An International Journal

Nature and Science

ISSN 1545-0740

Volume 8 - Number 7 (Cumulated No. 40), July 1, 2010

Marsland Press

P.O. Box 21126, Lansing, Michigan 48909, USA

525 Rockaway PKWY, #B44, Brooklyn, New York, 11212, USA

http://www.sciencepub.net
http://www.sciencepub.org
editor@sciencepub.net
naturesciencej@gmail.com

347-321-7172

Nature and Science

ISSN: 1545-0740

The ***Nature and Science*** is an international journal with a purpose to enhance our natural and scientific knowledge dissemination in the world under the free publication principle. Papers submitted could be reviews, objective descriptions, research reports, opinions/debates, news, letters, and other types of writings that are nature and science related. All manuscripts submitted will be peer reviewed and the valuable papers will be considered for the publication after the peer review. The Authors are responsible to the contents of their articles.

Introductions to Authors

1. General Information

(1) Goals: As an international journal published both in print and on internet, *Nature and Science* is dedicated to the dissemination of fundamental knowledge in all areas of nature and science. The main purpose of *Nature and Science* is to enhance our knowledge spreading in the world under the free publication principle. It publishes full-length papers (original contributions), reviews, rapid communications, and any debates and opinions in all the fields of nature and science.

(2) What to Do: *Nature and Science* provides a place for discussion of scientific news, research, theory, philosophy, profession and technology - that will drive scientific progress. Research reports and regular manuscripts that contain new and significant information of general interest are welcome.

(3) Who: All people are welcome to submit manuscripts in any fields of nature and science.

(4) Distributions: Web version of the journal is freely opened to the world, without any payment or registration. The journal will be distributed to the selected libraries and institutions for free. For the subscription of other readers please contact with: sciencepub@gmail.com; naturesciencej@gmail.com.

(5) Advertisements: The price will be calculated as US$400/page, i.e. US$200/a half page, US$100/a quarter page, etc. Any size of the advertisement is welcome.

2. Manuscripts Submission

(1) Submission Methods: Electronic submission through email is encouraged.

(2) Software: The Microsoft Word file will be preferred.

(3) Font: Normal, Times New Roman, 10 pt, single space.

(5) Manuscript: Don't use "Footnote" or "Header and Footer".

(6) Cover Page: Put detail information of authors and a short title in the cover page.

(7) Title: Use Title Case in the title and subtitles, e.g. "**Debt and Agency Costs".**

(8) Figures and Tables: Use full word of figure and table, e.g. **"Figure 1.** Annul Income of Different Groups**", Table 1. Annual Increase of Investment".**

(9) References: Cite references by "last name, year", e.g. "(Smith, 2003)". References should include all the authors' last names and initials, title, journal, year, volume, issue, and pages etc.

Reference Examples:

Journal Article: Hacker J, Hentschel U, Dobrindt U. Prokaryotic chromosomes and disease. Science 2003;301(34):790-3.

Book: Berkowitz BA, Katzung BG. Basic and clinical evaluation of new drugs. In: Katzung BG, ed. Basic and clinical pharmacology. Appleton & Lance Publisher. Norwalk, Connecticut, USA. 1995:60-9.

(10) Submission Address: naturesciencej@gmail.com, Marsland Press, PO Box 180432, Richmond Hill, New York 11418, USA, 347-321-7172.

(11) Reviewers: Authors are encouraged to suggest 2-8 competent reviewers with their name and email.

2. Manuscript Preparation

Each manuscript is suggested to include the following components but authors can do their own ways:

(1) Title page: Including the complete article title; each author's full name; institution(s) with which each author is affiliated, with city, state/province, zip code, and country; and the name, complete mailing address, telephone number, facsimile number (if available), and e-mail address for all correspondence.

(2) Abstract: Including background, materials and methods, results, and discussions.

(3) Keywords.

(4) Introduction.

(**5) Materials and Methods**.

(**6) Results**.

(**7) Discussions**.

(**8) Acknowledgments**.

(**9) References**.

Journal Address:

Marsland Press
PO Box 180432, Richmond Hill, New York 11418, USA
Telephone: 347-321-7172
Emails: editor@sciencepub.net;
naturesciencej@gmail.com;
Websites: http://www.sciencepub.net;

ISSN: 1545-0740

Nature and Science

ISSN: 1545-0740

Volume 8 - Number 7 (Cumulated No. 40), July 1, 2010

Cover Page, Introduction, Contents, Call for Papers, ns0807

Welcome to send your manuscript(s) to: editor@sciencepub.net.

CONTENTS

	conditions of Garhwal Himalaya, the experiment was undertaken at the HRC, Garhwal University Srinagar, Uttarakhand, India. The effect of different concentration of Indolebutyric acid (IBA) and different growing conditions have been examined for stimulatory effects adventitious root formation in stem cutting of Kagzi-lime. Properly prepared cuttings of about 22-24 cm length in the month of June were treated with different concentrations of IBA viz., 500, 1000, 1500 ppm for 5 second by concentrated solution dip method and planted in 3 different conditions namely open area, under partial shade and under low cost polyhouse. The cuttings treated with IBA 500 ppm, performed the best in all aspects, as root formation, length of root, thickening of root and leaf sprouting in shoot, whereas, the open area growing condition was found effective in increasing the success rate of the cuttings. All the rooting parameters performance was recorded highest under polyhouse condition. Overall treatment C_1M_3 (IBA 500ppm and polyhouse) combination was found best in all parameters taken. [Nature and Science 2010;8(7):8-11]. (ISSN: 1545-0740). **Keywords***:* Kagzi lime, cuttings, IBA, rooting, low-cost poly house, Garhwal Himalaya	
3	**Anthropometric Measurements and Appetite Related Hormones in Obesity Patients** Hamdy A. Ahmed* and Mohamed I.Aref** *Biochem., Dept., NRC. Dokki, Cairo, Egypt. **Clin., Pathology Dept., El Hussein Hosp., Fac., of Medicine, Al Azhar Uni. Cairo, Egypt. **Abstract:** Obesity and hyperlipidemia are two of the most highly distributed disorders that commonly associated with a cluster of progressive pathogenesis of many public health problems. This nutritional diseases increase the morbidity and the mortality of other diseases as cardiovascular diseases, atherosclerosis, hypertension, type2 diabetes, osteoarthritis and certain types of cancer. Thus the goal of the present study was to investigate the anthropometric measurements and biochemical parameters in case of obese patients before and after dietary treatments as well as the obesity related hormones in obese and in comparison with their corresponding healthy subjects. Serum leptin concentration increased significantly in obese subjects associated with a significant decrease in serum ghrelin concentration in obese group only. After dietary treatment serum leptin concentration decreased significantly in obese subjects while serum ghrelin increased significantly when compared with their values before treatments. There was a significant increase in serum glucose, insulin and insulin resistant (IR) value in obese subjects. While after dietetic treatment for obesity, there was a significant decrease in serum glucose, insulin and IR associated with a significant increase in serum adiponectin levels. In conclusion, the main features of obesity are leptin resistance and insulin resistance. Other hormonal influences are ghrelin and adiponectin which could be pathogenic factors for obesity. Hyperghrelinemia lead to hyperphagia and morbid obesity. While hypoadiponectinemia correlated to insulin resistance and diabetes type 2. So reducing body weight and controlling of hyperlipidemia enhancing insulin and leptin sensitivity through increase in adiponectin secretion, which has known as anti-diabetic, anti-inflammatory and anti-atherogenic hormone. [Nature and Science 2010;8(7):12-19]. (ISSN: 1545-0740). **Keywords:** Obesity; hyperlipidemia; anti-diabetic; anti-inflammatory; anti-atherogenic; hormone	Full Text
4	**Studies on some Benzopyran Derivatives with Expected Antimicrobial and Antiviral Activity** Hanaa.F.Roaiah [a], Sally.S.El-Nakkady [a], Weam.S.El-Serwy [a], Mohamed.A.A.Ali [b],A.H.Abd El-Rahman [c], Zeinab El-Bazza[d] a Natural Chemistry Products Department, National Research Center, Dokki, Cairo, Egypt b virology Department National Research Center, Dokki, Cairo, Egypt c Chemistry Department, Faculty of Science, Mansoura University, Egypt d Pharmaceutical Microbiological Lab, National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt **Abstract:** The naturally occurring furocoumarin (xanthotoxin) **(1)** yielded 3-(6-hydroxy-7-methoxy-1-	Full Text

	benzofuran-5-yl) acrylohydrazide **(2)** upon treatment with hydrazine hydrate. When **2** was treated with an equimolar amount of the appropriate isothiocyanates it gave the respective N-substituted – 2 - [3 - (6 – hydroxy – 7 – methoxy – 1 – benzofuran – 5 - yl) prop – 2 -enoyl] hydrazine carbothioamide **(3a-c)**, which when heated with sodium hydroxide, yellow mercuric oxide and phosphorus oxychloride respectively were transformed into 5-[2-(4-substituted-5-mercapto-4H-1,2,4-triazol-3-yl)vinyl]-7-methoxy-1-benzofuran-6-ol **(4a-c)**, 5-{2-[5-(substituted amino)-1,3,4-oxadiazol-2-yl] vinyl}-7-methoxy-1-benzofuran-6-ol **(5a-c)**, and 5-{2-[5-(substituted amino)-1,3,4-thiadiazol-2-yl] vinyl}-7-methoxy-1-benzofuran-6-ol **(6a-c)** respectively. The reaction of **3a** with ethyl bromoacetate gave N'-(3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene)-3-(6-hydroxy-7-methoxy-1-benzofuran-5-yl)acrylo hydrazide **(7)**. When **3a,b** was heated with ethyl cyanoacetate it yielded N-substituted-2-[4-(6-hydroxy-7-methoxy-1-benzofuran-5-yl)-2-imino-2H-pyran-6-yl]hydrazine-carbothioamide **(8a,b)** respectively. The prepared compounds were tested for their antimicrobial and antiviral activities. [Nature and Science 2010;8(7):20-29]. (ISSN: 1545-0740). **Keywords**: Benzopyran; Antimicrobial; Antiviral Activity	
5	**Effect of arginine on growth, nutrient composition, yield and nutritional value of mung bean plants grown under salinity stress** Amira M. S. Abdul Qados Botany Department, Princess Nora Bint Abdul Rahman University, P. O. Box 2508 Safaqes St. Granada Esq. Riyadh 13242 – 07229 K.S.A **Abstract:** Salinity is a major limitation to legume production in many areas of the world. The salinity sensitivity of mung bean was studied to determine the effect of salinity on vegetative growth (plant dry weight and plant height), yield components (plant height, pods number, pods weight, seeds number/pod, seeds weight/plant and biological yield/plant), nutritional value of produced seeds (N, P, K, Ca, Mg, Na, Cl, soluble carbohydrate, polysaccharides, total carbohydrate, proline, total amino acids and protein contents) and mineral contents in green shoot at harvest (N, P, K, and Na). Also, the role of arginine in alleviating the effect of salinity stress was studied. Mung bean seeds were planted in soils of different salinity levels. The concentration of the irrigation water used in this experiment were (0, 15000, 3000, 4500 and 6000 ppm). All growth parameters were significantly reduced with high salinity levels (4500 and 6000 ppm) while 1500 and 3000 ppm induced slight increase. Salinity stress also, induced significant increases in Na, Cl, Ca and Mg and decreased significantly N, P, and K contents. Salinity stress reduced most yield components and nutritional value of produced seeds. However, spraying plants with arginine could alleviate the harmful effect of salinity at all studied parameters. [Nature and Science 2010;8(7):30-42]. (ISSN: 1545-0740). **Key words**: Mung bean, Salinity, Arginine, growth, Yield, Mineral compositions	Full Text
6	**Emerging trend of urban green space research and the implications for safeguarding biodiversity: a viewpoint** Gairola, S. and M. S. Noresah School of Distance Education, Universiti Sains Malaysia 11800, MINDEN, Penang, Malaysia drsanjaygairola@gmail.com, noreshah@usm.my **Abstract:** It is abundantly clear that rapid urbanization is greatly transforming the spatial pattern of urban land use worldwide. Consequently, the resulting losses of urban green space at local to global level are continuously altering urban ecosystems. Recent research on this aspect has stressed on the importance of urban green spaces as well as their losses due to rapid urban growth. The importance of ecosystem services provided by urban green spaces for human well-being is gaining recognition and has been highlighted by most of the recent studies. In this short communication, we discuss the emerging trend of urban green space research and its implications for safeguarding biodiversity in line with the declaration by the United Nations that 2010 to be the International	Full Text

Year of Biodiversity. [Nature and Science 2010;8(7):43-49]. (ISSN: 1545-0740).
Keywords: Urban Green Space, Biodiversity, Ecosystem Services, Urban Dwellers, Sustainable Development

7

Effect of location and growth season on the productivity and quality of some range plants in Wadi Halazien in the North Western Coast in Egypt

El-Zanaty, R. I. A.⋆, A. A. Abdel-Hafez⋆, k. I. Abdel-Gawad⋆, M. H. M. El-Morsy** and H. M. A. Abusaief⋆
⋆Faculty Agriculture, Cairo University, Egypt
**Desert Research Center, Cairo, Egypt

Abstract: This study was conducted during spring and autumn seasons of 2007 and 2008 in wadi Halazien, at the North West Coast region of Matruh governorate in Egypt. The aim of this investigation was to survey and classify natural plants species and to study the effect of location and seasonal changes on range productivity and quality of pasture species. Seventy-two plant species belong to 29 families (41.67 % annuals, 1.39 % biennials and 56.94 % perennials) were found. *Polygonum equisetiforme* in top, *Deverra tortuosa* in ridge, *Chiliadenus candicans* in bed 1 and *Carduncellus eriocephalus* in bed 2 gave the highest abundance in spring 2007. Whereas, *Gymnocarpos decandrus* in top, *Leopoldia comosa* in ridge and *Carduncellus eriocephalus* in bed 1 and 2 gave the highest one in spring 2008. Total abundance in bed 2 significantly surpassed other sites in both years. Species richness and Simpson's index of diversity in spring increased than in autumn in all sites of both years. *Lycium shawii* in top, *Euphorbia dendroides* in ridge, and *Thymelaea hirsuta* in bed 1 gave the highest importance value in spring 2007. Generally, plant species of family *Caryophyllaceae* in top, *Euphorbiaceae* in ridge, *Thymelaeaceae* in bed 1 and *Apiaceae* in bed 2 gave the highest fresh and dry yields in spring 2007, while, *Thymelaeaceae* in top, ridge and bed 2 and *Cistaceae* in bed 1 gave the highest ones in spring 2008. *Gymnocarpos decandrus* in top, *Euphorbia dendroides* in ridge, *Thymelaea hirsuta* in bed 1 and *Atriplex nummularia* in bed 2 gave the highest fresh and dry yields in spring 2007. While, *Thymelaea hirsuta* in top, *Gymnocarpos decandrus* in ridge, *Fumana thymifolia* in bed 1 and *Carduncellus eriocephalus* in bed 2 had the highest ones in spring 2008. Seasonal fresh and dry yields in bed 2 significantly exceeded other sites in both years. *Fabaceae* gave the highest CP % and DCP % at all sites, except in ridge *Brassicaceae* gave the highest ones in spring 2008. *Erodium crassifolium* in top, *Achillea santolina* in ridge, *Lotus polyphyllus* in bed 1 and 2 gave the highest CP % and DCP % in spring 2008. Crude protein % and DCP % in spring significantly exceeded it in autumn at all sites in both years. Abundance, fresh and dry yields had a positive correlation with precipitation, and a negative correlation with temperature. [Nature and Science 2010;8(7):50-70]. (ISSN: 1545-0740).

Key words: The productivity; Wadi Halazien; North Western Coast

Full Text

8

Morphological and Isozyme diversity in the accessions of two cultivated species of barnyard millet

Deepti prabha[1], Y. K. Negi[2], V. K. Khanna[1]

1. College of Agriculture, G. B. Pant University of Ag. and Tech, U. S. Nagar (Uttarakhand), India;
2. SBS PG Institute of biomedical Sciences and Research, Balawala, Dehradun (Utarakhand), India

deepti_prabha@rediffmail.com; yknegi@rediffmail.com; vkkhanna@rediffmail.com

Abstract: Present study was conducted to access genetic diversity among 33 accessions of two cultivated species of barnyard millet i.e., *Echinocloa crus-galli* (15 accessions) and *E. frumentacea* (18 accessions). Though crop possesses great nutritional value, little attention has been paid for the improvement of this crop. Peroxidase and esterase analysis showed seven loci possessing 23 alleles in all 33 accessions. Esterase was found to be more useful to assess diversity with more polymorphism in comparison to peroxidase. Based on allozyme frequencies, all the accessions of both the species were grouped separately in to two different groups at a linkage distance of

Full Text

	6.2. The data would be important in detailing the level of variation and relationship within and between species to plan future domestication trials and to manage the wild species collection that is available in the gene banks. [Nature and Science 2010;8(7):71-76]. (ISSN: 1545-0740). **Key words**: Barnyard millet, genetic diversity, isozyme analysis, peroxidase, esterase	
9	**Cyanobacteria of a Tropical Lagoon, Nigeria**. Adesalu, Taofikat Abosede [1], Nwankwo, Dike Ikegwu.[2] [1]Department of Botany and Microbiology, University of Lagos, Nigeria. [2]Department of Marine sciences, University of Lagos, Nigeria. boseadesalu@yahoo.com. **Abstract**: Investigations for the first time into the blue green algae of Lekki lagoon were carried out for 24 months (June 2003- May 2005) at monthly intervals using standard plankton net of mesh size 55µm. One hundred and seventy nine species belonging to thirty genera were observed. The filamentous blue green algae *Oscillatoria* formed the most abundant genus making up twenty three species followed by *Phormidium* eighteen species. *Anabaena* and *Chroococcus* recorded thirteen species each while the genera, *Gleocapsa, Merismopedia* and *Microcystis* recorded ten, eight and twelve species respectively. Only one genus each of *Cyanosarcina, Calothrix* and *Scytonema* were encountered. Bloom forming species identified were *Microcystis aeruginosa, M. flos-aquae, M. wesenbergii* and *Anabaena flos-aquae.* In this study, thirty-nine new species were recorded for Lagos lagoon complex in which Lekki lagoon is one of it while *Cyanosarcina hueberliorum* is new record for Nigeria. [Nature and Science 2010;8(7):77-82]. (ISSN: 1545-0740). **Keywords:** Cyanophytes, tropical, bloom, Lagos lagoon complex	Full Text
10	**Lipid profile among chronic hepatitis C Egyptian patients and its levels pre and post treatment** Ehab H Nashaat, MD Associated professor of internal medicine ,Faculty of medicine ,Ain Shams university. ehabnashaat@hotmail.com **Abstract: Background:** Hepatitis C is a common infection in the Egyptian population, specially genotype 4 .It is well recognized in many studies that hepatitis C chronic infection is associated with hypolipidemia, so in our study we compare the lipid profile between 150 patients with chronic hepatitis C and 150 normal persons with comparable age, sex and body mass index (BMI). The fasting cholesterol ,low density lipoprotein (LDL), high density lipoprotein (HDL),and triglyceride were compared .Then 36 patients of them received treatment in the form of pegylated interferon and ribavirin and then the patients who achieved viral clearance was reevaluated as regard the lipid profile versus the patients who did not achieve viral clearance and the relpsers. In our study we found that patients with chronic hepatitis C had significant lower LDL, cholesterol, and triglycerides than normal persons with comparable age, sex and BMI .The treated patients with sustained virological response showed increased LDL, cholesterol, and triglycerides from baseline compared to patients without viral clearance and even 2of them had increased LDL more than 130 mg/dl and had increased in cholesterol level more than 200 which necessate treatment for dyslipidemia in order to prevent the risk of coronary heart disease. Conclusion : patients with chronic hepatitis C had high levels of LDL, cholesterol, and triglycerides than non infected persons and after viral clearance a significant number of patients showed LDL, cholesterol, and triglycerides rebound even to levels may be associated with increased risk for coronary heart disease, so lipids should be carefully followed up after successful clearance of hepatitis C infection . [Nature and Science 2010;8(7):83-89]. (ISSN: 1545-0740). **Keywords:** HCV infection,lipids profile ,pre and post treatment	Full Text
11	**Comparative studies on the Indian cultivated *Pleurotus* species by RAPD fingerprinting** Swarnendu Chandra, Kabita Ghosh and Krishnendu Acharya*	Full Text

Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700 019, West Bengal, India.
krish_paper@yahoo.com

Abstract: The oyster mushrooms (*Pleurotus*) are the second most important cultivated mushroom in the world. The genus consists of a number of species. The genetic divergent of eight Indian species of *Pleurotus* viz. *P. florida* – P1, *P. membranaeceus* – OE128, *P. sajor-caju* – PL1140, *P. djamor* – X375, *P. cystidiosus* – P19, *P. flabelletus* – PL50, *P. sapidus* – PL40 and *P. ostreatus* – PO1803 was determined based on random amplified polymorphic DNA (RAPD) pattern. Result showed that all the species tested could be differentiated by RAPD data and even one individual primer (OPD-07) could also discriminate all tested species. Genetic similarity analysis and grouping derived from RAPD markers reveals a high level of genetic diversity. Therefore the RAPD technique can provide a powerful tool to discriminate the species and the molecular information are useful for the breeding system. [Nature and Science 2010;8(7):90-94]. (ISSN: 1545-0740).
Keywords: Oyster mushroom, *Pleurotus* species, RAPD

12

Factors Affecting the distribution and abundance of Bottom Fauna in Lake Nasser, Egypt

Isaac A. Iskaros and M. El Dardir*
*National Institute of Oceanography and Fisheries, Aswan, Egypt
dr_mona_zaki@yahoo.co.uk

