-1}mg^{-1}$dry mycelium)
1	4.0	3.4	0.57	3.79
2	5.0	4.1	1.83	4.53
3	6.0	5.3	3.13	9.91
4	7.0	6.6	4.44	6.63
5	8.0	7.5	2.12	≤0.01
6	9.0	8.3	0.43	≤0.01
7	10.0	9.2	0.07	0.02

Table 3. Effect of temperature on the growth and protease production by *Aspergillus sydowii*.

Serial No.	Temperature (in ^{0}C)	Mycelium dry weight (mg mL^{-1})	Protease synthesis (U h^{-1}mg^{-1}dry mycelium)
1	20	1.26	0.03
2	25	4.73	2.88
3	30	4.11	4.27
4	35	6.38	6.58
5	40	0.34	0.04
6	45	0.07	0.00
7	50	0.00	0.00

Table 4. Effect of incubation period on the growth and protease production by *Aspergillus sydowii*.

Serial No.	Incubation Periods	Mycelium dry weight (mg mL^{-1})	Protease synthesis (U h^{-1}mg^{-1}dry mycelium)
1	2	0.76	2.00
2	4	3.44	8.9
3	6	5.89	11.95
4	8	7.11	5.00
5	10	5.55	3.56
6	12	2.95	0.04

Table 5. Effect of increasing concentration of casein on the growth and protease production by *Aspergillus sydowii*.

Serial No.	Concentration of Casein (%)	Final pH	Mycelium dry weight (mg mL^{-1})	Protease synthesis (Uh^{-1}mg^{-1}dry mycelium)
1	Control (0)	8.2	4.45	0.03
2	1	4.2	8.33	11.50
3	2	4.1	8.96	13.66
4	3	3.6	4.10	4.44
5	4	6.2	0.41	0.03
6	5	6.3	0.05	≤0.01

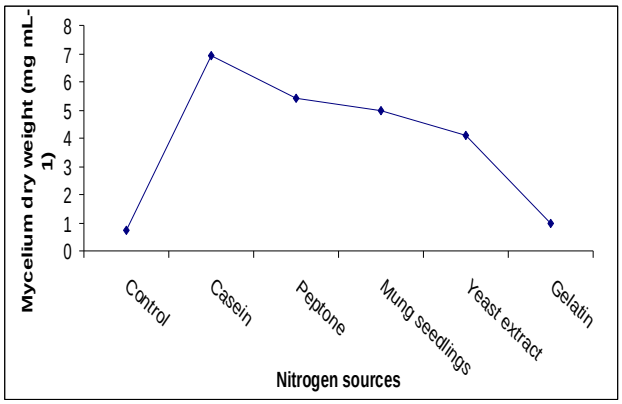

Figure 1. Effect of nitrogen sources on growth of *Aspergillus sydowii*.

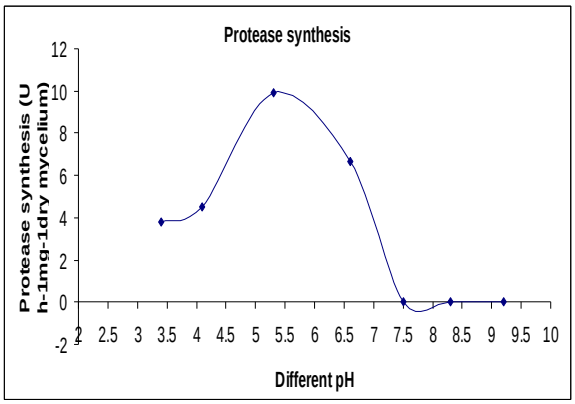

Figure 2. Effect of pH on the growth and protease production by *Aspergillus sydowii*.

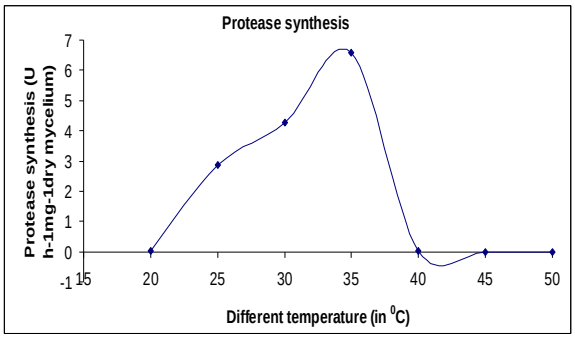

Figure 3. Effect of temperature on the growth and protease production by *Aspergillus sydowii*.