Abstract: This study aim to determine which factors regulate the benthic invertebrates in the offshore area of Lake Nasser. The area investigated represents about 80% of the total lake and that is not well utilized. Seven stations along the main channel of the lake and three main khors out of 85 were selected. Transparency, temperature, conductivity, dissolved oxygen, hydrogen ion concentration, some characteristics of bottom sediments, the population density and biomass of bottom fauna were measured. Only 10 species belonging to oligochaetes (3 species), chironomid larve (4 species) and molluscs (3 species) were recorded. The former were the most common group. The highest standing stock of bottom fauna was noticed in the main channel, particularly during spring (avg. 5846 org./m^2 and 29.6 g. f.w./m^2) associated with clay and silt grains representing (40.5 – 54.5%) and (37.0 – 46.0%), respectively; and subsequently high content of organic matter (8.0 – 12.5%). The three khors sustained low densities of bottom fauna and sediments constituted mainly of sand with low organic matter. [Nature and Science 2010;8(7):95-108]. (ISSN: 1545-0740).
Keywords: Lake Nasser, water quality, bottom sediments, bottom fauna, community structure

Full Text

13

Floristic Composition and Biological Spectrum of Vegetation in Alpine Meadows of Kedarnath: Garhwal Himalaya

*Kusum Pharswan *, J. P. Mehta and Subodh*
Department of Botany, Post Box-22, H. N. B. Garhwal University, Srinagar (Garhwal), 246174, India
*kkusumpharswan@gmail.com, Research fellow

Abstract: The present paper gives an account of flora of Kedarnath which have very harsh climatic conditions. 80 species, belonging 36 families were estimated from the study area. Notes on phenological pattern, life form (biological spectrum), plant type and uses have also been studied. Asteraceae was the dominant family (11.25%) recorded under present investigation. The genus and species are arranged alphabetically with in a family with correct nomenclature. The major class of life form was found to be Chamaephytic. Besides Chamaephytes (36.25%), the other life forms enumerated were Therophytes (28.75%), Cryptophytes (18.75%), Hemicryptophytes (11.25%) and Phenerophytes (5%). Most of the plant species had flowering and fruiting in rainy season, followed by summer season and very few species in winter season. [Nature and Science 2010;8(7):109-115]. (ISSN: 1545-0740).
Key words: Kedarnath, life forms, biological spectrum, floristic list, altitude

Full Text

The effects of Agnucaston and Metformin on the chromosomes of pregnant females and their embryos

Hanaa M.Roshdy

Cell Biology Department, National Research Centre, Giza, Cairo

Abstract: Background:Agnucaston (chaste berry fruits) and Metformin are the most common medications in the treatment of polycystic ovary syndrome (PCOS) and hormonal imbalance. The safe use of Agnucaston and Metformin in the virgin females and in the pregnant females and their embryos has not been adequately studied. **Aim** of the study is to evaluate the cytogenetic effects of Agnucaston and Metformin before and during pregnancy. **Materials and Methods**: The sample of this study was female mice (Virgin and pregnant) divided into two groups control (did not administer any medication) and treated group (administered Agnucaston and Metformin orally) by doses of 0.3 and 1.3 mg/kg/day respectively for (15) consecutive days. After one day from the last treatment the females were sacrificed and cytogenetic analysis were conducted. **Results**: females treated with Agnucaston(Virgin and pregnant) showed increase in frequencies of chromosomal aberrations significantly and also in their embryos but these increases were highly significant in pregnant females than virgin While, the females treated with Metformin there was a slight significant increase in the frequencies of the chromosomal aberrations in the pregnant females and embryos but there was no significant increase in the virgin females treated with Metformin before pregnancy. **Conclusion**: Our results indicate that Agnucaston has a mutagenic effects on the females (Virgin and pregnant) and on the embryos while Metformin has a slight mutagenic effects on the pregnant females and their embryos but does not have a mutagenic effects on the virgin females. [Nature and Science 2010;8(7):1-7]. (ISSN: 1545-0740).

Key words: (polycystic ovary syndrome), Agnucaston, Metformin, chromosomal aberrations, mice, embryos.

Introduction

Polycystic ovary syndrome (PCOS) a common endocrine-pathy and is the major cause of menstrual disorder and an ovulate infertility. It affects more than 15 to 20% of girls and young women in the world. Rotterdam (2004). The most common cause of (PCOS) is the hormonal imbalance in the brain and in the ovary at the same time. The pituitary gland in the brain produce excess Latinizing hormone (LH) which increases the production of male hormones (testosterone) by the ovary .This high level of male hormones increases the risk of metabolic syndrome (high blood pressure, high cholesterol and high insulin in the blood).Because the level of male hormones remain high, so some of it may be converted to estrogen causing increase in its levels in the blood without change in progesterone level causing hormonal imbalance ,leading to stop ovulation and irregular menstrual cycle.

The use of Agnucaston is the most common form in treatment of (PCOS). It is (a herbal medication) that contains the (chaste berry). Chasteberry has antioxidants, flavonoids and other chemical properties that act on the neurotransmitter dopamine in the brain which correct the hormonal imbalance and lower the level of Luteinizing hormone which also lower the level of testosterone in the blood so help to imbalance the level of progesterone and the level of estrogen in the blood, Milewicz. et al (1993) and Lauritzen et al (1997). Chaste berry is sometimes called (the women's herb). It is used in regulation of the menstrual cycle, premenstrual disorder, female infertility and treating polycystic syndrome. Chasteberry seems to affect many hormones that regulate women's reproductive cycles, Jarry et al (1994). Metformin is another medication which decreases insulin level. because the high insulin level initiates ovaries to produce more testerone hormone, Dunaif et al (1989). Only one of the two medications are required for the treatment of PCOS but most women are treated with the both in the same time.

At present no adequate data illustrates the safety use of Agnucaston and Metformin in the females before and during pregnancy is available. So, in the present study, we aimed to study the cytogenetic effects of Agnucaston and Metformin if given orally for about 15 consecutive days to the females before pregnancy and during pregnancy. In addition, their effects on their embryos .Results were compared with the controls.

2-Materials and Methods:

2.1.Materials:

2.1.1. Chemical drugs:

a) Metformin hydrochloride: was obtained from a pharmacy in Egypt under a trade name (Amophage). Its chemical name is (N, N-dimethy Limidodicarbonimidic

hydrochloride). It is not chemically or pharmacologically related to any other classes of oral anti-hyperglycemic agents. Its molecular formula is $C_{14}H_{11}N_5$.HCI and a molecular weight is165.63. It is freely soluble in water.

Amophage is used to management diabetes (type II diabetics as also used for treating the polycystic ovary syndrome (PCOS).

The recommended dose of Amophage is 500mg/kg/day for human,. Norman (2003).

b) Agnucaston**:**

Was obtained from (Bionorica) (Germany).

Agnucaston is a film coated tablets (125mg) contains: Dry extract of chaste tree fruit. Each tablet contains 125 mg of chastetree.It is soluble in water.

Chaste tree is sometimes called (the women's herb) it is used for menstrual disorders and polycystic syndrome (PCOS) Chaste berry seems to control production of many chemicals (dopamine) and hormones in the brain that regulate women's reproductive cycles. The recommended dose of Agnucaston is 125 mg/kg/day, for human,Hobbs and Blumenthal (1999).

2.1.2. Study sample:

Virgin female mice weighting 25-30 gm were acquired in pathogen-free, well-ventilated room in order to enable the animals to acclimatize to their environment. Drinking water and food supplied add libitum. They were divided into two groups; first group were used for studying the effects of the treatments (in virgin females) and the second group were used for studying the effect of the treatments (in pregnant females) as following:

1- The first group was divided into three parts. The first part of virgin females administrated orally a single dose of Amophage 1.3mg/kg/day. This dose equal to the recommended dose for human after modified to suit the small weight of albino mice according to Pagat and Barnes (1964). The second part of virgin females administrated orally a single dose of Agnucaston 0.3 mg/kg/day. This dose equal to the recommended dose for human after modified to suit the small weight of albino mice according to pagat and Barnes (1964). The third part virgin females served as control administrated distilled water.

2- The second group were females housed with adult males by ratio of 3.1 after one day of mating the females which exhibiting a vaginal plug were considered as pregnant. The day of the appearance of the vaginal plug was considered as the first day of pregnancy. The pregnant females were weighted and caged individually and divided into three parts: The first part administrated orally from the day (3) to the day (18) of gestation with a dose of (1.3 mg.kgm/day) Amophage. The second part administrated, orally from (3) to day (18) of gestation with a dose of (0.3 mg/kg/day) Agnucaston. The third part served as control were administrated distilled water orally. After (15) days of treatments with (Amophage and Agnucaston) the virgin and pregnant females were killed, the bone marrow of females were collected and (15) embryos were randomly selected from each part of pregnant females to study the chromosomal abnormalities.

2.2. Methods

2.2.1. Chromosomal preparations from bone marrow cells of females (virgin and pregnant).

Chromosomes from bone marrow cells were prepared according to the methods of HUS and patton (1969) and Yosida ***et al.*** (1971).

Bone marrow cells were collected in T.C.M. 199 culture media and colchicine was added (2m.L of 0.05 Colchicine) then, the cells were incubated at 37°C for 90 minutes. After centrifugation, 5ml of hypotonic solution of (0.56%) KCl was added and the pellet suspended and incubated at 37°C for 30 minutes. After centrifugation the cells were fixed in freshly prepared 3:1 methylalcohol-glacial acetic acid then two or three drops of cell suspension were dropped on a clean slide. Slides were stained with Giemsa stain 10% for 25 minutes.

2.2. 2. Chromosomes preparations from embryos cells:

Chromosomes preparations from embryonic cells were prepared according to Romagnano *et al.* (1995). Embryos livers were collected from each group and placed in 5ml T.C.M. 199 media, 2ml of 0.05 colchicine was added for each tube and incubated at 37°C for 90 minutes. An amount of 5ml of hypotonic solution of 0.56% KC1 was added to the pellet and the cells were incubated at 37°C for 20 minutes .5ml of fresh fixative (3 methyl alcohol: 1 glacial acetic acid) were added to the cells. Two or three drops from the cell suspension were added to the surface of clean slides, air-dried and stained with 5% Giemsa stain, and examined for chromosomal aberrations. 50 metaphase spreads were examined for each female and embryo, scoring the different types of chromosomal aberrations (structural and numerical).

2.2.3. Statistical analysis:

The data of chromosomal aberrations in the females and Embryos were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1999). Least significant differences were used compare between means according to Waller and Duncan (1969) at probability 5%.

3-Results

3.I. Chromosomal aberration:

3..1.1. In the virgin females:

Means ± L.SD values and results are given in Table (1). In the group of virgin females treated with Amophage for 15 consecutive days. The frequencies of the total number of structural and numerical aberrations were in the same limit of control group, there was no significant difference between Amophage group and control group (18, 7.67) and (17, 6.33) respectively. While, in the group of virgin females treated with, (Agnucaston) there were significant increases in the frequencies of structural and numerical aberrations compared with controls (26, 13) and (17, 6.33) respectively.

3.1.2. In the pregnant females:

Means, ± L.S.D. values and results are given in Table (2). In the group of pregnant females treated with Amophage from day (3) to day (18) of pregnancy. The frequencies of the total structural and numerical aberrations were increased significantly from that of the control group (17.67 and 8.33) and (14.33 and 7.33) respectively.

Also, in the group of pregnant females treated with Agnucaston the frequencies of the total structural and numerical aberrations were increased highly significantly from that of the control group (32.3 and 17.67) and (14.33 and 7.33) respectively.

Table (1): The effect of oral administration of Amophage and Agnucaston on (virgin females):

Treatments	Structural Aberrations								Numerical aberrations			
	Chromotid gaps	Chromotid break	Deletion	Fragment	Centromeric attenuation	Ring	Endometosis	Total structural aberrations	<40	>40	Polyploidy	Total numerical aberration
Control	3.33b± 0.58	0.67±0.58	1.67b± 0.58	2.33±0.58	3.33b± 0.58	0.00± 0.00	3.00b± 1.00	14.33c± 1.53	3.00b±1.00	4.33b± 0.58	0.00b±0.00	7.33c± 1.15
Amophage	4.00b± 1.00	1.00b± 0.00	2.67b± 1.53	2.00±1.00	3.67b± 0.58	0.33± 0.58	4.00b± 0.00	17.67b± 0.58	3.67b±0.58	4.67b± 0.58	0.00b±0.00	8.33b±1.15
Agnucaston	6.67a± 0.58	3.67a± 0.58	5.00a± 0.00	3.33±0.58	6.67a± 0.58	1.00± 1.00	6.00a± 1.00	32.33a± 0.58	7.33a±0.58	8.67a± 0.58	1.67a±0.58	17.67a ±0.58
L.S.D. at α 0.05	1.490	0.941	1.884	N.S.	1.53	N.S.	1.632	1.998	1.490	1.153	0.666	1.998

Means of different letters (a, b, c d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

Table (2): The effect of oral administration of Amophage and Agnucaston on pregnant females

Treatments	Numerical aberrations								Numerical aberrations			
	Chromatid gaps	Chromatid break	Deletion	Fragment	Centromeric attenuation	Ring	Endo metosis	Total structural aberrations	<40	>40	Polyploidy	Total numerical aberrations
Control	4.33b± 0.58	1.33b± 0.58	2.33c±0.58	2.67± 0.58	2.67b±0.58	0.00± 0.00	3.67± 0.58	17.00b± 1.00	2.67b± 0.58	3.67b± 0.58	0.00± 0.0()	6.33b± 1.15
Amophage	5.00b± 0.00	1.67b± 0.58	3.00b± 0.00	1.67± 0.58	3.00b±0.00	0.33± 0.58	3.33± 0.58	1.8.00b ±0.00	3.33b± 0.58	4.33b± 0.58	0.00± 0.00	7.67b± 0.58
Agnucaston	6.33a± 0.58	3.00a±0.00	4.00a±0.00	2.67± 0.58	4.67a±0.58	0.67± 0.58	4.67± 0.58	26.00a± 1.73	6.00a±l.00	6.33a± 0.58	0.67± 0.058	13.00a± 1.00
L.S.D. at α 0.05	**0.941**	**0.941**	**0.666**	**N.S.**	**0.941**	**N.S.**	**N.S.**	**2.307**	**1.490**	**1.153**	**N.S.**	**1.884**

Means of different letters (a, b, c d) in the same column are significantly different.
The column without letters is not significant. 50 metaphase were examined from each animals.

3.13. In the embryos:

Means ± L.S.D. values and results are given in Table (3).

Cytogenetic examination in embryos treated with (Amophage) showed a slight significant increase in the total number of structural and numerical aberrations as compared with embryo control group the frequencies of structural and numerical aberrations were (14.7 and 7.3) compared with that of control (12.3 and 6.0) respectively.

On the other hand, cytogenetic examination in embryos group treated with (Agnucaston) showed a highly significant increase in the total number of structural and numerical aberrations compared with the control group. The frequencies of structural and numerical aberrations were (24.3 and 12) compared with that of control (12.3 and 6.0) respectively.

Table (3): Embryo: The effect of oral administration of Amophage and Agnucaston on embryos.

	Structural Aberrations								Numerical aberrations			
Treatments	**Chromotid gaps**	**Chromotid break**	**Deletion**	**Fragment**	**Centromeric attenuation**	**Ring**	**Endo metosis**	**Total structural aberration**	**<40**	**>40**	**Polyploidy**	**Total numerical aberration**
Control	3.7b±0.6	0.3b±0.6	0.7b±0.6	2.3±0.6	3.0±1.0	0.0±0.0	2.3b±0.6	12.3c±l.2	2.7b±0.6	3.3c± 0.6	0.0±0.0	6.0c± 1.0
Amophage	3.3b±0.6	1 .Oab±0.0	1.7b±0.6	2.7±0.6	3.0±1.0	0.0±0.0	3.0b±0.0	14.7b±0.6	3.3b±0.6	4.0b± 0.0	0.0±0.0	7.3b± 0.6
Agnucaston	5.3a±0.6	1,7a±0.6	3.3a±0.6	3.7±-.6	4.7±0.6	1.0±0.0	4.7a±0.6	24.3a±0.6	5.3a±0.6	6.0±0.0	0.7±0.6	12.0a 0.0
L.S.D. at α 0.05	1.153	0.941	1.153	N.S.	N.S.	N.S.	0.941	! .632	1.153	0.666	N.S.	1.331

Means of different letters (a, b, c d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

3.2. Comparison between the effect of all groups (control and treated) before pregnancy and during pregnancy:

3.2.1. Comparison between control groups in the virgin and pregnant Females:

Means ± L.S.D. and results are given in Table (4). There were no significant differences between all the types of structural and numerical aberrations in the two control groups of the virgin and pregnant females.

3.2.2. Comparison between the effect of Amophage treatments on the females before and during pregnancy.

Means ± L.S.D. and results are given in Table (5) cytogenetic examination showed that there were no significant differences between all types of structural and numerical aberrations and also between the total structural and numerical aberrations in the two Amophage treatment groups of virgin and pregnant females. This means that the effect of Amophage is the same before and during pregnancy.

3.2.3. Comparison between the effect of Agnucaston treatments on the females before and during pregnancy:

Means ± L.S.D. and results are given in Table (6) when comparing the frequencies of the total chromosomal aberrations (structural and numerical) between the virgin females and pregnant females treated with Agnucaston we found that mothers (pregnant females) had more frequent chromosomal aberrations than those of virgin females. This means that the effect of Agnucaston is more frequent during pregnancy than before pregnancy.

3.2. Comparison between the effect of all groups (control and treated) before pregnancy and during pregnancy:

3.2.1. Comparison between control groups in the virgin and pregnant Females:

Means ± L.S.D. and results are given in Table (4). There were no significant differences between all the types of structural and numerical aberrations in the two control groups of the virgin and pregnant females.

3.2.2. Comparison between the effect of Amophage treatments on the females before and during pregnancy.

Means ± L.S.D. and results are given in Table (5) cytogenetic examination showed that there were no significant differences between all types of structural and numerical aberrations and also between the total structural and numerical aberrations in the two Amophage treatment groups of virgin and pregnant females. This means that the effect of Amophage is the same before and during pregnancy.

3.2.3. Comparison between the effect of Agnucaston treatments on the females before and during pregnancy:

Means ± L.S.D. and results are given in Table (6) when comparing the frequencies of the total chromosomal aberrations (structural and numerical) between the virgin females and pregnant females treated with Agnucaston we found that mothers (pregnant females) had more frequent chromosomal aberrations than those of virgin females. This means that the effect of Agnucaston is more frequent during pregnancy than before pregnancy.

Table (4): Comparison between control groups in the females before and during pregnancy.

Treatments	Structural Aberrations								Numerical aberrations			
	Chromotid gaps	Chromotid break	Deletion	Fragment	Centromeric attenuation	Ring	Endo metosis	Total structural aberration	<40	>40	Polyploidy	Total numerical aberration
Before pregnancy During pregnancy	4.33 ± 0.58 3.33 ± 0.58	1.33 ±0.58 1.67 ± 0.00	2.33 ± 0.58 3.00 ± 0.00	2.67 ± 0.58 1.67 ± 0.58	2.67 ± 0.58 3.00 ± 0.58	0.00 ± 0.00 0.33 ± 0.58	3.67 ± 0.58 3.33 ± 0.58	17.00 ± 1.00 18.00 ± 1.73	2.67± 0.58 3.33 ± 1.00	3.67± 0.58 4.33 ± 0.58	0.00 ± 0.00 0.00 ± 0.58	6.33 ±1.15 7.67 ± 1.00
L.S.D. at α 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Means of different letters (a, b, c d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

Table (5): Comparison between the effect of oral administration of Amophage in the females before and during pregnancy.

Treatments	Structural Aberration								Numerical aberration			
	Chromatid gaps	Chromatid break	Deletion	Fragment	Centromeric attenuation	Ring	Endo metosis	Total structural aberration	<40	>40	Polyploidy	Total numerical aberration
Before pregnancy During pregnancy	5.00± 0.00 4.00± 1.00	1.67± 0.58 1.00± 0.00	3.00± 0.00 2.67± 1.53	1.67± 0.58 2.00± 1.00	3.00± 0.00 3.67± 0.58	0.33± 0.58 0.33± 0.58	3.33± 0.58 4.00± 0.00	18.00± 0.00 17.670.58	3.33± 0.58 3.67± 0.58	4.33± 0.58 4.67± 0.58	0.00± 0.00 0.00± 0.00	7.67± 0.58 8.33.± 1.15
L.S.D. at α 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Means of different letters (a, b, c d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

Table(6): Comparison between the effect of oral administration of Agnucaston in the females before and during pregnancy.

Treatments	Structural Aberration								Numerical aberration			
	Chromotid gaps	Chromotid break	Deletion	Fragment	Centromeric attenuation	Ring	Endo metosis	Total structural aberration	<40	>40	Polyploidy	Total numerical aberration
Before pregnancy **During pregnancy**	6.33± 0.58 6.67± 0.58	3.00± 0.00 3.67± 0.58	4.00± 0.00 5.00± 0.00	2.67± 0.58 3.33± 0.58	4.67b±0.58 6.67a±0.58	0.67±0.58 1.00±1.00	0.67± 0.58 6.00± 1.00	26.00b±l.73 32.33a± 0.58	6.00±1.00 7.33±0.58	6.33b±0.58 8.67a±0.58	0.67± 0.58 1.67± 0.58	13.00b± 1.00 17.67a±0.58
L.S.D. at a 0.05	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**1.308**	**N.S.**	**N.S.**	**2.927**	**N.S.**	**1.308**	**N.S.**	**1.851**

Means of different letters (a, b, c d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

4-Discussion:

Polycystic ovary syndrome (PCOS) is one of the most common causes of menstrual disorder in the world and a leading cause of infertility. Polycystic ovary syndrome (PCOS) is caused by an imbalance in the hormones of the brain and ovary. The most important hormones that caused (PCOS) called the (LH) Luteinizing hormone and insulin hormone. When the levels of the two hormones were increased in the blood, extra production of testosterone by the ovary result which caused a menstrual disorder and infertility in the girls and women.

The most common drugs for the treatment of (PCOS) are Agnucaston and Amophage (Metformin hydrochloride). Agnucaston is a herbal drug contains a dry extract of the fruit of the chaste tree which taken to decrease the level of (LH) in the blood leading to regulate the menstrual periods and treatment of (PCOS). Also (Metformin hydrochloride) was used to increase the sensitively of the body to insulin hormone leading to decrease the insulin level in the blood.

The present study was carried out in order to evaluate the cytogenetic effects of Agnucaston and Metformin on the Bone marrow cells of virgin, pregnant females (mothers) and their embryos.

In the present study, the administrating of virgin females with (Amophage once daily for 15 days showed no significant increase in the chromosomal aberrations in the bone marrow cells compared with the control group.

While, the administration of virgin females with Agnucaston for 15days caused a significant increase in the chromosomal aberrations of bone marrow cells compared with the control group.These results is in agreement with George and George et al (2003) who observed that Metformin has a mutagenic or clastogenic effect when administrated orally to the human.

Wherever, negative results were obtained by Prilepkaya et al (2009) who found that no clastogenic or mutagenic effects was observed when Agnucaston administrated orally in a recommended dose to human females

In addition, cytogenetic and developmental toxicity in embryos may occur through a direct effect of some chemicals or hormones on the embryo, fetus or indirectly through toxicity of the drug to the mothers and the placenta, or most commonly as a combination of the two concepts. Maternal conditions are capable of adversely affecting the developing organism in the uterus Khera (1981) and (1984).

In our study, the administration of Metformin with a dose equal the recommended dose in human given to the female mice during pregnancy caused slightly significant increase in the chromosomal aberrations (structural and numerical) to the pregnant females and their embryos. These finding was in agreement with Glueck et al (2002) who observed that after oral administration of Metformin to the females during pregnancy no clastogenic effect on the females and their embryos was observed.

Also, in the present study, we found that when pregnant females were administrated orally with a dose of Agnucaston equal to the recommended dose in human during pregnancy caused a highly significant increase in the total chromosomal aberrations (structural and numerical) in the pregnant females and in the embryos.

These findings were in agreement with Jerry et al (1991) who reported that Agnucaston should be prevented during pregnancy because it caused embryo toxicity though the placenta.

Also, positive results were obtained by Jerry et at (1991) who observed that Agnucaston when administration to females during pregnancy caused an abortion and bleeding.

However, negative results were observed by Christiansen et al (2005) who found that Agnucaston did not cause any toxicity when administrated orally to the females during pregnancy.

In the present study when comparing the cytogenetic effect of oral administrations of the two drugs (Metformin and Agnucaston). We found that in the females treated with Metformin before and during pregnancy there were no significant difference in the frequencies of chromosomal aberrations (structural and numerical).However, in the females treated with Agnucaston there were significant differences in the frequencies of chromosomal aberrations (total structural and numerical).

These results were in agreement with Jakubowicz, et al (2002) who reported that Metformin has no toxic effects in the females (during pregnancy) .In addition, these results was in agreement with Hobbs and Blumeutual, (1999) who observed that agnucaston may has a toxic effects if taken during pregnancy.

In conclusion, our results indicated that Agnucaston had a significant mutagenic and cytotoxic effects on females (before and during pregnancy) and on the embryos. In the present study we found that the treatment of female mice with Metformin had no mutagenic or cytotoxic effects to the female mice (before pregnancy). However, the treatment with Metformin during pregnancy caused a slight significant increase in the total chromosomal aberrations of pregnant females and embryos. This may be due to cross of Metformin innto female placenta causing slight cytotoxic effects to the mothers and embryos. So, we concluded that (Metformin) should be taken under medical control but Agnucaston should be avoided during pregnancy and lactation.

5-**References**

Christiansen, Nybo A. *et al.* (2005). Evidence-based investigations and treatments of recurrent pregnancy loss. Fertility and sterility 83(4)827-839.

Dunaif S. Segal K.R.; Futterwet, W. (1989). Profound peripheral insulin resistance, indpendent of obesity in polycystic ovary syndrome Diabetes, 38: 1165-74.

George S.S., George K, *et al.* (2003). Sequential treatment of Metformin and clomiphene citrate in women with poly cystic syndrome. Human Repro 18, 299-304.

Glueck C.J., Wang P. and *et al.* (2002). Pregnancy out comes among women with polycystic ovary syndrome treated with Metformin. Human Reprod, 1.7: 2858-2864.

Hobbs, C. and M. Blumenthal (1999). Vitex agnus-castus: Aliteratural Review Herbal Gram (47).

HSU, T.I.C and Patton, J.L (1969). Bone marrow preparations for chromosome studies, In Bernischek comparative mammalian cytogenetics springerverlag, pp 454-460.

Jakubowicz D., Luorno M..I. *et al.* (2002). Effects of Metformin on early pregnancy loss in the polycystic ovary syndrome. J. Clin. Endocrinal metab, 87: 524-529.

Jarry, H.S. Leonliardt, C. *et al.* (1994). Invitro prolactin but not LH and FSH Release is inhibited by compounds in extracts of agnus castus: Exp Clin. Endocrinol 102(6): 448-454. Balen, A. Michelmore, K. (2002). What is Polycystic ovary syndrome Hum Repro, 17:2219-2227.

Jerry, H, Hoff W., et al (1991). Chasttree significantly inhibits the stress induced secretion of prolactin in male rats. Phytother. (12): 77-82.

Khera, K.S. (1981). Common fetal aberration and their teratogenic significance, a review. Fund Appl Toxical, 1: 13-18.

Khera, K.S .(1984). Maternal toxicity: a possible factor in embryo-fetal deaths and fetal malformations of rodent-rabbit. Teratology, 31:129-153.

Lauritzen, C. *et al.* (1997). Treatment of premenstrual tension syndrome with vitex agnus castus. Controlled, doubled blind study versus pyridoxine. Phytomedicine 4: 183-189.

Milewicz, A. *et al.* (1993). Vitex agnus custus extract in the treatment of luteal phase defects due to latent hyperprolactinemia results of controlled double-blind study Arzne imforsch 43(7): 752-756.

Norman R.J. (2003). Metformin in polycystic ovary syndrome: systematic Review and Meta. Analysis BMJ. 327-954.

Pagat and Barnes (1964). Eveluation of Drug activities, Vol. 1. Academic press.

Romagnano, A., Richer, C.L. and Perrove, MA. (1985). A direct technique for the preparation of chromosomes from embryo equine embryos. Can. J. Genet. Cytol, 27: 365-369.

Rotterdam, (2004). Consensus of diagnostic criteria and long-term health risks Related to polycystic ovary syndrome. Fartil-Steri 81-19-25.

Snedecor, G.W. and Cochran, W.G. (1990): Statistical methods 9th ed Lowa state Univ. Press, Lova, U.S.A.

Prilepskaya V., Ledina A.and *et al.* (2009). Vitex agnus castus. Successful treatment of moderate to severe premenstrual syndrome. Maturitas, spp. 555.563.

Waller, A. and Duncan, D.B. (1969). Multiple range and multiple test.. Bionetries, 11: 1-24.

Yosida, T. H; Truchiya, K and moiwaki, K (1971). Frequency of chromosome polymorphism of Rattusrattus collected in Japan. Chromosoma, 30:33.

4/15/2010

Effects of IBA on rooting performance of *Citrus auriantifolia* Swingle (Kagzi-lime) in different growing conditions

Bani Bhushan Bhatt[1]*, Yogendra Kumar Tomar[2]

1. Department of Horticulture, GBP UA&T, College of Forestry and Hill Agriculture, Hill campus Ranichauri, Tehri Garhwal. 249 199, India
2. Department of Horticulture, HNB Garhwal University, Srinagar Garhwal, Uttarakhand 246 174, India
bhushanbani@gmail.com

Abstract: Considering the unavailability of information of the effect of rooting hormones in combination with modified growing conditions on the rooting characteristics of *Citrus auriantifolia* Swingle cuttings under valley conditions of Garhwal Himalaya, the experiment was undertaken at the HRC, Garhwal University Srinagar, Uttarakhand, India. The effect of different concentration of Indolebutyric acid (IBA) and different growing conditions have been examined for stimulatory effects adventitious root formation in stem cutting of Kagzi-lime. Properly prepared cuttings of about 22-24 cm length in the month of June were treated with different concentrations of IBA viz., 500, 1000, 1500 ppm for 5 second by concentrated solution dip method and planted in 3 different conditions namely open area, under partial shade and under low cost polyhouse. The cuttings treated with IBA 500 ppm, performed the best in all aspects, as root formation, length of root, thickening of root and leaf sprouting in shoot, whereas, the open area growing condition was found effective in increasing the success rate of the cuttings. All the rooting parameters performance was recorded highest under polyhouse condition. Overall treatment C_1M_3 (IBA 500ppm and polyhouse) combination was found best in all parameters taken. [Nature and Science 2010;8(7):8-11]. (ISSN: 1545-0740).

Key words: Kagzi lime, cuttings, IBA, rooting, low-cost poly house, Garhwal Himalaya.

1. Introduction

Kagzi lime belongs to family Rutaceae, is one of the most important citrus fruit as a major source of Vitamin C and acidic acid (Souci *et al.*2000) grown throughout the world (Babu, 2001). In India, it grown in the states of Andra Pradesh, Karnataka, Maharashtra, Punjab, Rajasthan and Uttarakhand in a total area of 125457.00 ha from which about 1617783.00 tonnes of production is obtained annually (Salaria, 2006 and FAO, 2008). In Uttarakhand the Lime is grown in certain portion of Pauri, Chamoli, Rudraprayag and Dehradun districts of Garhwal and some parts of Kumaon region, but there are hardly any systemic plantations of kagzi lime orchards in the state. Besides having high nutritional value and table purpose use, kagzi lime is extensively used as rootstock for malta and santra.

Generally, kagzi lime is regenerated through seeds, but there is a problem of non-uniformity of progeny and high chance of viral disease contamination by this method (Babu, 2001). For overcoming this problem the vegetative multiplication through cutting is only practicable and widely used option for augmenting natural regeneration and for large scale cultivation programmes. Owing to high intensity of polyembroyny (90-100%) and least chance of contamination of viral diseases (Babu, 2001) in Kagzi-lime, the stem cutting is suitable method for regeneration for the species. It is inexpensive, rapid and simple and does not require the special techniques as required in other vegetative methods.

The stimulation of adventitious root formation in stem cuttings treated with auxins is well known (Blazich, 1988). In addition, the combination with other compounds has been shown to enhance the root formation (Kling and Meyer 1983, Singh and Singh 2005). Phloroglucinol (1,3,5-trihydroxibenzene) stimulated *in vitro* root formation in apple root stocks (James and Thurbon, 1979); it was also reported to act synergistically with IBA in apple (James and Thurbon, 1981), *Rubus* (James, 1979) and in *Prunus* (Jones and Hopgood 1979) species. Although, there is a lot of work done on different aspects of propagation of citrus fruits, however, the effects of IBA with different growing condition in stimulation of rooting of cuttings appear to be unknown.

Keeping these facts in view, the present study, deals with the use of IBA with slight modifications of growing conditions for rooting

parameter and success percentage in the stem cuttings of kagzi lime with view to developing a mass scale clonal multiplication technology package which is cheap and simple.

2. Material and Methods

2.1 Study area

The experiment was carried out at the Horticulture Research Centre (HRC), in Chauras campus of HNB Garhwal University, Srinagar (Garhwal). Geographically the experimental site is lying between 30° 12′ to 30° 13′ North latitude and 78° 45′ to 78° 50′ East longitude while altitudinally located at 570 m asl. The site in the valley area of Garhwal Himalaya and experience a wide range of temperature variation ranging from 0°C in winter to a maximum of 40°C during summer. The relative humidity varies from 39.24 to 79.83 % and mean annual rainfall from 2.50 to 235.24 mm.

2.2 Methodology

4-5 gunny bags of sandy loam soil were taken from HRC field, exposed to Sun for killing the insects, spores of pathogens and the weeds. Stones, gravels and weeds were removed manually. After 2-3 hours drying in Sun in the month of June, 1 part of FYM was mixed thoroughly with the 2 parts of well dried sandy loam soil. This prepared media was filed in perforated polythene bags of about 1kg capacity y (20-22 cm height x 8-10 cm diameter). IBA solution of 500, 1000 and 1500 ppm were prepared and kept in 1 L beakers. The juvenile branches of mature Kagzi-lime trees (8-10 years) were used to obtain the cuttings in the end of June 2003. Approximately 22-24 cm long cuttings having 6-8 nodes with 0.6 - 1.2 cm diameter were prepared from central and basal parts of the branch. Cuttings were defoliated for reducing the transpiration rate and allowing the closer spacing in the bags. The cuttings were arranged into the 4 bundles each with 81 cuttings. Three bundles of cuttings were treated with different concentrations of IBA viz., 500 ppm, 1000 ppm and 1500 ppm respectively. The basal parts (2-3 cm) of all the cuttings were dipped in different concentrations of IBA for 5 second, concentrated solution dip method, (Hartmann *et al.* 2002) at room temperature of 20 $\pm$ 2 ^{0}C (3). Fourth bundle of cuttings was used as control (simply dipped for 5 sec in plain tap water). The treated cuttings were planted in the 3 different growing conditions, viz, open sunny area (M_1), partial shade of big tree throughout the day (M_2) and polyhouse conditions (M_3) of 3m (l) x 2m (b) x 2m (h) size.

Standard methodology was used to record the observations on root characteristics (Hartmann *et al.* 2002). The experiment was laid out in the factorial randomised block design (FRBD) with 3 replications having 9 cuttings in each replication within each treatment combination. The analysis of the data was done as per the standard methods (Cochran ad Cox, 1992).

3. Results and Discussion

The rooting response of *Citrus auriantifolia* cuttings treated with different IBA concentration and growing conditions is shown in table 1. The first callusing was observed on 14th day after planting the cutting and observed till 130 days. The lower concentration of IBA (500ppm) was found more effective. The mean values indicate that the maximum number of sprouted cuttings after 130 days was recorded in the treatment C_1 (500 ppm of IBA) with 68.50% followed by C_2 (1000 ppm IBA) treatment with 51.83 %. While, least number of sprouted cuttings (37.%) were recorded in C_0 treatment (control). Present findings are in line with the some earlier reports in the literature (Verma *et al.* 2005) but contradictory to the findings of Singh and Singh (2005), who noticed maximum sprouting percentage in higher concentration (3000 ppm of IBA). The maximum mean sprouting percentage (60.50%) was observed under the treatment M_1 (open area) closely followed by the treatment M_2 (partial shade) with 48.54% whereas, the lowest was recorded in M_3 (polyhouse) condition. However, C_1M_3 (500ppm of IBA with polyhouse condition) treatment was found the best treatment combination with 83.33% of sprouted cuttings. This may be due to favourable climatic conditions to the survival of cuttings under polyhouse condition as well as the effect of rooting hormones in lower doses.

Mean values of table 1 also reveals that the maximum number of primary roots (8.76) was obtained under C_2 (1000 ppm IBA) treatment followed by treatment C_1 (500 ppm of IBA) with 7.54. Present findings of the experiment is supported and strengthened by the work of Singh and Singh (2005), who observed the 13.55 number of primary roots per cuttings after treated with 1000ppm of IBA and Kumar *et al.* (1995). Treatment M_3 (polyhouse) produce the maximum number of primary roots (11.24) followed by M_1 (open area) treatment with 7.08, while, cuttings growing under M_2 (partial shade) condition produced minimum number of primary roots (4.06) among all the growing conditions. The interaction between various IBA concentrations and different

growing conditions was also found to be significant. The maximum number of primary roots (18.66) were observed under C_2M_3 (1000 ppm of IBA with polyhouse condition) treatment combination and followed by the treatment C_3M_3 (1500 ppm of IBA with polyhouse) treatment with 11.66. The C_1M_2 and C_2M_2 combination shown equal number of primary root (2.62) induction. The better rooting and their development (500 ppm of IBA with polyhouse) might be attributed due to greater metabolic activity and maximum utilisation of sugar and starch after hydrolysis from stem.

The maximum length of the root (15.11 cm) was found under C_1 (500 ppm of IBA) treatment followed by C_0 (control) treatment with 13.44 cm. The results of present investigation are strengthened by Verma *et.al.* (2005), reported that maximum root length (15.27 cm) in citrus cuttings. Whereas the growing condition M_3 (polyhouse) was found best, to producing the maximum length of root (18.75 cm), while the M_1 (open condition) shown the minimum length of root (9.08 cm). Combination treatment C_1M_3 (500 ppm of IBA with polyhouse grown) and C_2M_3 (1000 ppm IBA with polyhouse) were found equally good in term of producing the maximum length of longest root (20.33 cm), while, minimum length of root (4.66 cm) was found under C_2M_1 (1000 ppm of IBA with open area grown) treatment combination.

Furthermore, it is very clear from table 1 that the treatment C_1 has the maximum effect on root thickness (0.24 cm) and significantly better than all other treatments. The present findings are conformed by Singh and Singh (2005), who also observed the maximum effects of IBA to obtain the maximum diameter of root (1.93 mm) among all other growth regulators like IAA and NAA. Growing of cuttings under different growing conditions, M_3 (polyhouse) condition was found most suitable to give the maximum diameter of thickest root (0.26 cm), while minimum diameter of thickest root (0.21 cm) was observed under M_1 (open area) condition. Treatment combinations C_2M_3 (1000 ppm of IBA with polyhouse) and C_3M_3 (1500 ppm of IBA with polyhouse) were found equally good for producing the maximum diameter of root (0.30 cm).

4. Conclusion

The results of investigation clearly reveal that the IBA 500 ppm is most effective in the stimulation of root system arising from cutting and development of roots of *Citrus auriantifolia* cutting, and can be used for mass scale multiplication. It was interesting to observe that open area growing condition alone gives good results but moreover, IBA 500 ppm gives good results with combination of polyhouse growing condition. The results of this investigation are expected to pave the way for substantially augmenting natural regeneration through seeds; in addition, this has the advantage clonal or true to type propagation of elite trees.

Table 1: Effect of different concentrations of IBA and various growing conditions on success percentage and rooting parameters of kagzi-lime cuttings.

IBA Conc.	Percentage of sprouted cuttings (%)				Number of primary root (cm)				Length of longest root (cm)				Diameter of thickest root (cm)			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
Control (Water)	61.00	33.33	15.33	36.55	7.33	7.66	5.66	6.88	10.33	11.33	18.66	13.44	0.20	0.20	0.20	0.20
500 ppm	72.16	50.00	83.33	68.50	11.00	2.62	9.00	7.54	14.00	11.00	20.33	15.11	0.26	0.20	0.26	0.24
1,000 ppm	50.00	55.50	55.50	53.67	5.00	2.62	18.66	8.76	4.66	11.66	20.33	12.22	0.20	0.20	0.30	0.23
1,500 ppm	58.83	55.33	38.83	51.00	5.00	3.33	11.66	6.88	7.33	7.33	15.66	10.11	0.20	0.20	0.30	0.23
Mean	60.50	48.54	48.25		7.08	4.06	11.24		9.08	10.33	18.75		0.21	0.20	0.26	
$CD_{0.05}$																
IBA Conc. (C)			3.07			1.37				2.26				0.016		
Growning conditions (M)			2.66			1.18				1.95				0.018		
C x M			5.32			2.37				3.92				0.032		

M_1= Open sunny area, M_2= Partial shade of big tree throughout the day, M_3= Polyhouse condition

Acknowledgements:
Authors gratefully acknowledged the Head, Department of Horticulture, HNB Garhwal University, Srinagar, and Dr V P Bhatt, Govt. P G College Gopeshawar for encouragement and Support throughout the study periods.

***Correspondence to:**
B B Bhatt, Ph.D.,
Department of Horticulture, GBP UA&T,
College of Forestry and Hill Agriculture, Hill campus Ranichauri, Tehri Garhwal. 249 199
bhushanbani@gmail.com, Phone: 09410328014

References

[1] Salaria AS. Horticulture at a glance; Fruit and plantation crop. Vol. I. Jain Brothers, 16/873, East Park Road, Karol Bagh, New Delhi. 2006: 18.

[2] FAO. Statistical database of the food and agricultural organisation, Rome, Italy. www.fao.org. 2008.

[3] Hartmann HT, Kester DE, Davies FT, Geneve RL. Techniques of propagation by cuttings. In: *Plant Propagation: Principles and Practices*. 6th ed., Prentice Hall of India, Pvt. Ltd., New Delhi. 2002: 321.

[4] Cochran WG, Cox, MG. Experimental design. John Wiley Sons, Inc, New York. 1992: 106-117.

[5] Verma SK, Singh H, Bhardwaj PN, Arya RR. Propagation of citrus species at Bhowali, Uttaranchal. *Prog. Hort.* 2005: **37 (2)** : 274-279.

[6] Singh AK, Singh R. Influence of growth regulating substances on rooting of cuttings of poinsettia cv. Flaming Sphere. *Prog. Hort.* 2005: **37 (1)** : 85-88

[7] Kumar R, Gill DS, Kaushik RA. Effect of indolebutyric acid, P-hydroxy benzoic acid and season on the propagation of lemon cv. Baramasi from cuttings. *Hariyana J. Hort. Sci.*, 1995:**24:** 13-18.