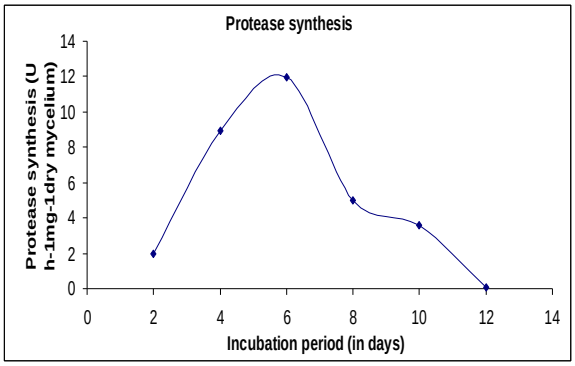

Figure 4. Effect of incubation period on the growth and protease production by *Aspergillus sydowii*.

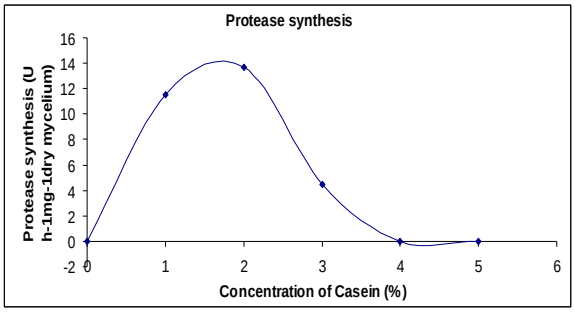

Figure 5. Effect of increasing concentration of casein on the growth and protease production by *Aspergillus sydowii*.

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DNA fingerprinting of Rape seed (*Brassica rapa* L.) varieties of Bangladesh using SSR markers

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Abstract: The identification and differentiation of the varieties through DNA fingerprinting using microsatellites (SSRs) are quite effective, when the variety specific primers are used. This is gaining importance particularly when the distinguishing a variety from others using morphological traits are becoming difficult due to use of limited elite varieties for new varieties. A set of microsatellite loci (B.n.12A, B.n.38A and B.n.59A1) has been investigate to distinguish the uniqueness of nine released rape seed (*Brassica rapa* L.) varieties in Bangladesh for the purpose of obtaining distinctness of the plant variety at molecular level. In the present study a total of nine rape seed (*Brassica rapa* L.) varieties have been used to characterize those groups. Upon PCR amplification, the alleles were separated on polyacrylamide gel using a sequencing gel electrophoresis system and visualized by silver-staining method. The loci were polymorphic in all the varieties. Differences were observed in heterozygosities in the studied varieties. The mean observed heterozygosity (Ho) and expected heterozygosity (He) were 0.124 and 0.507, respectively. Varied ranges of alleles occurred might be due to mutation of di-nucleotide repeat units which could also be indicative of varietal differences. Polymorphism Information Content (PIC) values in the present study were high which ranged from 0.481 to 0.667. UPGMA dendrogram based on Nei's (1972) genetic distance indicated differentiation of nine varieties of rape seed into two main clusters: Tori-7, BARI sharisha-9 and BARI sharisha-12 grouped in cluster 2 while others in cluster 1. In cluster 1 Agrani and Sampad grouped together in sub-cluster I and with minimal genetic distance (0.000). Safal also showed nil genetic distance with SS-75 and BARI sarisha-6. The varieties Sampad and Tori-7 showed the highest genetic distance value (3.860). Nine rape seed varieties in this study showed unique and differential DNA banding patterns across at least one and/or combination of three primers. The data obtained can be provided some levels of identity and protection against remaining and other practices are beyond ethics and rules.

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Keywords: SSR, Genetic diversity, Polymorphism Information Content (PIC), Rape seed

1. Introduction

In Bangladesh a good number of *Brassica* varieties have been released and registered for cultivation. The crop genus *Brassica* comprises of six economically important species with great genetic and morphological diversity. The origin, evolution, taxonomy, and genomic relationships of the crop Brassicas have been reviewed extensively. These are usually cultivated for their edible oils, the content of which varies between 33 and 37% of the grains. These seed oil however contains high erucic acids and high glucossinolates. In Bangladesh these varieties, particularly the one of *Brassica rapa* L. (Tori-7) is a very short duration crop (75-80 days). This variety is cultivated in marginal lands mostly as monocrop.

There are extensive *Brassica* breeding programmes from which large number of variants is being selected every year in Bangladesh. These along with the varieties already developed and registered for cultivation are very good sources of variable genes. It is not possible to differentiate all these materials only through morphological traits so attempts have been taken to use microsatellites for analysis of variation within and between populations. It is possible through this technique to calculate the genetic distances between individuals in order to infer levels of relatedness or even to determine parentage, which often represent an important basis for subsequent evolutionary or ecological analysis of phenotypical traits (Rahman et al., 2006, Lynch, 1990 and Bowcock et al., 1994).This assumption is further clear from the study of Song et al. (1999) where according to them many commercial soybean varieties arise from limited elite entries and are often indistinguishable based on these traits. They further opined that a system based on DNA markers could provide unique DNA profiles or fingerprints for

cultivars. This is more important in countries where the PVP has provisions for exemption for research purposes as in case of US PVP of 1994 which enables plant breeders to obtain Intellectual Property Rights for 20 years at the same time kept provisions for research sharing. Similar conditions exist in similar acts of many other countries. This requires the variety distinctness to be more unique and at the DNA level as in cases of forensic identification of distinctness by SSR.

In soybean, maize and potato there has been considerable works on the DNA profiling using either RAPD or SSR technique. The most important issue here is to distinguish the varieties not only at the morphological trait level but also at the DNA level. With increasing number cultivars in Bangladesh and the presently amended acts of Plant Variety and Farmers Right Protection Act (PVFRPA) where breeder's right has been given, the necessity of DNA level distinction has become necessary. With the increase number of varieties being in the farmers' field, there has been an interest to differentiate these varieties at molecular level so that the variety identification can lead to use of both morphological and molecular characters. The Ministry of Agriculture, Government of Bangladesh decided to characterize these varieties at their molecular level. The present study was an attempt to distinguish the uniqueness of the variety and establish genetic diversity of the nine of rape seed varieties in Bangladesh using a moderate number of primer pairs.

2. Materials and Methods

2.1 Collection of samples and isolation of genomic DNA:

Seeds of nine rape seed (*Brassica. rapa* L.) varieties were collected from Oilseed Research Center of Bangladesh Agricultural Research Institute [Sonali sarisha (SS-75), Kalayania (TS-72), Tori- 7, BARI sarisha-6, BARI sarisha-9 and BARI sarisha-12) and Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh (Sampad, Agrani and Safal)]. Seeds were germinated and grown at aseptic condition. Fresh leaf samples of 12-days-old seedling were used as the source of genomic DNA. Leaf tissues were cut into small pieces, homogenized and digested with extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 300 mM NaCl and 1% SDS, pH 8.0). After incubation for 20 minutes at 65 ^{o}C with intermittent swirling, the mixture was emulsified with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1 v/v/v). DNA was precipitated using two volume of absolute alcohol in presence of 0.3M sodium acetate and pelleted by centrifugation. The pellets were then washed with 70% ethanol, air dried and re-suspended in an appropriate volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH=8.0). DNA quality was checked by electrophoresis in a minigel and quantification was accomplished using a spectrophotometer (Spectronic® Genesis™, Spectronic Instruments Inc., USA).

2.2 Microsatellite markers and PCR amplification:

A set of five microsatellite loci (B.n.12A, B.n.35D, B.n.38A, B.n.59A1 and B.n.68/1) have been selected from the literature cited by Szewc-McFadden et al. (1996) to estimate the potential of these marker for variety identification. Finally three primers, B.n.12A, B.n.38A and B.n.59A1 were selected based on their performance for SSR data analysis (Table 01). Polymerase Chain Reactions were done in a volume of 10 µl containing 10X PCR Buffer, 0.25 mM each of the dNTPs, 2.5 µM of each primer, 1 unit ampli Taq DNA polymerase, 50 ng template DNA and a suitable amount of sterilize deionized water. Amplification were carried out in a oil free thermal cycler (Thermal cycler gradient, Eppendorf) with the following program: Initial denaturation at 94 ^{o}C for 3 min followed by 35 cycles at 95 ^{o}C for 30 sec, 58 ^{o}C for 45 sec, and 72 ^{o}C for 1 min and a final cycle at 72 ^{o}C for 7 min. PCR products were checked in 2% agarose gel.