[8] Blazich FA. Chemical and formulations used to promote adventitious rooting. In: Davies TD, Hassig BE and Sankhla N ed. Adventitious root formation in cuttings, Portland: Dioscorides Press. 1988: 132-149.

[9] Babu RSH. Limes and Lemons. In: Chadha, KL ed. Handbook of Horticulture. ICAR, New Delhi. 2001: 212.

[10] James DJ. The role of auxins and phloroglucinol in adventitious root formation in *Rubus* and *Fragaria* grown *in vitro*. J Hort Sci. 1979: **54**: 273-277.

[11] James DJ, Thurbon IJ. Rapid *in vitro* rooting of the apple rootstock M9. J Hort Sci. 1979: **54:** 309-311.

[12] James, D J and Thurbon, I J. Shoot and root initiation *in vitro* in the rootstock M9 and the promotive effects of phloroglucinol. J Hort Sci. 1981: **56**: 15-20.

[13] Jones OP, Hopgod ME. The successful propagation *in vitro* of two rootstock of *Prunus*: the plum rootstock Pixy (*P. insititia*) and the cherry rootstock F12/1(*P. avium*). J. Hort Sci. 1979:**54**: 63-66.

[14] Kling GJ, Meyer MMJr. Effects of phenolic compounds and indoleacitic acid on adventitious root initiation in cutting of *Phaseolus aureus, Acer saccharinum, Acer griseum*. J. Hort Sci 1983:**18**: 352-354.

[15] Souci SW, Fachmann W, Kraut H. Food composition and nutrition tables. 6th edn. Medpharm Scientific Publishers, Stuttgart. 2000.

04/04/2010

Anthropometric Measurements and Appetite Related Hormones in Obesity Patients

Hamdy A. Ahmed* and Mohamed I.Aref**
*Biochem., Dept., NRC. Dokki, Cairo, Egypt. **Clin., Pathology Dept., El Hussein Hosp., Fac., of Medicine, Al Azhar Uni. Cairo, Egypt.

Abstract: Obesity and hyperlipidemia are two of the most highly distributed disorders that commonly associated with a cluster of progressive pathogenesis of many public health problems. This nutritional diseases increase the morbidity and the mortality of other diseases as cardiovascular diseases, atherosclerosis, hypertension, type2 diabetes, osteoarthritis and certain types of cancer. Thus the goal of the present study was to investigate the anthropometric measurements and biochemical parameters in case of obese patients before and after dietary treatments as well as the obesity related hormones in obese and in comparison with their corresponding healthy subjects. Serum leptin concentration increased significantly in obese subjects associated with a significant decrease in serum ghrelin concentration in obese group only. After dietary treatment serum leptin concentration decreased significantly in obese subjects while serum ghrelin increased significantly when compared with their values before treatments. There was a significant increase in serum glucose, insulin and insulin resistant (IR) value in obese subjects. While after dietetic treatment for obesity, there was a significant decrease in serum glucose, insulin and IR associated with a significant increase in serum adiponectin levels. In conclusion, the main features of obesity are leptin resistance and insulin resistance. Other hormonal influences are ghrelin and adiponectin which could be pathogenic factors for obesity. Hyperghrelinemia lead to hyperphagia and morbid obesity. While hypoadiponectinemia correlated to insulin resistance and diabetes type 2. So reducing body weight and controlling of hyperlipidemia enhancing insulin and leptin sensitivity through increase in adiponectin secretion, which has known as anti-diabetic, anti-inflammatory and anti-atherogenic hormone. [Nature and Science 2010;8(7):12-19]. (ISSN: 1545-0740).

Keywords: Obesity; hyperlipidemia; anti-diabetic; anti-inflammatory; anti-atherogenic; hormone

1. Introduction

Obesity is the increased accumulation of fat cells in the body in the form of adipose tissues. It is associated with many diseases, particularly cardiovascular diseases, type 2 diabetes, breathing difficulties during sleep, certain types of cancer, and osteoarthritis. Obesity is most commonly caused by a combination of excessive dietary calories, lack of physical activity, and genetic susceptibility, though a limited number of cases are due solely to genetics, medical reasons, or psychiatric illness. Overeating either due to hormonal imbalance or to bad food behavior and some others diet problems, increase body weight and deposition of fat in the body, when the increment of fat exceed the normal weight by 20% lead to the production of obesity *Haslam and James, (2005) & Yu* et al., (2006).

Adipocytes take up and store free fatty acids as neutral fat. Body fat is located in the subcutaneous adipose tissue, and in visceral adipose tissues such as peritoneal adipose tissues.There are two types of adipose tissues in the body the white and brown adipose tissues. The white adipose tissue has been considered to stored fat and it is reported to have a secreting function for various chemical factors as leptin hormone, adiponectin hormone and tumor necrosis factor TNF-α, these chemical factors are called adipocytokines. Brown adipocytes; possess mechanisms that regulate metabolic pathways that are under the control of the sympathetic nervous system or endocrine system.*Kawada et al., (2001), Ahima, (2006) & James, (2008).*

Leptin hormone is produced by white adipose tissue and it is the product of obese (ob) gene. When there is a lot of adipose tissue, production of leptin increase to activate the satiety centers in the hypothalamus and reduce food intake. Conversely, when the reserves of adipose tissue decreases due to limited availability of food, leptin levels decrease and appetite increases *Martin et al., (2008).* So leptin is important as a regulator for appetite, whole body energy balance and body composition. So leptin administration has been shown to reduce fat mass, food intake hyperglycemia and hyperinsulinemia. Martin et al., (2008) & *Paz-Filho et al., (2009).*

Ghrelin is the first circulating hunger hormone. Synthesis of ghrelin occurs predominantly in epithelial cells lining the fundus of the stomach , in the placenta, kidney, pituitary and hypothalamus *Mundinger et al., (2006). Mondal et al., (2005)* defined ghrelin, as the ligand for the growth hormone secretagoue receptor, potently stimulates secretion of growth hormone. Also ghrelin affects on energy metabolism and food intake, so it was identified as a prominent target for development of anti-obesity

treatments.Mondal et al., (2005), Mundinger et al., (2006) & Gueorguiev et al., (2009).

Insulin hormone is produced by β-cells in the pancreas. Its primary role is to regulate blood glucose levels. Insulin acts on liver to increase glucose uptake and formation of glucose-6-phosphate with activate glycogen synthesis. In adipose tissue, insulin stimulate glucose uptake and then glucose is converted to glycerol and combines with free fatty acids(FFAs) to form triacylglycerols. While in muscles, insulin stimulates uptake of glucose and amino acids to form glycogen and protein. Also decreases protein catabolism *Semenkovich, (2006).*

Adiponectin is a new member of adipocytokines and it is produced solely in adipocytes then secreted into serum and in white adipose tissue. Adiponectin has anti-diabetic, anti-inflammatory and anti-atherogenic effects. It correlates negatively with percent body fat, waist/hip ratio and plasma insulin, and positively with insulin sensitivity .*Fu et al., (2005) & ADA, (2007).*

The aim of the work is to find a relation between obesity related hormone and anthropometric measurements.

2. Material and Methods

This study was conducted with 30 male subjects, fifteen obese patients were selected from "Obesity Outpatient Clinic, while healthy normal subjects were random. All subjects were asked for the demographic, anthropometric, healthy and dietary information according to questionnaire form.

The selected subjects were as follow:-

1-Normal healthy control group: contain fifteen healthy adult males in normal body weight with Body Mass Index (BMI) (22.01±0.32kg/m^2) which was calculated from the equation according to Raatz et al., (2005). BMI= body weight in (kg)/ body height in $meter^2$.

2- Obese group: contain fifteen obese adult males with BMI (36.75±0.94 kg/ m^2) at zero time of experiment.

Methods

Anthropometric measurements:- are the most commonly used methods for diagnosis of obesity. Body weight was measured to nearest 0.1kg and body height to nearest 0.5cm with a digital scale with a standiometer (scaletronix, Holtain Ltd, Crymmych, Pembs, UK). Subjects were shoeless during measuring. Body mass index (BMI) was calculated from the equation according to Raatz et al., (2005) as described before. Body fat mass (BFM) was calculated from the sum of 4 skinfold measurements, triceps, biceps, subscapular and suprailiac, as described by Durnin and Womersley, (1974). By using skinfold dialmax caliper (Bel-Art Products Pequannock, USA)

Serum measurements:

Determination of glucose concentration:

Serum level of glucose was determined by enzymatic colorimetric method according to Teuscher and Richterich, (1971).

Determination of insulin concentration:-

Serum insulin level was determined by Radioimmuno-assay method according to Deberg et al., (1998) by using Sorin kit. Cat. No. P2796

Determination of insulin resistance (IR):-

Insulin resistance was estimated by using HOMA (homeostasis model assessment) Garces et al., (2005) from the following equation:

IR=fasting insulin (μIU/ml) ×fasting glucose (mmol/l)/22.5

Determination of adiponectin concentration:-

Serum adiponectin level was determined by Enzyme immunoassay method according to Yamauchi et al., (2003) by using SPI-BIO Human adiponectin enzyme immunoassay kit,.

Determination of leptin concentration:-Serum

leptin level was determined by Radioimmunoassay method according to Ahren, (1997) by using Linco's Human Leptin-RIA-Sensitive product

Determination of ghrelin concentration:

Serum ghrelin level was determined by Enzyme immuno-assay method according to Grassi and Pradelles, (1991) by using SPI-BIO Human ghrelin enzyme immuno-assay kit.

3. Results

Table (1) Anthropometric measurements in human subjects showed a significant increase in body weight, BMI and BFM in obese subjects compared with healthy control subjects and after dietary treatment their values decreased significantly by 16.39%, 25.44% and 16.70% respectively compared with corresponding values before treatments.

Table (2) showed the concentration of serum glucose, insulin, insulin resistance (IR) and adiponectin in human subjects. Concerning to the concentration of fasting serum glucose and of insulin,it recorded 82. 47 ± 0.61 mg /dl & 10.09 ±0.24 μIU/ml respectively in normal healthy subjects.There was a significant increase in serum glucose by 15.19%, insulin by 79.68% and IR by 108.96% in obese subjects compared with healthy

control subjects associated with a significant decrease in serum adiponectin levels by 39.38% in the same manner. While after dietetic treatment for obesity there was a significant decrease in serum glucose, insulin and IR associated with a significant increase in serum adiponectin levels in comparison with the corresponding values before treatments. Consuming the diet regimen for reducing weight in association with reduced blood glucose to no significant difference with the control, while insulin level decreased significantly (P<0.01) compared to its normal level, the percentages of decrement were 11.08% and 24.98% for glucose and insulin respectively of treated obese subjects in comparison with their corresponding values of obese before treatment.

Table (3): The results leptin and ghrelin in human subjects showed that serum leptin concentration increased significantly in obese and subjects by 202.75% and 181.79% respectively, associated with a significant decrease in serum ghrelin concentration in obese group only by 42.52% in comparison with the corresponding values of healthy control subjects. The normal ratio of the two-serum appetite hormones leptin: ghrelin was about 1:12 in healthy subjects. Their mean values were recorded 7.25±0.19ng/ml and 87.79±10.27pg/ml, respectively. After dietary treatment serum leptin concentration decreased significantly while serum ghrelin increased significantly in obese subjects when compared with their values before treatments. When obese subjects received the dietetic treatment of obesity. The value of leptin decreased significantly (28.75%), while ghrelin level significantly increased (39.38%,) in comparison with the values before treatment (15.64±0.41 ng/ml), (70.01±16.12 pg/ml) respectively.

Table (1): Anthropometric measurements; body weight (kg) body mass index BMI (kg/m^2)and body fat mass BFM (%) of obese male subjects before and after treatments compared with healthy male subjects.

Groups / Parameters	Healthy group	Obese group		Obese group
		before	Before	
Body Weight (Kg)	b 81.80±2.45	a 101.67±1.92	b 85.00±1.78	13.56
BMI (kg/m2)	c 22.01±0.32	a 36.75±0.94	b 27.40±0.63	4.78
BFM (%)	c 19.36±0.30	a 29.82±0.40	b 24.84±1.62	4.09

Values expressed as mean ± S.E. n=15
There was no significant difference between means have the same superscript in the same row at P<0.01

Table (2): Concentrations of serum glucose (mg/dl), insulin (µIu/ml), insulin resistance (IR) and adiponectin (µg/ml) of obese male subjects before and after treatments compared with healthy male subjects.

Groups / Parameters	Healthy group	Obese group		L.S.D 0.01
		Before	Before	
Glucose (mg/dl)	b 82.47±0.61	a 95.00±2.48	a 95.00±2.48	8.50
Insulin (µIu/ml)	c 10.09±0.24	a 18.13±0.65	a 18.13±0.65	2.38
Insulin resistance (IR)	c 2.05±0.65	a 4.27±0.22	a 4.27±0.22	0.76
Adiponectin (µg/ml)	a 18.30±9.80	b 5.70±2.00	b 5.70±2.00	8.11

Values expressed as mean ± S.E. n=15
There was no significant difference between means have the same superscript in the same row at P<0.01

Table (3): Concentrations of serum appetite related hormones; leptin (ng/ml) and ghrelin (pg/ml) of obese male subjects before and after treatments compared with healthy male subjects.

Groups / Parameters	Healthy group	Obese group		L.S.D 0.01
		Before	Before	
Leptin (ng/ml)	d 7.25±0.19	a 21.95±0.30	c 15.64±0.41	2.23
Ghrelin (pg/ml)	a 87.79±10.27	c 50.23±12.03	b 70.01±16.12	10.03

Values expressed as mean ± S.E. n=15
There was no significant difference between means have the same superscript in the same row at P<0.01

4. Discussion:

Body mass index (BMI) (kg/m^2) is useful anthropometric method and correlates closely with adiposity. Therefore, BMI increases in obese patient due to increase in adipose tissues mass. In addition, skin fold thickness as measured at different sites mainly in triceps, biceps, subscapular and superailiac, then Body fat mass (BFM) measured to give an account on the percentage of fat in body. These parameters are important for diagnosis of obesity. Reducing in body weight is positively correlates with (BMI) and (BFM).

Our results indicated that Body weight, BMI and BFM significantly increased in obese subjects compared with healthy control subjects and after dietary treatment their values decreased significantly compared with corresponding values before treatments. There was association between obesity with body mass index BMI. BMI and BFM were significantly higher in obese, while after weight reduction program there was a significant decrease in both BMI and BFM compared to normal healthy control.Hu et al., (2001), Mendez-Sanchez et al., (2002) & Mohn et al., (2004) . Another study of Gonzalez et al., (2007) reported that the obese subjects had significantly higher ($P<0.003$) weight, BMI and percentage of body fat than normal healthy control group.

After weight reduction regimen, theresignificant changes in body composition variables as body weight, and BMI in obese subjects when compared with normal healthy control subjects. In addition, plasma insulin reduced. Patalay et al.,(2005)& Shih et al., (2006). The reduction of insulin level after weight reduction may be decrease inhibition of hormone-sensitive lipase within the adipocytes, this lead to increase release of fat from the adipocytes, and this associated with decrease in skinfold thickness, a measurement of body fat mass (BFM).

Concerning to the concentration of fasting serum glucose and of insulin .The result showed a significant increase in the glucose and insulin levels, the percentages of increment were being 15.19%&79.68% for glucose and for insulin respectively, compared to the mean values of healthy control subjects. From the results of fasting serum glucose, insulin and insulin resistance (IR) in human ,it is clear that, serum glucose level normally maintained within narrow limits by mainly release of insulin in case of normal body weight. High serum fasting glucose associated with the change of insulin levels and is a definite sign of IR, which associated with marked overweight and dietary impairment factors that increase serum lipids profile.

Insulin resistance commonly observed in obesity and this may be due to increased plasma free fatty acids. Insulin resistance cause lack in appropriate insulin signaling which develop abnormalities in glucose metabolism and decrease glucose uptake into cells (hyperglycemia). Hyperglycemia is sensed by pancreatic beta cells, which increase insulin secretion to compensate for elevated blood glucose level, this lead to hyperinsulinemia characteristic of IR in obesity. Weight reduction in obese subjects ,reduced IR and increase insulin sensitivity, which enhance glucose uptake into cells and control hyperglycemia and hyperinsulinemia.

Moreover, from our results it is clear that, serum adiponectin level positively correlates with insulin sensitivity and negatively with insulin resistance. Since obesity is associated with hyperinsulinemia and insulin resistance adiponectin level decreases. Weight reduction as it modulates insulin resistance and increases sensitivity of insulin seems to enhance secretion of adiponectin from fat cells. Also adiponectin level decreased due to the presence of IR. After controlling by diet and medication insulin sensitivity increases and subsequently enhance adiponectin secretion. Increase plasma fatty acids as in obesity lead to insulin resistance. The mechanism of IR production explained as impairing the insulin-signaling pathway due to increasing accumulation of triacylglycerols in tissue. Moreover, through the metabolism of triacylglycerols intermediate metabolites formed such as diacylglycerols that is a potent activator of Protein Kinase-C (PKC-α) and (NF-KB) pathways. PKC is an enzyme that phosphorylates serine residue in insulin receptor and/or (IRS-1), the phosphorylation of these molecules lead to its destruction and impairs insulin signaling pathway and result in IR.Boden,and,Laakso,(2005). The results of Kristina and Steven, (2006) confirmed that obesity was associated with elevated plasma free fatty acids(FFAs) that lead to insulin resistance. The mechanisms whereby fatty acid as induce insulin resistance mediated by translocation of the PKC-α isoform from the cytosol to the membrane compartment resulting in impairment of insulin receptor substrate-associated phosphatidyl-inositol-3-kinase activity (IRS-PI3-K) which, is important in activation of insulin receptor and insulin signaling pathway. Lowering plasma fatty acid as level by controlling diet or regular exercise prevents activation of NF-KB pathway and had a benefit to increase insulin sensitivity and improving regulation of glucose level.

Our results indicated that serum adiponectin level was low in individuals with obesity compared with healthy control subjects, and this may be due to decreased adiponectin gene expression in these diseases. While weight reduction was associated with

increase in circulating adiponectin and may improve lipids profile in obese subjects.Schulze et al.,(2005). Boden and Laakso, (2005) confirmed that the plasma adiponectin level negatively correlated with obesity, percentage of body fat and plasma insulin level. But, it positively correlated with insulin sensitivity and insulin-mediated glucose uptake. The hyperinsulinemia caused by obesity-induced insulin resistance, together with enhanced TNF-α expression from adipose tissue may contribute to reduce adiponectin secretion. It has been suggested that visceral adipose tissue as in abdominal obesity may produce substances that decrease adiponectin mRNA. This reduction may be due to decreased in expression of adiponectin receptor R1 in adipose tissue, which recorded to increase by weight reduction. Maria et al.,(2006)& Lin et al., (2007).Fasting glucose, insulin and HOMA-IR significantly increased when compared with control group. The produced hyperglycemia was explained by impairment of glucose metabolism as measured by the two lipogenic genes (glycerol-3-phosphate dehydrogenase and fatty acids synthetase) since their level seemed to increased by high fat diet compared to control. Milagro et al., (2006) ,Gonzalez et al.,(2007),Rector et al., (2007)&Shargorodsky et al.,(2009) .The hypothesis of hyperglycemia associated with obesity-induced diets, which can produce type2 diabetes. This was explained as accumulation of adipose tissues in obesity increased the secretion of inflammatory cytokines from adipocytes as TNF-α and IL-1β, which responsible for decrease insulin sensitivity and affect β-cells function. In addition, hyperleptinemia present in obesity inhibited the auto defense of pancreatic cells against inflammatory cytokines, which subsequently produced IR.Sauter et al.,(2008).

The results showed that serum leptin concentration increased significantly, associated with a significant decrease in serum ghrelin concentration ,but after dietary treatment serum leptin concentration decreased significantly while serum ghrelin increased significantly in obese subjects when compared with their values before treatment.

With respect to our findings of tested appetite related hormones in human subjects ,it is clear that, serum leptin concentrations are positively correlates with BMI and BFM. Therefore, its level increases in obesity due to increase in body fat percentages. While by weight reduction, leptin level decreases due to reduce in BMI and BFM. Since hyperinsulinemia increase lipids deposition in adipose tissues which increase leptin secretion.

On the other hand, serum ghrelin concentration increases in case of negative energy balance. Since obese patients are usually in positive energy balance thus ghrelin level decrease in obese patients. Another explanation is that leptin level increase in obesity and decrease ghrelin secretion from stomach, while by reducing weight, ghrelin level increase due to decrease in leptin level by weight reduction.Insulin resistance and hyperleptinemia closely correlated together, since IR with hyperinsulinemia increased lipids storage in adipose tissue with increased secretion of leptin from adipocytes.Chu et al.,(2000)& Hintz et al., (2003).

Our results of serum leptin in association with the results of serum insulin and IR as shown in Table (2) go hand in hand with the results of Doehner et al., (2002) who reported that increase in plasma leptin level causes attenuation of insulin-induced activities in human hepatic cells and may leads to IR. Leptin down regulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), a key step of the insulin receptor-signaling cascade. Also leptin counteracted the other steps of this signaling cascade, like the association of insulin-receptor substrate-1 with the adapter molecule growth factor-receptor-bound protein 2. Leptin also inhibited glycogen synthesis in skeletal muscles. Furthermore, a functional leptin receptor was discovered in the pancreas, which exerts a direct inhibitory effect on insulin secretion. Obese subjects were almost hyperleptinemic, but the hypothalamus is unable to transduce this leptin signals to reduce body weight and termed leptin resistance. Leptin resistance also prevents exogenous leptin administration from promoting weight loss. Hewson et al., (2002).