2.3 Determination of Microsatellite allele lengths:

PCR products were electrophoresed on a 6% denaturing polyacrylamide gel containing 19:1 Acrylamide: Bis acrylamide and 8M urea. Electrophoresis was done using the Sequi Gen GT sequencing gel eletrophoresis system (BIO-RAD Laboratories, Hercules, CA). A pre-run of the gel for 30 minutes at 120W was followed by a final run at 60W and 50 ^{o}C upon loading of denatured PCR products for a specified period depending on the size of amplified DNA fragment (usually 1 hour for 100 bp). After completion of electrophoresis, the DNA fragments were visualized following the Promega (Madison, WI) Silver-staining protocol. The size (in nucleotides) of the most intensely amplified band for each microsatellite marker was determined based on its migration relative to molecular weight (mw) size markers (100bp DNA ladder, Genei, India).

2.4 Analysis of microsatellite data:

The bands representing particular alleles at the microsatellite loci were scored manually and designated the bands as A, B, C etc. from the top to the bottom of the gel. The genotypes of different strains were scored as AA, BB, CC etc. for homozygous or as AB, AC, BC etc. for heterozygous. A single genotypic data matrix was constructed for all loci. The software DNA FRAG version 3.03 (Nash, 1991) was used to estimate marker length and allelic length.

Table 1. Details of the microsatellite markers used in this study

Sl. No.	Locus	Forward Primer	Reverse Primer	Ann.T.
1	B.n.12A*	gcc-gtt-cta-ggg-ttt-gtg-gga	gag-gaa-gtg-aga-gcg-gga-aat-ca	58 ^{o}C
2	B.n.19A	cac-agc-tca-cac-caa-aca-aac-cta	ccc-cgg-gtt-cga-aat-cg	58 ^{o}C
3	B.n.38A*	caa-ggc-caa-aag-tgt-cca-t	acg-ctg-tct-tca-ggt-ccc-act	58 ^{o}C
4	B.n.59A-1*	tgg-ctc-gaa-tca-acg-gac	ttg-cac-caa-caa-gtc-act-aaa-gtt	58 ^{o}C
5	B.n.68A-1	tcg-cat-gct-cct-cta-gac-tcg	ttt-agc-acg-gga-atg-tca-gg	58 ^{o}C

Ref: Szewc-McFadden et al. (1996)

3. Results and Discussion

SSR profiles of nine rape seed (*Brassica rapa*) varieties tested with three primers are presented in Figure 1 in which three consecutive lanes presented each of the varieties and each lane with single individual. Allelic variation and diversity index (PIC) are shown in Table 2. All three microsatellite markers were found to be polymorphic, revealing a total of nine alleles with an average number of three alleles per locus in the nine rape seed (*Brassica rapa*) varieties examined (Table 2). At the B.n.12A locus, a total of three different alleles were identified among the nine rape seed genotypes ranging in size from 271 bp to 321 bp. Likewise, 3 alleles (size ranging from 149 bp-153 bp and 431 bp-450 bp) were detected at the locus B.n.38A and B.n.59A1 respectively (Table 2). Varied ranges of alleles occurred might be due to mutation of di-nucleotide repeat units which could also be indicative of varietal differences (Molla et al., 2007). The SSR are usually used widely for the analysis of the variations within and between populations. The distance calculations are based usually on the proportion of shared alleles (Lynch, 1990 and Bowcock et al., 1994) This method followed implicitly the infinite allele model, thus assuming independence of alleles and ignoring mutational processes, which can result in a biased distances especially when alleles are highly polymorphic. The identified SSR primers sometimes may not only indicate the locus of a single genome when the genomic constitution of the variety or the species is of long association and might have the possibility of sharing the gene(s) of one genome with that of the other accrued in the same genome through mutation of any dimension. This is more common when the tetraploidy happens in a population where the number of locus of the same gene(s) became double the number in diploid, and may also became three instead of four because of the nature of gene balance and gene recombination in the tetraploidy, which is part of the mutation by number of genome.