Fasting plasma ghrelin concentration was significantly lower in obese subjects compared with healthy normal control subjects. Ghrelin negatively correlated with BMI, percent of body fat, fasting plasma insulin and leptin concentrations. Weight loss increased plasma ghrelin level and increased ghrelin secretion from the epithelial cell of stomach since ghrelin level positively correlated with negative energy balance.Once weight reduced through marked food restriction exogenous administration of leptin is able to increase energy expenditure and permit weight loss suggesting that some factors or processes associated with weight loss improve leptin sensitivity.Inui et al.,(2004)& Straznicky et al., (2005).

The results obtained by Wang et al., (2005) support our data since they found hyperleptinemia in mice fed diet-induced obesity and indicated that this hyperleptinemia cannot deplete fat in adipocytes. The ability of adipocytes to undergo hypertrophy and hyperplasia despite hyperleptinemia implies that a powerful leptinergic blockage protects their vital fat-storing function from the antilipogenic action of leptin. This leptinergic blockage is essential for

obesity. Two likely mechanisms capable of blocking the paracrine action of increasing leptin level secreted by adipocytes, first, a large increase in expression of post receptor inhibitor of leptin, which appear in adipocytes by the sixth day of high fat feeding diet, at this point leptin level slightly elevated. Later, as the hyperleptinemia become more intense, the decline in lepr-b mRNA becomes substantial, and by the end of experiment lepr-b mRNA reaches undetectable levels. Thus, a combination of post receptor and receptor leptin blockage appears to minimize potential leptinergic interference with fat storage.

Leptin and adiponectin exert opposing effects on glucose metabolism, fat oxidation and insulin sensitivity. The ratio of leptin to adiponectin investigated as a potential atherogenic index; leptin to adiponectin ratio was 8-fold higher in obese subjects compared to non-obese and strongly correlated with other atherogenic metabolic markers such as BMI and skin-fold thickness. Koebnick et al., (2007).

Another study of Huang et al., (2008) confirmed that obese subjects are almost hyperinsulinemic. Since insulin stimulates leptin production from adipocytes via an indirect mechanism dependent on lipogenesis, most reducing weight regimens (through caloric restriction) cause lipolysis and this lead to acute leptin suppression and increased leptin sensitivity.

5. References

1. 1 ADA American Diabetes Association, (2007): Diagnosis and classification of diabetes mellitus. Diabetes Care; 30:S42–S47.
2. Ahima, R.X., (2006): Adipose Tissue as an Endocrine Organ. Obesity; 14: 242S–249S.
3. Ahren, B., (1997): Radioimmunoassay of plasma leptin in mice: influence of age, high-fat diet, and fasting. Am. J. Physiol.; 273:R113-R120.
4. Boden, G. and Laakso, M., (2005): Lipids and glucose in type 2 diabetes: what is the cause and effect? Diabetes Care; 27:2253-2259.
5. Chu, N.F., Wang, D.H., Shieh, S.M. and Rimm , E.B., (2000): Plasma leptin concentrations and obesity in relation to insulin resistance syndrome components among school children in Taiwan. The Taipei Children Heart Study; 24, 10, 1265-1271.
6. Deberg, M., Houssa, P., Frank, B.H., Sodoyez-Goffaux, F. and Sodoyez, J.C., (1998): Highly specific radio-immunoassay for human insulin based on immune exclusion of all insulin precursors. Clin. Chem.; 44:1504-1513.
7. Doehner, W., Rauchhaus, M., Godsland, I.F., Egerer, K., Niebauer, J., Sharma, R., Cicoira, M., Florea, V.G., Coats, A.J.S. and Anker, S.D., (2002): Insulin resistance in moderate chronic heart failure is related to hyperleptinemia, but not to norepnephrine or TNF-alpha. Intr. J. Cardiol.; 83:73-81.
8. Durnin, J.V. and Womersley, J., (1974): Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16-72 years. Br. J. Nutr.; 32:77-97.
9. Fu, Y., Luo, N., Klein, R.L. and Garvey, W.T., (2005): Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. J Lipid Res; 46: 1369-1379.
10. Garces, C., Gutierrez-Guisado, J., Benavente, M., Cano, B., Viturro, E., Ortega, H. and Oya, M., (2005): Obesity in Spanish school children: Relationship with lipid profile and insulin resistance. Obesity; 13:959-963.
11. Gonzalez, F., Rote, N.S., Minium, J., O'Leary, V.B. and Kirwan, J.P.,(2007): Obese Reproductive-Age Women Exhibit a Proatherogenic Inflammatory Response During Hyperglycemia Obesity; 15, 2436-2444.
12. Grassi, J. and Pradelles, P.H., (1991): Compounds labeled by the acetyl cholinesterase of Electrophorus Electricus. Its preparation process and it use as a tracer or marquer in enzymo-immunological determinations. United States patent, N° 1,047,330.
13. Gueorguiev, M., Lecoeur, C., Meyre, D., Benzinou, M., Mein, C.A., Hinney, A., Vatin, V., Weill, J., Heude, B., Hebebrand, J., Grossman, A.B., Korbonits, M. and Froguel, P., (2009): Association Studies on Ghrelin and Ghrelin Receptor Gene Polymorphisms With Obesity. Obesity; 17(4), 745-754.
14. Haslam, D.W. and James, W.P., (2005): "Obesity". Lancet; 366 (9492): 1197-209.
15. Hewson, A.K., Tung, L.Y., Connell, D.W., Tookman, L. and Dickson, S.L., (2002): "The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic". Diabetes; 51 (12): 3412-3419.
16. Hintz, K.K., Aberle, N.S. and Ren, J., (2003): Insulin resistance induces hyperleptinemia, cardiac contractile

dysfunction but not cardiac leptin resistance in ventricular myocytes. International Journal of Obesity 27, 1196-1203.
17. Hu, F.B., Chen, C., Wang, B., Stampfer, M.J. and Xu, X., (2001): Leptin concentrations in relation to overall adiposity, fat distribution, and blood pressure in a rural Chinese population; 25:(1), 121-125.
18. Huang, W., Metlakunta, A., Dedousis, N., Ortmeyer, H.K., Stefanovic-Racic, M. and O'Doherty R.M., (2008): Leptin Augments the Acute Suppressive Effects of Insulin on Hepatic Very Low Density Lipoprotein (VLDL) Production in Rats. Endocrinology; 10.1210/en. 1271-1280.
19. Inui, A., asakawa, A., Bowers, C.Y., Mantovani, G., Laviano, A., Meguid, M.M. and Fujimiya, M., (2004): Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ The FASEB Journal; 18:439-456.
20. James, W.P.T., (2008): The epidemiology of obesity: the size of the problem. J Intern Med; 263:336-352.
21. Kawada,T., Takahashi, N. and Fushiki, T., (2001): Biochemical and physiological characteristics of fat cell. J. Nutr. Sci. Vitaminol.; 47: 1-12.
22. Koebnick, C., Shaibi1, G.Q., Kelly, L., Roberts, C.K., Lane, C.J., Toledo-Corral, C., Davis1, J.N., Byrd-Williams, C., Weigensberg, M.J. and Goran, M.I., (2007): Leptin-to-adiponectin ratio as independent predictor of insulin sensitivity during growth in overweight Hispanic youth. J. Endocrinol. Invest.; 30: RC13-RC16.
23. Kristina, M.U. and Steven, E.K., (2006): The Role of Insulin Resistance in Nonalcoholic Fatty Liver Disease. The Journal of Clinical Endocrinology & Metabolism; 91: (12) 4753-4761.
24. Lin, E., Phillips, L.S. and Ziegler, T.R., (2007): Increases in adiponectin predict improved liver, but not peripheral, insulin sensitivity in severely obese women during weight loss. Diabetes; 56:735-742.
25. Maria, S.R., Aina, S.L., Steen, B.P., Jens, M.B., Mads, R. and Bjorn, R., (2006): Adiponectin receptors in human adipose tissue: Effect of obesity, weight loss and fat depots. Obesity; 14:28-35.
26. Martin, S.S., Qasim, A. and Reilly, M.P., (2008): Leptin Resistance: A Possible Interface of Inflammation and Metabolism in Obesity-Related Cardiovascular Disease. J Am Coll Cardiol; 52:1201-1210.
27. Mendez-Sanchez, N., Gonzalez, V., King-Martinez, A.C., Sanchez, H. and Uribe, M., (2002): Plasma Leptin and the Cholesterol Saturation of Bile Are Correlated in Obese Women after Weight Loss. J. Nutr.; 132:2195-2198.
28. Milagro, F.I., Campión, J. and Martínez, J.A., (2006):Weight Gain Induced by High-Fat Feeding Involves Increased Liver Oxidative Stress. Obesity; 14, 1118-1123.
29. Mohn, A., Catino, M., Capanna, R., Giannini, C., Marcovecchio, M. and Chiarelli, F., (2004): Increased Oxidative Stress in Prepubertal Severely Obese Children: Effect of a Dietary Restriction-Weight Loss Program. The Journal of Clinical Endocrinology & Metabolism; 90, (5) 2653-2658.
30. Mondal, M.S., Date, Y., Yamaguchi, H., Toshinai, K., Tsuruta, T., Kangawa, K. and Nakazato, M., (2005): Identification of ghrelin and its receptor in neurons of the rat arcuate nucleus. Regul. Pept; 126: 55-59.
31. Mundinger, T.O., Cummings, D.E. and Taborsky, G.J., (2006): Direct stimulation of ghrelin secretion by sympathetic nerves. Endocrinology; 147(6):2893-901.
32. Patalay, M., Lofgren, I.E., Freake, H.C., Koo, S.I. and Fernandez, M.L., (2005): The lowering of plasma lipids following a weight reduction program is related to increased expression of the LDL receptor and lipoprotein lipase. J. Nutr.; 135:735-739.
33. Paz-Filho, K., Esposito, B., Hurwitz, A., Sharma, C., Dong, V., Andreev, T., Delibasi, H., Erol, A., Ayala, M.-L. and Wong, (2009): Leptin and insulin sensitivity: reply to Oral and Burant. Am J Physiol Endocrinol Metab; 296(2): E396 - E396.
34. Raatz, S., K., Torkelson, c. J., Redmon, J. B. and Reck, K. P., (2005): Reduced glycemic index and glycemic load diets do not increase the effects of energy restriction on weight loss and insulin sensitivity in obese men and women 1,2. J. Nutr.; 135: 2387-2391.
35. Rector, R.S., Warner, S.O. and Liu, Y., (2007): Exercise and diet induced weight loss improves measures of oxidative stress and insulin sensitivity in adults with characteristics of the metabolic syndrome. Am J Physiol Endocrinol Metab; 293:E500-E506.
36. Sauter, N.S., Schulthess, F.T., Galasso, R., Castellani, L.W. and Maedler, K., (2008): The anti-inflammatory cytokine

interleukin-1-receptor antagonist protects from high-fat diet-induced hyperglycemia. Endocrinology; 149(5):2208-2218.
37. Schulze, M.B., Shai, I., Rimm, E.B., Li, T., Rifai, N. and Hu, F.B., (2005): Adiponectin and future coronary heart disease events among men with type 2 diabetes. Diabetes; 54: 534-9.
38. Semenkovich, C.F., (2006): Insulin resistance and atherosclerosis Clin. Invest.; 116(7): 1813-1822.
39. Shargorodsky, M., Boaz, M., Goldberg, Y., Matas, Z., Gavish, D., Fux, A. and Wolfson, N., (2009): Adiponectin and vascular properties in obese patients: is it a novel biomarker of early atherosclerosis? International Journal of Obesity; 33, 553-558.
40. Shih, L.Y., Liou, T.H., Chao,J.C.H., Kau,H.N., Wu, Y.J., Shieh, M.J., Yeh, C.Y. and Han, B.C., (2006): Leptin, Superoxide Dismutase, and Weight Loss: Initial Leptin Predicts Weight Loss. Obesity; 14, 2184-2192.
41. Straznicky, N.E., Lambert, E.A. and Lambert, G.W., (2005): Effects of dietary weight loss on sympathetic activity and cardiac risk factors associated with the metabolic syndrome. J Clin Endocrinol Metab.23:565-578.
42. Teuscher, A. and Richterich, P., (1971): Enzymatic method of glucose. Schweiz.Med.Wschr.; 10: 345-390.
43. Wang, M.Y., Orci, L., Ravazzola, M. and Unger. R.H., (2005): Fat storage in adipocytes requires inactivation of leptin's paracrine activity: Implications for treatment of human obesity. Proc Natl Acad Sci; U S A. 13; 102(50): 18011-18016.
44. Yamauchi, T., Kamon, J., Waki, H., Imai, Y., Shimozawa, N., Hioki, K., Uchida, S., Ito, Y., Takakuwa, K., Matsui, J., Takata, M., Eto, K., Terauchi, Y., Komeda, K., Tsunoda, M., Murakami, K., Ohnishi, Y., Naitoh, T., Yamamura, K., Ueyama, Y., Froguel, P., Kimura, S., Nagai, R. and Kadowaki, T., (2003): Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J Biol Chem.; 278, 2461-2468.
45. Yu, R., Kim, C.S., Kwon, B.S. and Kawada, T., (2006): Mesenteric adipose tissue-derived monocyte chemoattractant protein-1 plays a crucial role in adipose tissue macrophage migration and activation in obese mice. Obesity; 14:1353-1362.

5/1/2020

Studies on some Benzopyran Derivatives with Expected Antimicrobial and Antiviral Activity

Hanaa.F.Roaiah [a], Sally.S.El-Nakkady [a], Weam.S.El-Serwy [a], Mohamed.A.A.Ali [b],A.H.Abd El-Rahman [c], Zeinab El-Bazza[d]

a Natural Chemistry Products Department, National Research Center, Dokki, Cairo, Egypt
b virology Department National Research Center, Dokki, Cairo, Egypt
c Chemistry Department, Faculty of Science, Mansoura University, Egypt
d Pharmaceutical Microbiological Lab, National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt

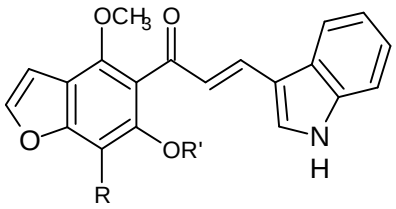
Abstract: The naturally occurring furocoumarin (xanthotoxin) **(1)** yielded 3-(6-hydroxy-7-methoxy-1-benzofuran-5-yl) acrylohydrazide **(2)** upon treatment with hydrazine hydrate. When **2** was treated with an equimolar amount of the appropriate isothiocyanates it gave the respective N-substituted – 2 - [3 - (6 – hydroxy – 7 – methoxy – 1 – benzofuran – 5 - yl) prop – 2 -enoyl] hydrazine carbothioamide **(3a-c)**, which when heated with sodium hydroxide, yellow mercuric oxide and phosphorus oxychloride respectively were transformed into 5-[2-(4-substituted-5-mercapto-4H-1,2,4-triazol-3-yl)vinyl]-7-methoxy-1-benzofuran-6-ol **(4a-c)**, 5-{2-[5-(substituted amino)-1,3,4-oxadiazol-2-yl] vinyl}-7-methoxy-1-benzofuran-6-ol **(5a-c)**, and 5-{2-[5-(substituted amino)-1,3,4-thiadiazol-2-yl] vinyl}-7-methoxy-1-benzofuran-6-ol **(6a-c)** respectively. The reaction of **3a** with ethyl bromoacetate gave N'-(3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene)-3-(6-hydroxy-7-methoxy-1-benzofuran-5-yl)acrylo hydrazide **(7)**. When **3a,b** was heated with ethyl cyanoacetate it yielded N-substituted-2-[4-(6-hydroxy-7-methoxy-1-benzofuran-5-yl)-2-imino-2H-pyran-6-yl]hydrazine-carbothioamide **(8a,b)** respectively. The prepared compounds were tested for their antimicrobial and antiviral activities. [Nature and Science 2010;8(7):20-29]. (ISSN: 1545-0740).

Key words: Benzopyran; Antimicrobial; Antiviral Activity

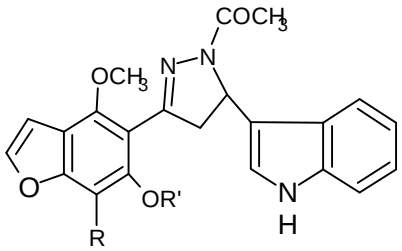
1. Introduction

This work deals with the synthesis of some benzofuran derivatives derived from naturally occurring xanthotoxin for biological evaluation.

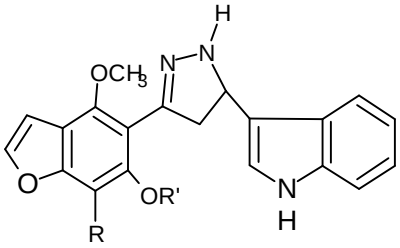
A wide variety of pharmacological properties was shown to be associated with benzofuran derivatives [1, 2]. Various thiosemicarbazides and their cyclized products e.g.triazoles, oxadiazoles, thiazolidinones and thiadiazoles are also associated with a broad spectrum of biological properties including anticonvulsant[3], anti-inflammatory[4-7], antitumor, antiviral[8], analgesic, ulcerogenic, lipid peroxidation[9], antimicrobial[10] and anti-HIV activities[11,12]. The chalcones **Ia-d**, pyrazolinyl derivatives **IIa-d**, N-acetyl pyrazolinyl derivatives **IIIa-d**, N-phenyl pyrazolinyl derivatives **IVa-d** and isoxazolinyl derivatives **VIa-d** possess moderate activity towards the Gram +ve bacteria and yeast in a concentration of 200µg/disk compared with Chloramphenicol 10µg/disk. Also, these compounds possess slight activity towards Gram -ve bacteria used. On the other hand, when these compounds were subjected to the U.V, light (366 nm), the activity was found to be higher than those compounds not subjected to the U.V. light [13].



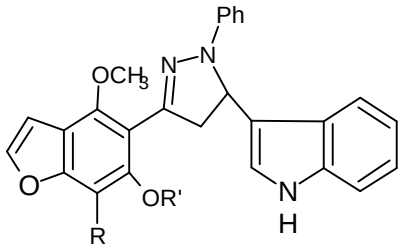
I



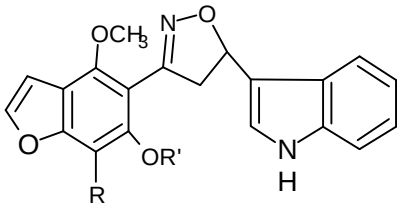
II



III



IV



VI

I,II,III,IV and VI

a,R=R'=H

b,R=OCH_3;R'=H

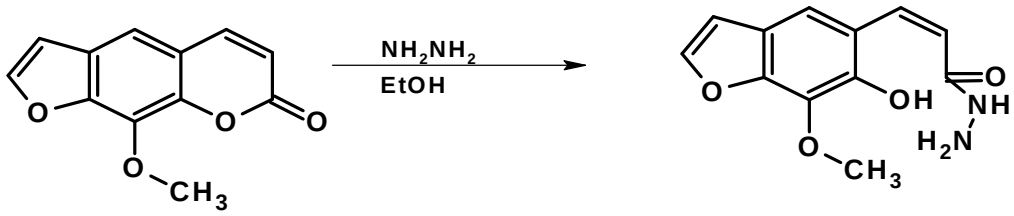
c,R=H;R'=CH_3

d,R=OCH_3;R'=CH_3

Figure 1. Furocoumarin Structure

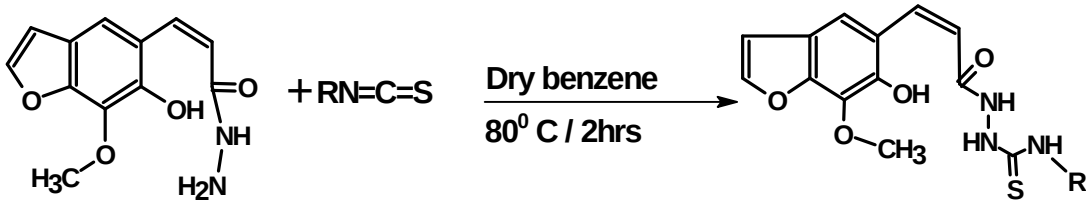
2. Results and Discussion

Xanthotoxin **(1)** (9-Methoxy-7H-furo [3, 2-g] chromen-7-one) yielded 3-(6-hydroxy-7-methoxy-1-benzofuran-5-yl) acrylohydrazide **(2)** upon treatment with hydrazine hydrate [14].



1 **2**

When compound **2** was treated with isothiocyanates derivatives namely, (benzyl-, ethyl- and cyclohexyl isothiocyanate), N-substituted–2-[3-(6–hydroxy–7–methoxy–1– benzofuran–5-yl) prop–2-enoyl] hydrazido carbothioamides**(3a-3c)** were obtained.



2 **3a-c**

The IR spectra of compounds **3a-c** showed the presence of C=S group at 3063-3225 cm^{-1}.

5-[2-(4-Substituted-5-mercapto-4H-1,2,4-triazol-3-yl)vinyl]-7-methoxy-1-benzofuran-6-ols**(4a-4c)** were formed by the cyclization of compounds **3a-c** by heating in aqueous sodium hydroxide.

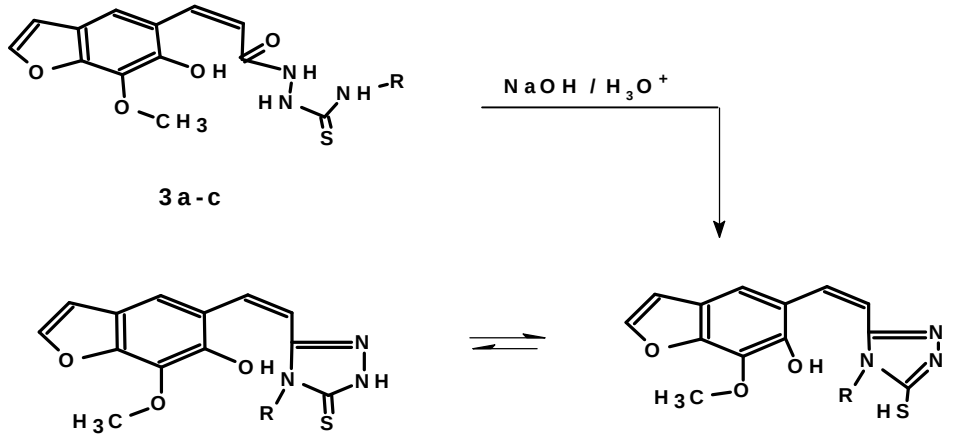
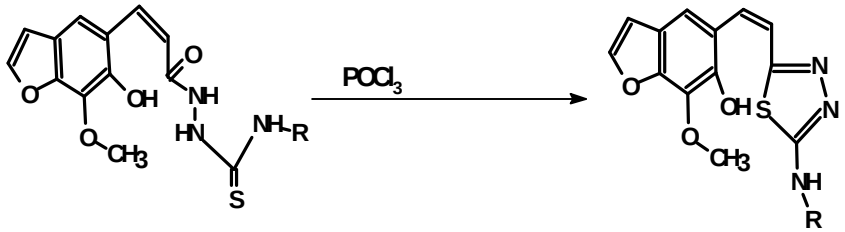


Figure 2.

The H^1-NMR of compounds **4a-c** confirmed the absence of the NH protons, and their IR spectra also showed the absence of the C=O and C=S groups.
On the other hand desulfurization of compounds **3a-c** by yellow mercuric oxide in boiling ethanol yielded 5-{2-[5-(substituted amino)-1,3,4-oxadiazol-2-yl]vinyl}-7-methoxy-1-benzofuran-6-ols **(5a-c).**

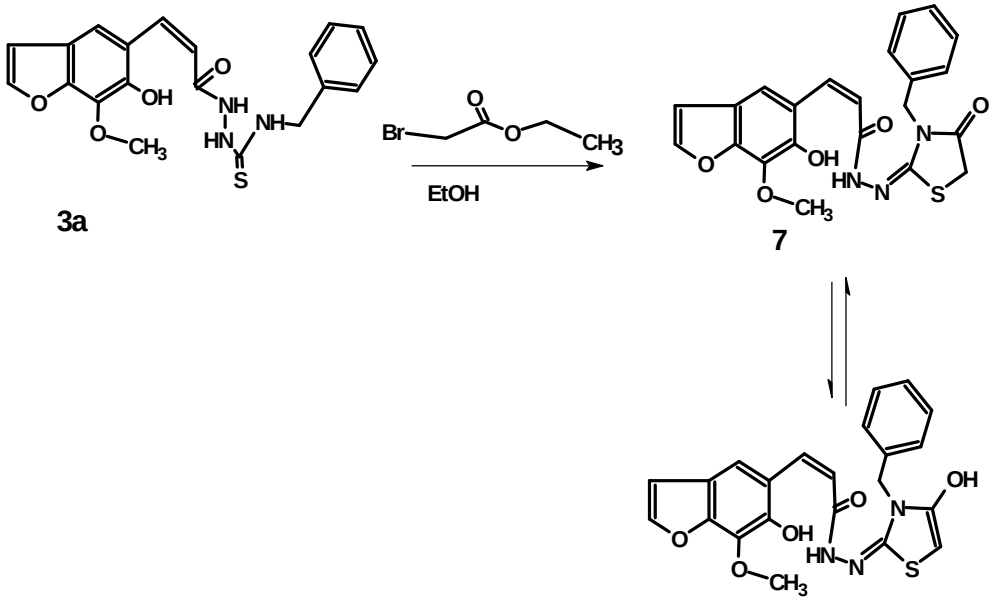
3a-c **5a-c**

The IR spectra of **5a-c** revealed the absence of C=O and C=S absorption.
Treatment of compounds **3a-c** with phosphorus oxychloride and heating yielded the corresponding 5-{2-[5 amino)-1,3,4-thiadiazol-2-yl]vinyl}-7-methoxy-1-benzofuran-6-ols **(6a-c).**



3a-c **6a-c**

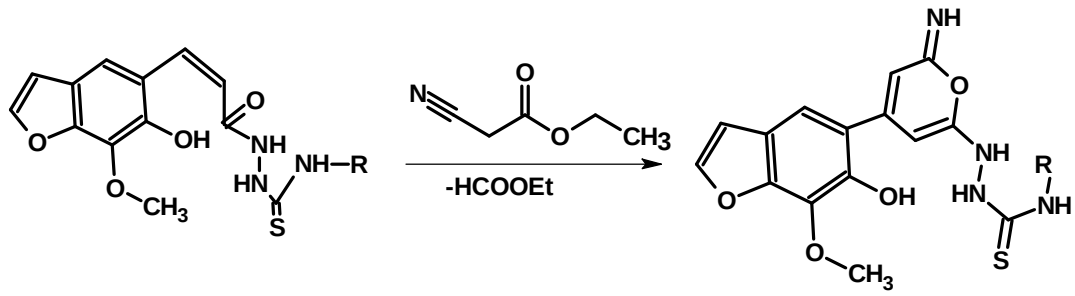
The IR spectra of **6a-c** revealed the absence of C=O and C=S absorption. The reaction of thiourea derivative **3a** with ethylbromoacetate yielded N'-(3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene)-3-(6-hydroxy -7-methoxy-1-benzofuran-5-yl)acrylo hydrazide **(7).**



3a **7**

The IR spectra of **7** revealed the absence of C=S absorption.

The reaction of **3a,b** with ethylcyanoacetate yielded N-substituted-2-[4-(6-hydroxy-7-methoxy-1-benzofuran-5-yl)-2-imino-2H-pyran-6-yl] hydrazinecarbothioamide **(8a,b).**



3a,b **8 a,b**

a, R=PhCH$_2$-
b, R=CH$_3$ CH$_2$-
c, R=C$_6$H$_{11}$-

The IR spectra of **8a, b** revealed the absence of C=O absorption

3. Biology

3.1 Antimicrobial activity test

The antimicrobial activity screening test of compounds 3(a-c), 4a, 4c, 5b, 5c, 6a, 6c, 7, and 8a, b against G +ve, G –ve and fungi was carried out by using the disk-diffusion method with some modifications [15]. Whatman No.1 filter paper disks (0.5 cm) were sterilized by autoclaving at 121 ^{0}C for 15 min and then impregnated with the tested compounds (3 x 10^{-6} g.or 300 µg/disk) (using chloroamphenical as control 300 µg/disk). The disks were placed on the surface of the cold solid medium in petri-dishes, inoculated with the tested micro-organisms, then incubated at 5^{0}C for permit good diffusion and then transferred to an incubator at 37 ^{0}C for 24 h for bacteria (1 and 2) and at 28^{0}C for 24h for fungus (3) (cf.Table1).

3.2 Antimicrobial activity data conclusion of the data obtained in table (1)

The preliminary antimicrobial activity screening for the prepared compounds were tested using one local strain as G+ve, one local strain as G-ve bacteria and one local strain as fungus together with chloramphenicol as control.

From the data obtained (table 1) it is clear that only compounds 3c, 5c (thiourea and oxadiazole derivatives) were found to be highly active against G+ve bacteria compared with the reference antibiotic chloramphenicol.

Compounds 3a and 8b also possess moderate activity towards G+ve bacteria while the other compounds are slightly active towards the same organism. On the other hand, compounds 3c and 5c possess moderate activity towards G-ve bacteria compared with the control, while the other compounds possess slight activity towards the same organism. Moreover, compounds 3a, 3b, 4c, 6c, 8a and 8b showed moderate activity towards the fungus compared with the reference, while the other compounds possess slight activity towards the same organism.

In general, only compounds 3c and 5c showed high activity towards G+ve bacteria and moderate activity towards G-ve bacteria.

N.B the activity of our target compounds, namely triazole, oxadiazole, thiadiazole, thiazolidine and pyrane derivatives will be studied in details against clinical pathogenic microorganisms and also their MIC will be published separately in the near future.

3.3 Antivirus activity

Cells and viruses

Herpes Simplex Virus type 1(HSV-1) was used for antiviral bioassay. The virus was isolated and propagated in the Virology Laboratory, Department

of Water Pollution Researches, and National Research Center.

African green monkey kidney cells (Vero) was used for HSV-1 propagation and bioassay. Cells were grown in minimum essential medium and supplemented by 1% antibiotic-antimycotic mixture (GIBCO-BRL), 8% fetal bovine serum (Sigma) and the pH was adjusted to (7.2-7.4) by 7.5% sodium bicarbonate solution. Cells were grown as monolayer sheets and dissociation by trypsin-versene solution (0.15% trypsin and 0.04% ethylene diamine tetraacetic acid, EDTA 2Na). The dissociated cells were subcultured in a 96-well plate to measure the cytotoxicity of the prepared compounds (Silva et al., 1997). Cytotoxicity assay were carried out for the prepared samples to determine the safe concentrations to be used for antiviral bioassay.

Plaque infectivity reduction assay (PIRA):

A 6-well plate was cultivated with Vero cell culture (105cell/ml) and incubated for 2 days at 370 C. The virus was diluted to give 107 PFU/ml final concentrations and mixed with the tested compounds at different concentrations and incubated overnight at 40• C. Growth medium was removed from the multiwell plates and virus-compound mixtures were inoculated (100µl /well). After 1h contact time, the inoculum was aspirated with 3ml of MEM and 1% agarose overlaid the cell sheets. The plates were left to solidify and then incubated at 37•C until the development of virus plaques. Cells were fixed in 10% formalin solution for 2hr, and stained with crystal violet stain. Control virus and cells were treated identically without compounds. Virus plaques were counted and the percentage of reduction was calculated (Silva et al., 1997).

4. Experimental

All melting points were uncorrected. Elemental analysis was carried out in the microanalytical unit of the National Research Centre. **IR** spectra were recorded on a Mattson-5000 FTIR spectrometer using KBr wafer technique.^{1}**H-NMR** spectra were determined on a varian-Gemini-300 MHz. And Jeol-Ex-300 MHz NMR spectrometer using TMS as an internal standard with (chemical shift. δ = 0 ppm). Mass spectra were determined on Finnigan mat SSQ 7000 mode: EI,70Ev (Thermo Inst.Sys.Inc.,USA). The purity of the synthesized compounds was tested by thin layer chromatography (TLC), Merck plates.

4.1. Synthesis of N-substituted – 2 - [–3 -(6– hydroxy – 7 – methoxy – 1 –benzofuran – 5 - yl) prop– 2 -enoyl] hydrazine carbothioamides (3a-c).

To a suspension of **2** (0.01 mol) in dry benzene (50 ml), the appropriate isothiocyanate (0.01 mol) was added. The reaction mixture was heated at 80^{O}C with stirring for 2 hrs and then left overnight at room temperature. The solid so obtained was filtered off and crystallized from ethanol to give **3a-c** (cf.Table 2).

4.2. Synthesis of 5-[2-(4-substituted-5-mercapto-4H-1, 2,4-triazol-3-yl) vinyl]-7-methoxy-1-benzofuran-6-ols (4a-c).

Compound of **3a-c** (0.01mol) in sodium hydroxide (5ml, 2N) was refluxed under stirring for 10 hours. The reaction mixture was then cooled and neutralized with dilute hydrochloric acid. The precipitate thus obtained was filtered off, washed with water several times, dried and crystallized from ethanol to give **4a-c**(cf.Table 2).

4.3. Synthesis of 5-{2-[5-(substituted amino)-1,3,4-oxadiazol-2-yl] vinyl}-7-methoxy-1-benzofuran-6-ols (5a-c).

Compounds of **3a-c** (0.002 mol) were refluxed with excess yellow mercuric oxide (0.015 mol) in ethanol (30ml) for 4-6 hours. The reaction mixture was allowed to cool to room temperature (to allow the sedimentation of the black mercuric sulphide),filtered and mercuric sulphide was washed with ethanol.The filtrate and alcoholic washing were combined, treated with water until a permanent turbidity existed and allowed to stand overnight .The product was separated and crystallized from ethanol to give **5a-c** (cf.Table 2).

4.4. Synthesis of 5-{2-[5-(substituted amino)-1,3,4-thiadiazol-2-yl] vinyl}-7-methoxy-1-benzofuran-6-ols (6a-c).

Phosphorous oxychloride (15ml) was added to the appropriate compound **3a-c** (0.005 mol) and the mixture was heated under reflux for 2-4 hours. The mixture was then evaporated in vacuo and the residue was washed with dilute ammonium hydroxide solution and water, dried and crystallized from ethanol to give **6a-c**(cf.Table 3).

4.5. Synthesis of N'- (3-benzyl-4-oxo-1, 3-thiazolidin-2-ylidene)-3-(6-hydroxy-7-methoxy-1-benzofuran-5-yl)acrylo hydrazide (7).

A mixture of **3a** (0.01 mol), ethyl bromoacetate

(0.01 mol) and anhydrous sodium acetate (0.015 mol) in absolute ethanol (30ml) was refluxed for 3h. The reaction mixture was cooled, diluted with water and allowed to stand overnight. The solid so obtained was filtered off, dried and crystallized from ethanol to give **7** (cf.Table 3).

4.6. Synthesis of N-substituted-2-[4-(6-hydroxy-7-methoxy-1-benzofuran-5-yl)-2-imino-2H-pyran-6-yl]hydrazinecarbothioamides (8a,b).

A mixture of **3a,b** (0.01 mol) and ethyl cyanoacetate (0.01 mol) in ethanol (30 ml) containing few drops of glacial acetic acid was refluxed for 4 hours. The precipitate that formed was filtered off, dried and crystallized from the proper solvent to give **8a,b**(cf.Table 3).

5. Results and Discussion

Cytotoxicity was carried out to determine the non cytotoxic doses of the prepared compounds to be used for antivirus bioassay. The results showed that no toxic effects were observed for all samples at dilution > 1:8 which permit the safe use of different concentrations of the tested materials for antiviral bioassays.

The results revealed that oxadiazole carboxylic acid was of highest activity against HSV-1 than all the other tested materials. It was noticed that cyclohexyl group increased the potential of some tested compounds for anti-HSV-1 activity as shown in the case of cyclohexyl thiadiazole, cyclohexyl triazole, cyclohexyl oxadiazole as shown in fig (A,B and C , respectively) .

Table 1. The prelininary antimicrobial screening test for the prepared compounds using chloroamphenical as control

Sample No.	*Micro-organism*		
	G + ve (1)	G – ve (2)	Fungi (3)
Control	++++	++++	++++
3a	++	+	++
3b	+	+	++
3c	+++	++	+
4a	+	+	+
4c	+	+	++
5b	+	+	+
5c	+++	++	+
6a	+	+	+
6c	+	+	++
7	+	+	+
8a	+	+	++
8b	++	+	++

Micro-organisms**:**

1-Bacillus Subtilies (G + ve)

2- Escherichia Coli (G – ve)

3-Aspergillus Niger (Fungus)

The inhibition zones were measured in the following manner:

Inhibition zone +++= highly active (> 12 mm)

Inhibition zone ++= moderately active (9-12 mm)

Inhibition zone += slightly active (6-9 mm)

Inhibition zone - = non sensitive (0.5 mm)

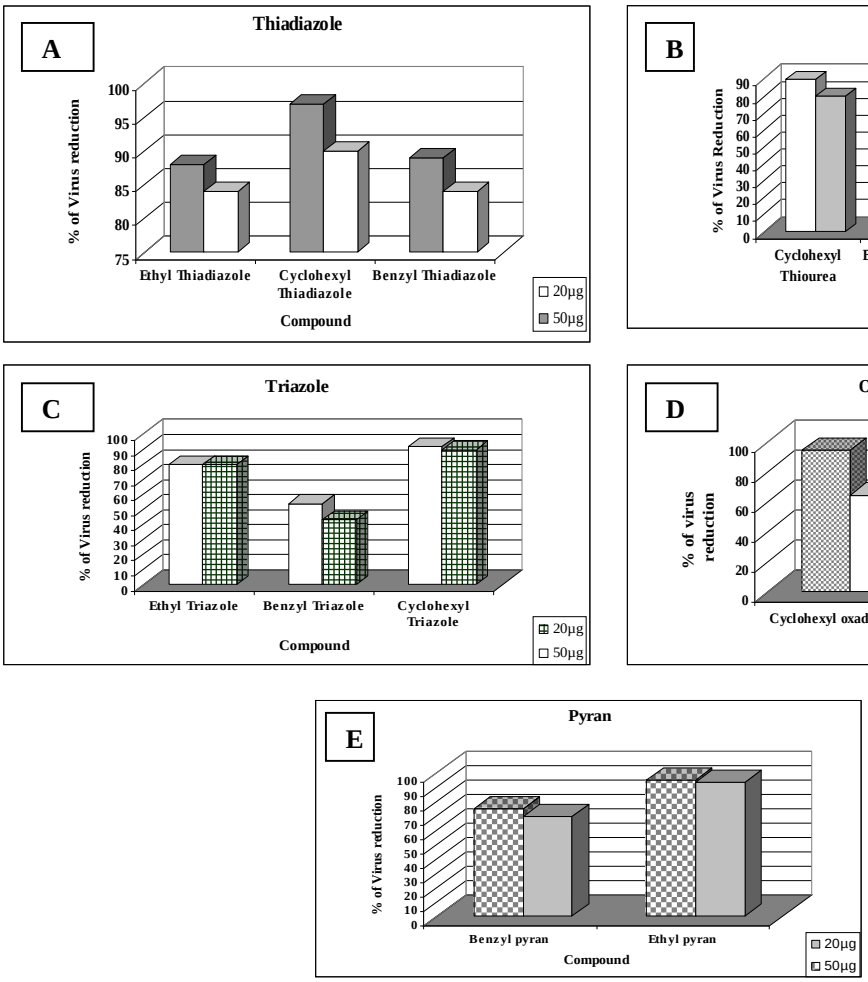
Table 2. Physical and spectral data of the newly synthesized compounds (3a-c)-(5a,b).

Compound no.	Mp (^{0}C) Yield(%)	Molecular formula (M.wt)	%Analysis Calcd/(Found) C	H	N	IR(ν ,cm^{-1}) and ^{1}H-NMR (DMSO-d$_6$)	MS (M$^+$)
3a	198-200 (95)	$C_{20}H_{19}N_3O_4S$ (397.449)	10.57 10.42	4.82 4.72	60.44 60.34	IR: 1335 (C=S), 3063-3255 (3NH), 3343 (OH). ^{1}H-NMR: 3.99 (s, 3H,OCH_3), 4.72 (d, 2H,CH_2Ph) , 7.25 (d, 1H furan H-3, $J_{H.H}$= 2.3 H_Z), 7.87 (d, 1H furan H-2, $J_{H.H}$= 2.3 H_Z) , 7.31-7.36 (m, 8H,aromatic protons+2H olefinic protons), 7.41- 7.82 (3br.s, 3H,3NH).	397
3b	>300 (85)	$C_{15}H_{17}N_3O_4S$ (335.379)	12.53 12.46	5.11 5.05	53.72 53.65	IR: 1305(C=S),3128-3204 (3NH), 3250(OH). ^{1}H-NMR: 1.08 (t,3H,CH_3),3.50 (m,2H,CH_2), 7.79-7.82 (3br.s,3H,3 NH), 9.06 (br.s,1H,OH).	335
3c	217-220 (85)	$C_{19}H_{23}N_3O_4S$ (389.470)	10.79 10.68	5.95 5.88	58.59 58.43	IR: 1341(C=S),3138-3013 (3NH) and 3243 cm^{-1} (OH). ^{1}H-NMR: 3.98 (s,3H,OCH_3), 1.27-2.21 (m,10H of cyclohexyl) , 4.07 (m,1H of cyclohexyl), 6.82 and 7.75 (2d,2H furan H-3,H-2,$J_{H.H}$=2.3Hz),7.80-7.82(3br.s,3H,3NH) , 6.83 and 6.99 (2d,2H olefinc protons),9.13 (br.s,1H,OH),	389
4a	>300 (95)	$C_{20}H_{17}N_3O_3S$ (379.433)	11.07 11.00	4.52 4.46	63.31 63.20	IR: 1619(C=N),3238(OH). ^{1}H-NMR: 3.98 (s, 3H,OCH_3), 7.25 (d, 1H furan H-3, $J_{H.H}$= 2.3 Hz), 7.88 (d, 1H furan H-2, $J_{H.H}$= 2.3 Hz), 7.29-7.33 (m, 5H,aromatic protons and 2H	379

						olefinic protons), 12.75 (s, 1H,SH).	
4b	205-210 (98)	$C_{15}H_{15}N_3O_3S$ (317.364)	13.24 13.11	4.76 4.65	56.77 56.67	IR: 1630(C=N),3238(OH) . ^{1}H-NMR: δ1.02(t,3H,CH_3),2.50(q,2H,CH_2),3.92(s,3H,O CH_3),6.8-7.81 (m,5H,aromatic protons + olefinic protons), 12.6 (br.s, 1H, OH) , 13.4(s,1H,SH).	317
4c	170-174 (98)	$C_{19}H_{21}N_3O_3S$ (371.454)	11.31 11.21	5.70 5.65	61.44 61.32	IR: 1636(C=N),3347(OH). ^{1}H-NMR: 3.3(m,1H,cyclohexyl),1.03-1.77 (m,10H,cyclohexyl), the OH signal gave one D_2O exchangeable signal at δ9.14.	371
5a	196-201 (60)	$C_{20}H_{17}N_3O_4$ (363.367)	11.56 11.46	4.72 4.61	66.11 66.03	IR: 1670(C=N), 3323(NH), 3343(OH). ^{1}H-NMR:	363
5b	214-216 (65)	$C_{15}H_{15}N_3O_4$ (301.297)	13.95 13.82	5.02 4.96	59.79 59.68	IR: 1623(C=N),3349 (NH) , 3396(OH).	301
5c	208-212 (60)	$C_{19}H_{21}N_3O_4$ (355.388)	11.82 11.74	5.96 5.87	64.21 64.10	IR: 1656(C=N),3267 (NH) , 3304(OH).	355

Table 3. Physical and spectral data of the newly synthesized compounds (6a-c),7, and (8a,b).