Brassica campestris or *B. rapa* is a diploid with AA genome of 2n=2x= 20, while *B. napus* (2n=2x=38) or rapeseed is an amphidiploid with *B. rapa* as one of its putative parents. The other parent is *B. oleracea* having CC genome of 2n=2x=18. These informations on the basic diploid also indicate that the 20 chromosome of *B. rapa* or 18 chromosome of *B. oleracea* have also absorbed the chromosome of two types of presently unknown sources. In case of the *B. rapa*, the selection for seed in the Mediterranean, Near east and India resulted into *oleifera*, *dichotoma* and *trilocularis* which have further given rise to *chinensis*, *pekinensis* and *nippossinica* having same number of chromosome with same AA designation, because they do easily get crossed with fertile fruits and seeds. This indicates the flexibility in its genomic constitution with absorbed micro-mutants.

Table 2: Size and frequency of alleles and diversity index at three microsatellite loci in nine rapeseed (*Brassica rapa* L.) varieties

Locus	Allele Size (bp)	Allele frequency	Diversity Index (PIC=1-$\sum Xi^2$)
B.n.12A	321	0.6852	0.481
	312	0.1667	
	271	0.1481	
B.n.38A	153	0.1111	0.667
	151	0.7778	
	149	0.1111	
B.n.59A1	450	0.2222	0.667
	443	0.4444	
	431	0.3333	

The PIC value which is the reflection of allele diversity was also estimated. The average PIC value was 0.605 and it ranged from 0.481 (B.n.12A) to 0.667 (B.n.38A and B.n.59A1). Lower PIC values were observed might be due to use of limited number of inbred rape seed varieties showing less number of alleles in our study. Gene diversity (heterozygosity) is presented in Table 3. The mean observed heterozygosity (Ho) and expected heterozygosity (He) were 0.124 and 0.507, respectively which might be due to small number of alleles (3) per locus.

Table 3. Summary of heterozygosity statistics for all loci

Locus	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
B.n.12A	0.630	0.370	0.510	0.499	0.4808	0.272
B.n.38A	1.000	0.000	0.623	0.377	0.3704	0.000
B.n.59A1	1.000	0.000	0.346	0.654	0.6420	0.000
Mean	0.877	0.124	0.493	0.507	0.4977	0.091
St. Dev	0.214	0.214	0.139	0.139	0.1366	0.157

* Expected homozygosity and heterozygosity were computed using
** Nei's (1973) expected heterozygosity

SSR genotypic data from a number of loci have the potential to provide unique allelic profiles or DNA fingerprints for precisely establishing genotypic identity. Comparisons between SSR band positions against each marker in this study are shown in Table 4. The band patterns corresponding to individual variety may help to recognize the variety in question. When one primer would not distinguish individual variety from others, another primer should be considered and sometimes combination of more than one primer should be taken into account. Thus additional primer or set of primers might be needed to test to identify all expected varieties. Among nine alleles detected, three were specific to two rape seed varieties. One specific allele was detected in the variety Sampad (B.n.38A/149) and BARI sarisha-9 (B.n.38A/153) (Table 2). The three microsatellite primer pairs were able to identify and discriminate six rape seed varieties.

Table 4: Analysis of three microsatellite loci for nine rape seed varieties

Sl. no.	Cultivars	Band positions due to primers (bp)											
		B.n.12A				B.n.38A				B.n.59A1			
1	Agrani	321			AA		151		BB	450			AA
2	Kalayania (TS-72)	321	312		AB		151		BB	450			AA
3	Sampod		312	271	BD			149	CC		443		BB
4	Safal	321			AA		151		BB		443		BB
5	Sonali sarisha (SS-75)	321			AA		151		BB		443		BB
6	Tori-7	321		271	AD		151		BB		443		BB
7	BARI sarisha-6	321			AA		151		BB		443		BB
8	BARI sarisha-9	321	312	271	ABD	153			AA			431	CC
9	BARI sarisha-12	321	312	271	ABD		151		BB			431	CC