Compound no.	Mp (^{0}C) Yield(%)	Molecular formula (M.wt)	%Analysis Calcd/(Found)			IR(ν ,cm^{-1}) and ^{1}H-NMR (DMSO-d_6)	MS (M^+)
			C	H	N		
6a	185-190 (78)	$C_{20}H_{17}N_3O_3S$ (379.433)	11.07 10.95	4.52 4.43	63.31 63.26	IR: 1624(C=N),3217 (NH) , 3434(OH). ^{1}H-NMR: 3.94 (d,2H,CH_2Ph), 3.99 (s,3H,OCH_3),7.31-7.72 (m,5H,aromatic protons and 2H olefinc protons),7.26 (d,1H furan H-3,$J_{H.H}$= 2.3 Hz),7.85 (d,1H furan H-2,$J_{H.H}$= 2.3Hz),8.00(br.s,1H,NH),9.12 (br.s,1H,OH).	379
6b	>300 (60)	$C_{15}H_{15}N_3O_3S$ (317.364)	13.24 13.16	4.76 4.67	56.77 56.67	IR: 1622(C=N),3140 (NH) , 3387(OH). ^{1}H-NMR: 1.11 (t,3H,CH_3), 2.49 (m,2H,CH_2) , 4.00 (s,3H,OCH_3), 6.84-7.84 (m,3H,aromatic protons +2H olefinic protons).	317
6c	185-191 (65)	$C_{19}H_{21}N_3O_3S$ (371.454)	11.31 11.21	5.70 5.65	61.44 61.39	IR: 1634(C=N),3188 (NH) , 3241(OH). ^{1}H-NMR: 1.22-1.9(m,10H,cyclohexyl),3.43 (m,1H,cyclohexyl),3.99 (s,3H,OCH_3),6.5-7.9 (m,5H,aromatic protons + olefinic protons).	371
7	224-229 (75)	$C_{22}H_{19}N_3O_5S$ (437.470)	9.61 9.52	4.38 4.21	60.40 60.29	IR: 1609 (C=N),1712 (C=O), 3031(NH). ^{1}H-NMR: 4.79 (s,2H,CH_2Ph),7.25-7.38 (m,8H,aromatic protons+2H olefinic protons).	437
8a	192-199 (75)	$C_{22}H_{20}N_4O_4S$ (436.485)	12.84 12.92	4.62 4.75	60.54 60.61	IR:1353 (NC=S), 3028-3253 (NH).^{1}H-NMR:4.7 (d,2H,CH_2Ph) ,5.7-8.6 and 9.8 (br.s,4H,4NH),9.5(br.s,1H,OH),7.0-7.6 (m,8H,aromatic protons + 1H olefinic proton).	436
8b	209-213 (75)	$C_{17}H_{18}N_4O_4S$ (374.415)	14.96 14.82	4.85 4.75	54.53 54.61	IR: 1368 (NC=S), 3019-3280 (NH).^{1}H-NMR:1.21 (t,3H,CH_3) ,3.46 (q,2H,CH_2), 6.8-7.87 (m,3H aromatic protons + 1H olefinic proton),9.06-9.11 (4br.s,4H,4NH).	374



Anti-HSV 1 activity of the selected prepared compounds.
A. Thiadiazole (6a-c)
B. Thiourea (3a-c)
C. Triazole (4a-c)
D.Oxadiazole (5a,c)
E.Pyran (8a,b)

References

1-Hayakawa,I.; Shioya,R.; Agatsuma,T.; Furukawa,H; and Sugano,Y.; *Bioorganic & Medicinal Chemistry Letters, **2004** , 14, 3411-3414.*

2-Masubuchi,M.; Ebiike,H.; Kawasaki,K.; Sogabe,S.; Morikami,K.; Shiratori,Y.; Tsujii,S.; Fujii,T.; Sakata,K.; Hayase.M.; *et al, Bioorganic & Medicinal Chemistry, **2003**,11, 4463-4478.*

3-Almasirad, A.; Tabatabai,S.A.; Faizi,M.; Kebriaeezadeh,A.; Mehrabi,N.; Dalvandi,A.; and Shafiee,A.; *Bioorganic & Medicinal Chemistry Letters*, in Press, **2004**.

4-Burbuliene,M.M.; Jakubkiene,V.; Mekuskiene,G.; Udrenaite, E.; Smicius,R.; and Vainilavicius,P.; *II Farmaco*, **2004**,59, 767-774.

5-Jakubkiene,V.; Burbuliene,M.M.; Mekugkiene,G.; Udrenaite,E.; Gaidelis,P.; and Vainilavieius,P.;*II Farmaco*, **2003**,58, 323-328.

6-Cristina L.A.; Renata ,L. ; Antonio, L.F.;Faria,V.R.; Wanderley,A.G.; Afiatpour, P.;Camelo,E.;

Ximenes,P.A.; Srivastava,R.M.; Oliveira,C.F.; Medeiros,M.V.; Antunes,E.; and Brondani,D.J.;*II Farmaco*, **2000**, 55, 719-724.

7- Sawhney,S.N.; and Sharma,P.K.; *Bioorganic& Medicinal Chemistry Letters*, **1992**,3,1551-1554.
8-AI-Soud,Y.A.; AI-Dweri,M.N.; and AI-Masoudi,N.A.; *II Farmaco*, **2004**,59, 775-783.
9-Amir,M.; and Shikha,K.; *European Journal of Medicinal Chemistry*, **2004**,39, 535-545.
10- Sahin,G.; Palaska,E.; Ekizoglu ,M.;and Ozalp,M.; *II Farmaco*, **2002**,57, 539-542.
11-EI-Emam,A.A.; AI-Deeb,O.A.; AI-Omar,M.; and Lebrnaim,J.;*Bioorganic & Medicinal Chemistry*, **2004**,12, 5107-5113.
12-Takayama,H.; Shirakawa, S.; Kitajima,M.; Aimi,N.; Yamaguchi,K.; Hanasaki,Y.; Ide,T.; Katsuura,K.; Fujiwara,M.; ljichi,K.; et al.*Bioorganic & Medicinal Chemistry Letters*, **1996**, 6, 1993-1996.
13-El-Shihi,T.H.; Abdel Latif,N.A.;and El-Sawy, E.R.;*Egypt.J.Chem*., **2005**,48,365-376
14-Brokke, M.E.; Christensen, B.E.; *J.Org.Chem*., **1958**,23,589.
15-Abou-Zeid, A.A.;Abdel-Hamid,M.M.; and Shehata,Y.M.;*Zeitschrift für Alig.Microbiology*; **1976**,16(9),337.

4/9/2010

Effect of arginine on growth, nutrient composition, yield and nutritional value of mung bean plants grown under salinity stress

Amira M. S. Abdul Qados
Botany Department, Princess Nora Bint Abdul Rahman University, P. O. Box 2508 Safaqes St. Granada Esq. Riyadh 13242 – 07229 K.S.A

Abstract: Salinity is a major limitation to legume production in many areas of the world. The salinity sensitivity of mung bean was studied to determine the effect of salinity on vegetative growth (plant dry weight and plant height), yield components (plant height, pods number, pods weight, seeds number/pod, seeds weight/plant and biological yield/plant), nutritional value of produced seeds (N, P, K, Ca, Mg, Na, Cl, soluble carbohydrate, polysaccharides, total carbohydrate, proline, total amino acids and protein contents) and mineral contents in green shoot at harvest (N, P, K, and Na). Also, the role of arginine in alleviating the effect of salinity stress was studied. Mung bean seeds were planted in soils of different salinity levels. The concentration of the irrigation water used in this experiment were (0, 15000, 3000, 4500 and 6000 ppm). All growth parameters were significantly reduced with high salinity levels (4500 and 6000 ppm) while 1500 and 3000 ppm induced slight increase. Salinity stress also, induced significant increases in Na, Cl, Ca and Mg and decreased significantly N, P, and K contents. Salinity stress reduced most yield components and nutritional value of produced seeds. However, spraying plants with arginine could alleviate the harmful effect of salinity at all studied parameters. [Nature and Science 2010;8(7):30-42]. (ISSN: 1545-0740).

Key words: Mung bean, Salinity, Arginine, growth, Yield, Mineral compositions

1. Introduction

Salinity is a common a biotic stress factor seriously affecting crop production in different regions, particularly in arid and semi – arid regions. It is estimated that over 800 million hectare of land in the world are affected by both salinity and sodicity (Munns, 2005). There are various detrimental effects of salt stress in crop plants, responsible for severe decrease in the growth and yield of plants. Osmotic stress (drought problem), ion imbalance, particularly with Ca, K, and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress (Zhu, 2001; Munns, 2002; Munns et al., 2006 and Eker et al., 2006). Salt stress, like many a biotic stress factors, reduces the ability of plants to take up water, leading to growth reduction as well as metabolic changes similar to those caused by the water stress (Munns, 2002). High salt concentration in root affects the growth and yield of many important crops (Alam et al., 2004; Taffouo et al., 2004). The salinity may reduce the crop yield by upsetting water and nutritional balance of plant (Khan et al., 2007 and Taffouo, 2009).

Agricultural soils have many types of salt ions. However, NaCl is usually the damaging and predominant salt (Turan et al., 2007a). Although, adaptation of plants to salinity is associated with osmotic adjustment (Turan et al., 2007b), they widely differ from the exerted to which they accumulate inorganic ions (Munns, 1993). Osmotic regulators in plants include K, soluble sugar, proline and betaine (Le Rudulier, 2005). These molecules are important physiological indicator for evaluating osmotic adjustment ability (Zhu, 2002).

Mung bean (*Vigna radiate* L.Wilczek) is a summer pulse crop with short duration (70 – 90 days) and high nutritive value. It has many effective uses, green pods in cooking as peas, sprout rich in vitamins and amino acids. This crop can be used for both seeds and forage since it can produce a large amount of biomass and then recover after grazing to yield abundant seeds (Lawn and Ahn, 1985) and can be used in broilers diets as a non – traditional feed stuff (El-Karamany et al., 2003).

Arginine is one of the essential amino acids (considered the main precursor of polyamines which produced by decarboxylation of arginine via arginine decarboxylase to form putrescine (Evans and Malmberg, 1989; Bocherueu, 1999). Polyamines and their precursor arginine have been implicated as vital modulators in a variety of growth, physiological and developmental processes in higher plants (Glastone and Kaur-sawhny, 1990). Polyamines are involved in the control of cell cycle, cell division, morphogenesis in phytochrome and plant hormone mediated process and the control of plant senescence, as well as in plant response to various stress factors (Walters, 2000 and Abdel Monem, 2007). The application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean (Nassar et al., 2003); in wheat

Abd ElMonem, 2007) and El-Bassiouny et al., 2008). Moreover Hassanein et al., (2008) and Khalil et al., (2009) recorded the positive role of arginine in alleviating the inhibition occurs as the result of exposing plants to stress.

The objective of the present study was to evaluate the response of mung bean to grown under saline irrigation and to study the role of arginine in alleviating the harmful effects of salinity stress.

2. Material and Methods

The experimental plant used in this investigation was mung bean (*Vigna radiata* var. kawmy-1). The chemical used in the present work was arginine (one of the essential amino acids); it was supplied from SIGMA – ALDRICH.

A pot experiment was carried out in the screen greenhouse of National Research Centre, Dokki, Giza, Egypt. This experiment was carried out to study the effect of spraying mung bean plants with different concentrations of arginine (0.0, 1.25, 2.50 and 5.00 mM) on growth, yield and chemical composition of yielded seeds of mung bean under different salinity levels (1500, 3000, 4500 and 6000 ppm). The salt components of salt mixture are shown in Table 1.

Table (1): The component of salt mixture used for chloride Stalinization expressed as % of total salt content as described by Stroganov (1962):

$MgSO_4$	$CaSO_4$	NaCl	$MgCl_2$	$CaCO_3$
10	1	78	2	9

The components of specific anions and cations in chloride mixture expressed as percentage of total mill equivalent

Table (2): The components of specific anions and cations in chloride mixture expressed as percentage of total mill equivalent

Na^+	Mg^{+2}	Ca^{+2}	SO_4^{-2}	Cl^-	CO_3^{-2}
38	6	6	5	40	5

A homogenous lots of mung bean seeds variety kawmy-1 was sown in pots (50 cm in diameter) containing equal amounts of mixture sandy and clay soil (2:1). The pots were divided into five groups according to irrigation with different levels of saline solutions by using Stroganov nutrient solutions. Each group was divided into four sub-groups according to the concentration of arginine (0.0, 1.25, 2.5 and 5.00 mM). Each sub-group sprayed with one concentration at 21 days after sowing and the spraying was repeated after one week from the first spray. Every treatment consisted of 5 replicates distributed in a completely randomized design. The pots were irrigated with equal volumes of the various salinity levels, after 21 from sowing. Irrigation was run as follow 3 times with saline solutions and one with tap water. Fertilization was done with the recommended dose i.e. (phosphorous / pot as triple phosphate, nitrogen / pot as urea and potassium / pot as potassium sulphate) during preparation of pots and after sowing. After 15 days from sowing thinning was carried out, so as five uniform seedlings were left in each pot for studying the effect of different treatments on the yield of mung bean cultivar.

Five mung bean plants from each pot were cut from ground surface at 75 days after sowing. Plant height and dry weight of mung bean of shoot were determined. Dry weight was determined after drying in a forced oven at 70˚C till constant weight. Measurement of yield and its components was recorded at harvest.

Chemical composition of seeds and green shoots at harvest:

The dried seeds were finally ground. A total soluble carbohydrate was determined using modifications of the procedures of Yemm and Willis, (1954) and Handel (1968). Total carbohydrate content was determined calorimetrically according to Dubois et al., (1956). Polysaccharides were calculated by the difference between total carbohydrates and total soluble carbohydrates. Total free amino acids and proline contents were determined calorimetrically according to Hassanein, (1977) for extraction (Muting & Kaiser, 1963) and Bates et al. (1973). Total N and protein contents were determined by the Kjeldahl method of Pirie (1955). The nutrient elements K, Na, Mg, Ca, Cl and P were determined according to the method described by Chapman and Pratt (1978) in seeds and green leaves. Also, the protein electrophoratic pattern of yielded grains was recorded according to Reuveni et al., (1992) with some modifications.

Statistical analysis:

The data recorded were subjected to the statistical analysis by M-STAT-C statistical analysis program (MSTAT, 1988). Least significant difference test was applied at 0.05 probability level to compare mean treatments.

3. Result Analysis

Mung bean growth:

Data in Table 3 clear that, the irrigation of mung bean with saline water (1500 and 3000 ppm) increased significantly plant height and dry weight /plant as compared with control plant. While the higher levels of salinity (4500 and 6000 ppm) decreased significantly these parameters as compared with untreated plant. The reduction in vegetative growth due to high salinity effect is in harmony with previous investigators (Taffouo et al., (2004) on some legumes plants Mohamedin et al. (2006) on sunflower and Touffouo et al. (2009) on cowpea plants.

The inhibition effects of salinity on growth parameters of mung bean plants might be due to salinity which inhibits the growth through reduced water absorption, reduced metabolic activities due to Na^+ and Cl^- toxicity and nutrient deficiency caused by ionic interference (Ghoulam et al., 2002 and DeLacerda et al., 2003).

The results in same table also showed that, spraying mungbean with arginine (1.25, 2.5 and 5 mM) reduced significantly plant height of mung bean plants as compared with control plant. Increasing arginine concentration reduced gradually plant height of mung bean plant. Plant dry weight increased significantly over the control plant. These results are in good harmony with those obtained by Abd El-Monem (2007) and El-Bassiouny et al., (2008) on wheat plants.

The results also showed that, spraying mung bean plants with different concentrations of arginine could alleviate the harmful effect of salinity on plant height and plant dry weight). The highest value of plant dry weight was recorded at 2.5 mM arginine. These results are in agreement with those obtained by Abd El-Monem (2007) who found that, arginine at 2.5 mM was the optimum concentration in the alleviation the harmful effects of stress. Also, Xu et al. (2001) and Nassar et al. (2003) concluded that exogenous application of polyamine (end product of arginine) to several plant species have been shown to promote cell division, cell differentiation and general growth promotion. They can also help to stabilize membrane and wall properties (Velikov et al., 2000) and protect plant against environmental stress (Mo and Pua, 2002).

Mung bean yield and its components:

Data in (Table 4) showed the plant height at harvest, number and weight pods per plant, pods weight per plant, seeds number /pod, seeds and biological yield per plant affected by salinity irrigation and spraying plants with arginine concentrations. All these criteria decreased under different salinity irrigation. The plant height, pods number/plant and seeds number /pod were significantly decreased under all salinity levels. Increasing salinity levels induced gradual reduction as compared with untreated plant. While, pods and seeds weights/plant increased significantly under lower levels of salinity (1500 and 3000 mg/l) and decreased under the higher concentrations (4500 and 6000 mg/l). Biological yield was increased significantly under 1500 mg/l and gradually reduced under all other salinity levels. For instance the reduction in the plant height, pods number /plant and seeds number/pod reached to 63.84%, 59.66% and 12.2% at 6000 mg/l respectively. The lowest level of salinity (1500 mg/l) increased the weight of pods and seeds /plant and the biological yield of mung bean by 24.52%, 27.16% and 14.99% respectively. The highest level of salinity reduced the parameters by 58.65%, 43.21% and 37.10 % respectively. These results agree with those obtained by Mass and Grieve (1990) on both durum and bread wheat; Sharma and Gill (1994) on mustard yield; Abd El-Halim et al. (1995) on wheat; El-Bassioumy et al. (1999) on sunflower; El-Bassiouny and Bekheta (2001) on wheat and Zadeh and Naeini (2007) on canola. The depressive effect of salinity on yield may be attributed to the inhibitory effect of salinity on vegetative growth (Table 3). In this connection Abd El-Halim et al. (1995) concluded that, the reduction of wheat grain yield per plant due to salinization might be due to the harmful effect of salt stress on growth, the disturbance in mineral uptake and/or enhancement of plant respiration. Moreover, Taffouo et al. (2009) reported that, the significant decrease of yield components observed under salt stress in cowpea would be partly related to a significant reduction of foliar chlorophyll contents (more than 50%) and K^+ concentration in saline medium.

Results in same table also show that, exogenous application of arginine under salinity level caused increase in all parameters of yield components as compared to the corresponding salinity level. Krishnamurthy (1991) reported that, when putrescine (arginine forming substance) was exogenously supplied on the salt stressed plant, the grain yield of rice increased. This increment could be due to antisenescence effect of putrescine. El-Bassiouny and Bekheta (2001) proved that, putrescine is intimately involved in salt treated wheat plant thereby regulating growth, development and grain yield. Nassar et al. (2003) concluded that, arginine induce early flowering and fruiting of bean plants respectively.

Chemical analysis of yielded seeds:

The obtained results in Table (5) show that irrigation of mungbean with different salinity levels decrease total soluble carbohydrates, polysaccharides, total carbohydrates, total amino acid contents and protein percentage of mung bean seeds as compared to plants irrigated with non saline water (control). The magnitude of reduction was increased with increasing

salinity level. The reduction in total soluble carbohydrates, polysaccharides and total carbohydrates in mung bean seeds could be attributed to the nutritional imbalance and specific toxic effect of salinity as recorded by Nou et al. (1995); hyperosmotic stress and reduced photosynthesis Abd El-Wahab (2006).

Moreover, total amino acid content in mung bean seeds found to be adversely affected due to salinity effect. The same result was obtained by Sarwat and Sherif (2007) who stated that, amino acid appeared to be decreased with salinity depending on the concerned amino acid response on barley.

The reduction in protein percentage in seeds of mung bean plants irrigated with salinity may be attributed to the reduction in the total nitrogen (Table 6) content as salinity increased. This result was in line with El-Hindi and El-Ghamry (2005) on cherry gold plants and Abd El- Wahab (2006) on Foeniculum vulgare.

The results in same table clear that Proline content of mung bean seeds increased gradually with increasing salinity levels as compared with those plants grown on non saline water. This result demonstrate that, the physiological function of proline accumulated in mung bean under salinity stress may not be just behave as an osmolyte and protectant but may also have other roles related to stress. The result of proline in mung bean seeds is in good agreement with those obtained by Nuran and Cakirlar (2002) on maize; Abd El-Hamid et al. (2003) on *Pancratium maritimum*; Shi and Sheng (2005) and Mohamedin et al. (2006) on sunflower.

Table 5 also shows the role of arginine in ameliorate the adverse effect of salinity on all chemical composition of mung bean seeds. Arginine treatment of mung bean plants increased significantly total soluble carbohydrate, poly saccharides, total carbohydrates, proline, total amino acid and protein contents of mung bean seeds under saline and non saline irrigation. The magnitude of increment was much pronounced by using 2.5 mM arginine under all salinity levels. These results could be supported by the results obtained by Abd El-Monem (2007); El-Bassiouny et al. (2008) and Hassanein et al. (2008) who indicated that arginine was the most effective compound in increasing soluble carbohydrate, poly saccharides, total carbohydrates, proline, total amino acid and protein contents of wheat plants and grains under normal or stressed condition.