The variety Agrani and Kalayania (TS-72) could be easily identified by the primer B.n.12A and B.n.59A1, in which Agrani showed diploid condition at locus B.n.12A (Table 4). In combination of two alleles B.n.12A 321+271 and B.n.59A1443 also identified Tori-7. Besides, BARI sarisha-12 only differed by the combination of B.n.12A and B.n.59A1 exhibiting 151 and 431 bp (Table 4). Our results represent one of the first attempts to find out a small set of microsatellite makers to discriminate rape seed varieties of Bangladesh providing meaningful data that can be enlarged by additional rape seed varieties and new microsatellite markers. Microsatellites are considered appropriate for variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently (Smith et. al. 1996). In a study, a minimum number of three microsatellite markers was sufficient for rapid and unambiguous discrimination of olive varieties (Dunja et. al., 2002).

UPGMA dendogram based on Nei's (1972) genetic distance indicated segregation of nine varieties of rapeseed into two main clusters: Tori-7, BARI sharisha-9 and BARI sharisha-12 grouped in cluster 2

while others in cluster 1 (Fig. 1). In cluster 1 Agrani and Sampad grouped together in sub-cluster I with minimal genetic distance (0.000). The highest genetic distance value (3.860) was observed between Sampad and Tori-7 (Table 5). This distance is possible because the variety Sampad is of yellow sarson ecotype having self-compatibility as the nature of pollinating behaviour. The material was first introduced in Bangladesh from Czechoslovakia and subsequently with adequate selection pressure the variety was released by Bangladesh Agricultural University, Mymensingh. It is also interesting to note that the Agrani and Sampad falls in the same group also because, possibly the latter one is selection from the former.

The results of the present study could be applied as a preliminary instruction to maintain the appropriate identity of rape seed varieties and in broad sense, to protect the plant varieties of Bangladesh. The SSR profiles could distinguish some of the rape seed varieties. The unidentified varieties should be characterized at more loci and a set of least number of informative loci to be identified for variety identification. The data obtained can be used for varietal survey and the construction of a database of all rape seed varieties grown in Bangladesh, providing also additional genetic information of the agronomic and quality characteristics of rape seed varieties.

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Table 5. Summary of Nei's (1972) genetic distance values between nine rapeseed (*Brassica rapa* L.) varieties for all loci

Varieties	Agrani	Kalayania (TS-72)	Sampad	Safal	Sonali sarisha (SS-75)	Tori-7	BARI sarisha-6	BARI sarisha-9	BARI sarisha-12
Agrani	****								
Kalayania (TS-72)	0.090	****							
Sampod	0.000	2.025	****						
Safal	0.4055	0.602	1.018	****					
Sonali sarisha (SS-75)	0.405	0.602	1.018	0.000	****				
Tori-7	0.443	0.610	**3.860**	0.449	0.443	****			
BARI sarisha-6	0.405	0.602	1.018	0.0000	0.000	0.443	****		
BARI sarisha-9	1.677	1.992	2.4086	1.677	1.677	0.549	1.677	****	
BARI sarisha-12	0.697	0.670	2.185	0.697	0.697	0.077	0.697	0.562	****

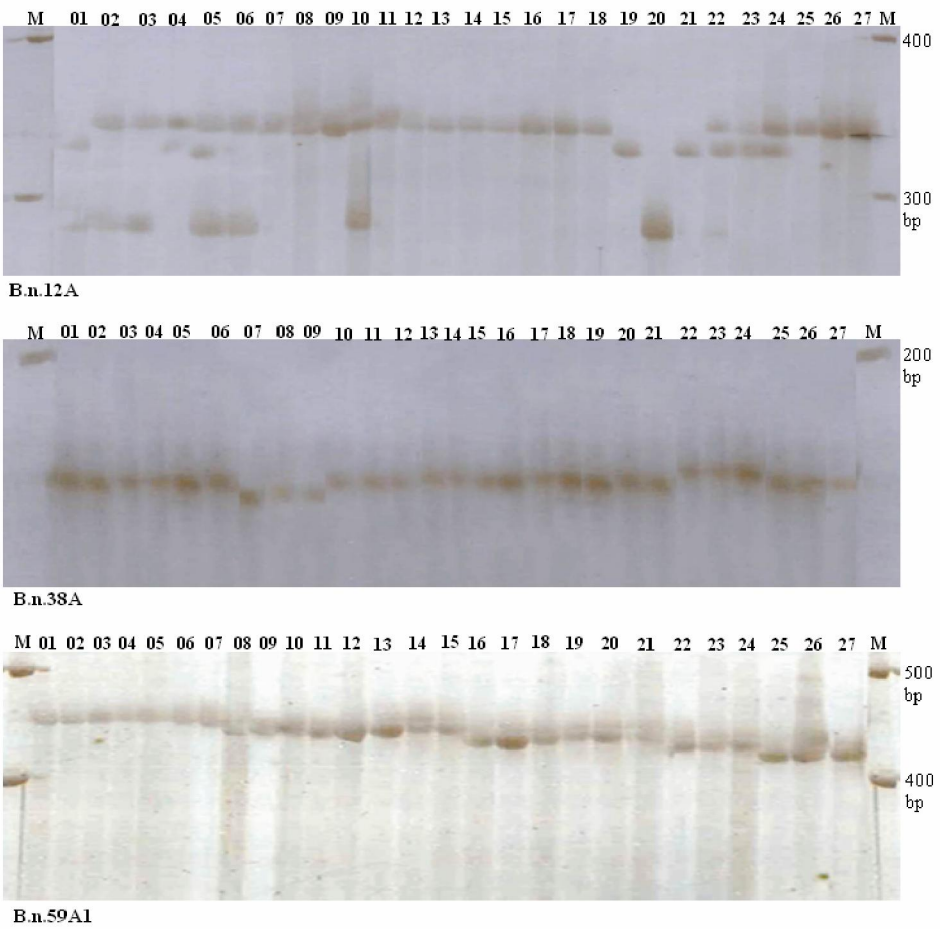

Figure 1. Microsatellite profiles of nine rape seed (*Brassica rapa* L.) varieties across three loci, B.n.12A, B.n.38A and B.n.59A1. Lanes, 1-3 = Agrani; 4-6 = Kalayania (TS-72); 7-9 = Sampad; 10-12 = Safal; 13-15 = Sonali sarisha; 16-18 = Tori 7; 19-21 = BARI sharisha-6; 22-24 = BARI sharisha-9; 25-27 = BARI sharisha-12. M: Molecular wt. Marker (100 bp DNA ladder)

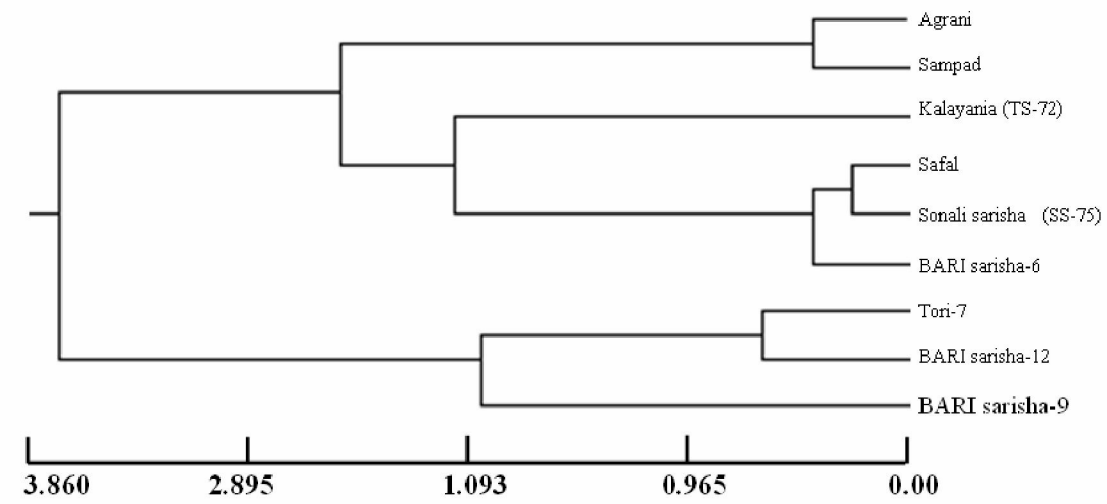

Figure 2. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between five Rapeseed varieties according to microsatellite analysis.

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