Macro elements in mungbean leaves and seeds at harvest:

N, P and K concentrations in mung bean green seeds (Table 6) and leaves (Table 7) were significantly decreased under different saline irrigation compared with control plants. Data also in Table (7) show that, Na content of green leaves and Na, Ca, Mg and Cl concentrations of seeds (Table6) increased significantly in response to irrigation with all salinity levels as compared to leaves or seeds irrigated with tap water. Confirm these results Pessarakli (1991) and Al-Rawahy et al. (1992) who stated that, salinity can reduce N accumulation in plants. Many scientists attributed the reduction of N concentration to Cl antagonism of NO_3^- uptake Lea-Cox and Syverten (1993). This may be attributed to increase in Cl uptake which accompanied by a decrease in NO_3 concentration (Bar et al., 1997). Phosphate availability is reduced in saline soils not only because of ionic strength effect that reduce the activity of phosphate but also because phosphate concentration in soil solution is tightly controlled by sorption processes and by the low solubility of Ca and P minerals. Therefore, it is understandable that phosphate concentration in field grown agronomic crops decreased as salinity increased (Mohamedin et al., 2006).

In response to K results Grattan and Grieve (1999) reported that under saline conditions, high level of external Na not only interfere with K^+ acquisition by roots but also may disturb the integrity of root membranes and alter selectivity.

The obtained results of Na and Cl concentrations in mung bean seeds are in good harmony with those obtained by Francois (1996); Barrett-Lennard (2003) and Shi & Sheng (2005) on sunflower. Salinity has an antagonistic effect on the uptake of Ca and Mg which caused by displacing Ca in membranes of root cells (Yermiyahu et al., 1997); and (Asik et al., 2009) on wheat.

External application of arginine reduced significantly Na and Cl concentrations, in leaves and seeds of mung bean, while increased the uptake of N, P K in leaves and N, K, Ca, Mg and P in mung bean seeds and furthermore the K/Na ratio increased under all salinity levels compared to corresponding control. The magnitude of reduction was increased with increasing arginine concentration. These results are in good harmony with those obtained by Sharma et al. (1997) they reported that foliar application of putrescine (one product of arginine) enhance the uptake of K, Ca and Mg but decreased Na and Cl uptake in chick pea plant. Santa-Cruzet al. (1997); Mansour and Al-Mutawa (1999) and El-Bassiouny and Bekheta (2001) suggested that the main role of all arginine products (putrescine, spermidine and spermine) in salt treated plants in the long term is to maintain a cation-anion balance in plant tissues by stabilizing membrane at high external salinity.

Protein electrophoratic pattern:

Results in Table (8) show that, the changes in protein electrophoretic pattern of mung bean seeds sprayed with arginine (1.25, 2.5 and 5 mM) and irrigated with different levels of salinity (1500, 3000, 4500 and 6000 ppm).

In the control mung bean seeds sowing without treatment, the separation of 8 protein bands were

apparent, their molecular weights ranged between 117 KDa and 8 KDa. Irrigation of wheat plants with different salinity levels (2000, 4000, 6000 and 8000 ppm) showed an increase in the number of protein bands to 17, 17, 17 and 18 bands, respectively. The result also showed the disappearance of protein bands at molecular weights 36 and 14.5 KDa at all salinity levels. These results indicate that the seeds of plant irrigated with different salinity levels characterized by disappearance of certain bands and the appearance of new ones as compared with that of the untreated grains, Table (8). In this respect Kermode (1997) and Bekheta and El-Bassiouny (2005) concluded that one of the important mechanism involved in the cell protection against salinity stress is the induction of de novo synthesis of a set of new protein. Therefore, in the present study, salinity stress in general induced synthesis of a new set of protein bands (3 bands) at molecular weights 100, 70 and 44 KDa at all salinity levels, and at molecular mass 36 KDa at 8000 ppm only.

In this respect, HSP 70 a group of HSPs accumulated in response to drought stress (Pareek et al., 1995). Such proteins are referred to as associated proteins. Moreover, Close (1996) and Han et al (1997) concluded the presence of some protein band at different molecular weight have a protective role in under water loss due to their function as an trap ion in dehydrating cells and sequestering ions as they become concentrated.

Irrigation of mung bean with different concentrations of arginine induced the appearance of protein band at molecular weight 80 KDa. In this respect, Kuznetsov and Shevyakova (1997) stated that putrescence (the final product of arginine decarboxylation) could change the stability and substrate specificity of protein kinase/phosphayase systems to modify the properties of polypeptides and acting as substrates for phosphorylation and dephosphorylative enzymes and affect the stability of protein molecules in plants. It is worthy to mention that, arginine treatments induced the appearance of new protein bands at molecular weight 131.00 KDa. This band disappeared when plants irrigated with different salinity levels.

Moreover, there are a protein bands appeared at molecular weights 92, 90, 71.5, 60, 32.5, 16.5 and 8 KDa are appeared in plants sprayed with all arginine concentrations and irrigated with all salinity levels. Arginine also showed disappears of protein band at molecular weight 19 KDa under all salinity levels, and at molecular weights 117 and 104 KDa under control, 4500 and 6000 ppm. These results are in good harmony with those obtained by (Abd El-Monem, 2007, El-Bassiouny *et al.*, 2008 and Khalil *et al.*, 2009) who indicated that, arginine treatments induced the appearance of new protein bands at molecular weights 222.0, 214.6, 131.8, 93.1, 78.7, 50.7, 34.6 and 14.1 KDa in wheat plants. Also Bekheta and El-Bassiouny concluded that the plant treated with putrescine and irrigated with different salinity levels showed that the improving salt tolerance by adding putrescine increased the intensity of salt responsive proteins at molecular weights 91, 70, 36, 21, 17 and 15 KDa).

Table (3): Effect of different concentrations of arginie on plant height and dry weight of mungbean at 75 DAS grown under salinity stress.

Treatment		Shoot length (cm)	Dry weight/plant (g)
Salinity (ppm)	Arginine (mM)		
Zero	**Zero**	32.67	4.29
	1.25	39.00	5.42
	2.50	38.00	4.48
	5.00	37.00	4.03
1500	**Zero**	45.00	3.89
	1.25	46.00	5.54
	2.50	30.67	3.18
	5.00	29.00	2.14
3000	**Zero**	35.33	3.11
	1.25	42.00	5.09

		2.50	28.67	3.20
		5.00	26.00	2.00
4500		**Zero**	33.00	2.97
		1.25	39.00	3.43
		2.50	25.00	2.41
		5.00	22.00	1.74
6000		**Zero**	20.67	1.85
		1.25	23.67	2.11
		2.50	21.33	1.65
		5.00	19.33	1.63
LSD at 5%				**0.12**
Mean of main effects:				
Salinity (ppm)		**Control**	36.67	4.55
		1500	37.67	3.69
		3000	33.00	3.35
		4500	29.75	2.64
		6000	21.25	1.81
LSD at 5%			**1.31**	**0.04**
Arginine (mM)		**Zero**	33.33	3.22
		1.25	37.93	4.32
		2.50	28.73	2.99
		5.00	26.67	2.31
LSD at 5%			**1.14**	**0.05**

Table (4): Effect of different concentrations of arginine on mungbean yield and its components grown under salinity stress.

Character Treatment		**Plant ht. at harvest (cm)**	**Pods no/ plant**	**Pods wt. (g/plant)**	**Seeds no/pod**	**Yields/plant (g)**			**HI %**
Salinity (ppm)	**Arginine (mM)**					**Seed**	**Straw**	**Bio.**	
Zero	**Zero**	59.00	6.00	2.08	4.07	1.62	8.38	10.00	16.20
	1.25	43.67	6.58	2.46	4.37	1.88	8.90	10.78	17.44
	2.50	42.00	8.67	2.91	4.89	2.03	9.86	11.89	17.07
	5.00	43.33	5.08	1.81	2.80	1.26	8.74	10.00	12.60
1500	**Zero**	50.67	5.67	2.59	4.95	2.06	7.16	9.22	22.34
	1.25	41.33	7.33	2.82	5.42	2.36	8.09	10.45	22.58
	2.50	40.00	7.33	3.09	4.68	2.55	8.12	10.67	23.90
	5.00	40.00	3.92	2.20	3.34	2.01	7.10	9.11	22.06
3000	**Zero**	47.33	4.83	2.36	3.90	1.88	7.23	9.11	20.64
	1.25	41.33	6.50	2.58	4.63	2.22	8.11	10.33	21.49
	2.50	34.33	6.75	2.75	3.49	2.52	7.03	9.55	26.39

	5.00	24.00	3.17	1.84	2.95	1.20	8.13	9.33	12.86
4500	**Zero**	39.67	3.58	1.59	2.80	1.07	8.04	9.11	11.75
	1.25	40.00	3.92	1.96	3.52	1.34	8.44	9.78	13.70
	2.50	30.67	5.00	2.12	2.86	1.56	7.44	9.00	17.33
	5.00	31.33	2.33	1.33	2.17	0.87	7.02	7.89	11.03
6000	**Zero**	21.33	2.42	0.86	2.56	0.92	7.86	8.78	10.48
	1.25	34.00	3.08	0.99	3.09	1.06	8.27	9.33	11.36
	2.50	28.67	3.42	1.11	2.62	1.27	7.95	9.22	13.77
	5.00	28.00	1.33	0.64	1.34	0.55	6.89	7.44	7.39
LSD at 5%		**2.14**	**0.42**	**0.12**	**0.54**	**0.19**	**0.22**	**0.12**	**5.72**
Mean of main effects:									
Salinity (ppm)	**Control**	47.00	6.58	2.32	4.03	1.70	8.97	10.67	15.83
	1500	43.00	6.06	2.68	4.60	2.25	7.62	9.86	22.72
	3000	36.75	5.31	2.38	3.74	1.96	7.63	9.58	20.34
	4500	35.42	3.71	1.75	2.84	1.21	7.74	8.95	13.45
	6000	28.00	2.56	0.90	2.40	0.95	7.74	8.69	10.75
LSD at 5%		**1.49**	**0.20**	**0.05**	**0.21**	**0.09**	**0.14**	**0.10**	**2.91**
Arginine (mM)	**Zero**	43.60	4.50	1.90	3.66	1.51	7.73	9.24	16.28
	1.25	40.07	5.48	2.16	4.21	1.77	8.36	10.13	17.32
	2.50	35.13	6.23	2.40	3.71	1.99	8.08	10.07	19.69
	5.00	33.33	3.17	1.56	2.52	1.18	7.58	8.75	13.19
LSD at 5%		**0.88**	**0.17**	**0.05**	**0.22**	**0.08**	**0.09**	**0.05**	**2.35**

Table (5): Effect of different concentrations of arginine on chemical constitute in mungbean seeds grown under salinity stress.

Treatment		**Chemical constitute in grains (mg/100 g grain weight)**					**Protein (%)**
Salinity (ppm)	**Arginine (mM)**	**Total soluble Sugar**	**Poly sacharides**	**Total charb.**	**Total amino acids**	**Prolin**	
Zero	**Zero**	21.25	32.06	53.31	50.90	11.82	20.73
	1.25	26.75	38.44	65.19	55.65	13.83	22.06
	2.50	28.95	44.67	73.62	61.90	16.65	25.12
	5.00	27.50	42.94	70.44	52.85	13.48	30.25
1500	**Zero**	21.55	31.99	53.54	44.10	12.44	23.69
	1.25	28.25	43.94	72.19	54.45	14.81	26.25
	2.50	30.25	45.62	75.87	55.85	17.80	27.13
	5.00	24.10	38.88	62.98	51.20	14.52	29.06
3000	**Zero**	17.15	25.23	42.38	43.15	13.48	20.70
	1.25	21.55	34.57	56.12	50.90	16.96	22.81
	2.50	23.35	36.02	59.37	54.35	18.11	24.19
	5.00	22.40	34.66	57.06	46.25	15.73	26.50
4500	**Zero**	15.75	23.63	39.38	40.30	14.70	18.13

	1.25	18.70	27.80	46.50	44.45	18.15	20.69
	2.50	21.10	31.71	52.81	53.50	18.61	22.81
	5.00	19.10	26.34	45.44	41.48	17.83	26.06
6000	**Zero**	15.03	22.47	37.50	38.30	16.96	16.75
	1.25	17.65	25.54	43.19	41.50	19.59	18.38
	2.50	19.35	29.84	49.19	51.95	20.63	20.44
	5.00	18.50	29.75	48.25	42.52	19.00	23.25
LSD at 5%		**0.86**	**2.78**	**3.37**	**3.36**	**0.47**	**1.44**
Mean of main effects:							
Salinity (ppm)	**Control**	26.11	39.53	65.64	55.33	13.95	24.54
	1500	26.04	40.11	66.15	51.40	14.89	26.53
	3000	21.11	32.62	53.73	48.66	16.07	23.55
	4500	18.66	27.37	46.03	44.93	17.32	21.92
	6000	17.63	26.90	44.53	43.57	19.05	19.71
LSD at 5%		**0.54**	**1.53**	**1.74**	**1.05**	**0.15**	**1.03**
Arginine (mM)	**Zero**	18.15	27.07	45.22	43.35	13.88	20.00
	1.25	22.58	34.06	56.64	49.39	16.67	22.04
	2.50	24.60	37.57	62.17	55.51	18.36	23.94
	5.00	22.32	34.51	56.83	46.86	16.11	27.02
LSD at 5%		**0.35**	**1.14**	**1.38**	**1.38**	**0.19**	**0.59**

Table (6): Effect of different concentrations of arginine on macro elements in mungbean seeds grown under salinity stress.

Treatment		**Macro elements (mg/g grains dry weight)**							**K/Na**
Salinity (ppm)	**Arginine (mM)**	**N**	**P**	**K**	**Na**	**Ca**	**Mg**	**Cl**	
Zero	**Zero**	3.37	0.61	0.49	1.59	0.80	1.20	0.16	0.31
	1.25	3.53	0.65	0.56	1.38	1.40	2.00	0.15	0.41
	2.50	4.02	0.70	0.61	1.10	1.60	2.60	0.15	0.55
	5.00	4.84	0.73	0.62	1.03	2.00	2.20	0.17	0.60
1500	**Zero**	3.79	0.56	0.39	1.72	1.60	2.20	0.19	0.23
	1.25	4.20	0.60	0.44	1.62	1.90	3.00	0.17	0.27
	2.50	4.34	0.65	0.45	1.38	2.10	3.90	0.15	0.33
	5.00	4.65	0.67	0.55	1.14	2.30	3.30	0.21	0.48
3000	**Zero**	3.32	0.47	0.34	1.95	1.80	2.80	0.22	0.17
	1.25	3.65	0.50	0.35	1.82	2.40	3.60	0.22	0.19
	2.50	3.87	0.60	0.38	1.62	2.60	4.10	0.20	0.24
	5.00	4.24	0.57	0.39	1.34	2.80	3.60	0.23	0.29
4500	**Zero**	2.90	0.38	0.24	2.54	2.10	3.10	0.23	0.10
	1.25	3.31	0.41	0.27	2.08	2.80	4.20	0.23	0.13

	2.50	3.65	0.50	0.27	1.89	2.90	4.80	0.21	0.14
	5.00	4.17	0.51	0.29	1.61	3.10	3.80	0.24	0.18
6000	**Zero**	2.68	0.32	0.18	2.93	2.40	3.60	0.24	0.06
	1.25	2.94	0.34	0.20	2.24	2.95	4.60	0.24	0.09
	2.50	3.27	0.40	0.22	2.08	3.20	5.10	0.21	0.10
	5.00	3.72	0.44	0.25	1.82	3.40	4.20	0.25	0.14
LSD at 5%		**0.23**	**0.08**	**0.06**	**0.08**	**0.27**	**0.14**	**4.19**	**0.71**
Mean of main effects:									
Salinity (ppm)	**Control**	3.94	0.67	0.57	1.28	1.45	2.00	0.16	0.47
	1500	4.25	0.62	0.46	1.47	1.98	3.10	0.18	0.33
	3000	3.77	0.54	0.37	1.68	2.40	3.53	0.22	0.22
	4500	3.51	0.45	0.27	2.03	2.73	3.98	0.23	0.14
	6000	3.15	0.38	0.21	2.27	2.99	4.38	0.24	0.10
LSD at 5%		**0.17**	**0.06**	**0.04**	**0.06**	**0.20**	**0.25**	**2.09**	**0.03**
Arginine (mM)	**Zero**	3.21	0.47	0.33	2.15	1.74	2.58	0.21	0.17
	1.25	3.53	0.50	0.36	1.83	2.29	3.48	0.20	0.22
	2.50	3.83	0.57	0.39	1.61	2.48	4.10	0.18	0.27
	5.00	4.32	0.58	0.42	1.39	2.72	3.42	0.22	0.34
LSD at 5%		**0.09**	**0.03**	**0.02**	**0.03**	**0.11**	**0.06**	**1.72**	**0.02**

Table (7): Effect of different concentrations of arginine on macro elements in leaves of mungbean at harvest grown under salinity stress.

Treatment		**Macro elements (%)**			**Na (ppm)**
Salinity (ppm)	**Arginine (mM)**	**N**	**P**	**K**	
Zero	**Zero**	2.58	0.19	2.14	265.50
	1.25	2.99	0.22	2.38	175.50
	2.50	2.65	0.20	2.26	306.50
	5.00	2.42	0.19	2.19	448.00
1500	**Zero**	2.72	0.21	2.24	383.50
	1.25	3.12	0.24	2.76	273.00
	2.50	2.77	0.21	2.65	367.50
	5.00	2.55	0.20	2.41	653.00
3000	**Zero**	2.37	0.17	2.30	633.00
	1.25	2.81	0.19	2.43	416.50
	2.50	2.42	0.16	2.03	586.50
	5.00	2.33	0.16	1.96	852.00
4500	**Zero**	2.01	0.15	2.21	823.50
	1.25	2.27	0.18	2.32	601.00
	2.50	2.01	0.15	1.93	685.00

	5.00	1.83	0.14	1.77	352.00
6000	**Zero**	1.77	0.13	2.19	1064.00
	1.25	1.92	0.15	2.24	854.50
	2.50	1.57	0.13	1.76	970.00
	5.00	1.41	0.13	1.55	1352.00
LSD at 5%		**ns**	**ns**	**ns**	**114.20**
Mean of main effects:					
Salinity (ppm)	**Control**	2.66	0.20	2.24	298.88
	1500	2.79	0.22	2.52	419.25
	3000	2.48	0.17	2.18	622.00
	4500	2.03	0.16	2.06	615.38
	6000	1.67	0.14	1.94	1060.1
LSD at 5%		**ns**	**ns**	**ns**	**68.40**
Arginine (mM)	**Zero**	2.29	0.17	2.22	633.90
	1.25	2.62	0.20	2.43	464.10
	2.50	2.28	0.17	2.13	583.10
	5.00	2.11	0.16	1.98	731.40
LSD at 5%		**ns**	**ns**	**ns**	**51.07**

Table (8):Effect of diferent concentration of arginie on electrophoratic pattern of mungbean seeds grown under saliniy stress.

Mwt	Salinity mg/l														
	control	1.25 mM arginine	2.5mM arginine	5mM arginine	1500	1.25 mM arginine	2.5mM arginine	5mM arginine	3000	1.25 mM arginine	2.5mM arginine	5mM arginine	4500	1.25 mM arginine	2.5mM arginine
117.0	11.00				3.11	3.89	3.11		3.14	2.25	2.66				
104.0	11.64				4.36	5.44	5.74	3.58	3.68	4.62	5.27		3.16	3.21	3.58
100.0					7.65	3.24	3.14	2.98	5.17	5.64	6.54	6.94	3.68	5.61	5.82
92.0		3.59	5.11	5.28	4.65	4.04	4.21	4.46	3.68	3.82	4.25	5.11	3.54	4.85	4.46
90.0		7.24	9.65	9.81	2.11	3.78	4.36	4.08	3.98	3.77	3.82	4.75	4.87	4.52	5.08
80.0		5.98	5.24	4.35		4.36	4.82	4.36		3.24	4.52	5.63		5.36	4.36
71.5		6.24	4.50	4.27	4.21	4.74	5.14	9.25	3.36	4.55	6.12	7.41	3.25	4.72	5.25
70.0					8.24	8.62	8.91	9.14	8.97	9.15	9.25	9.62	9.35	9.56	10.14
60.0		4.03	5.19	6.80	5.24	6.83	6.97	4.96	4.82	5.13	5.31	5.64	3.95	4.12	4.96
51.5	12.53	7.68	3.62	5.14	5.67	4.33	5.14	5.31	5.45	5.22	4.32		4.94	5.04	5.31
44.0					6.82	5.44	5.98	5.11	10.09	9.87	8.53	7.52	12.55	10.73	9.11
36.0															
32.5		8.08	9.72	10.51	9.76	10.75	10.89	11.23	11.66	10.31	9.66	9.80	12.54	9.85	10.23
30.3	18.69	14.98	12.40	10.50	8.64	6.88	6.45	7.28	8.11	8.66	7.85	7.51	7.35	7.84	8.72
25.5	11.98	8.40	6.70	8.66	5.87	3.54	3.14	5.71	7.83	6.24	5.67	6.58	8.32	6.08	5.71
23.0	12.98	4.22	6.28	7.25									6.14	5.22	4.64
19.0	10.22				6.87				5.74				4.37		
16.5		10.05	11.14	10.67	8.69	9.17	9.87	10.21	7.82	7.98	8.63	8.75	5.62	4.03	3.91
14.5	10.96	14.31	12.23	9.65		8.71	7.26	6.98		4.36	2.75	6.43		3.14	2.96
8.0		5.20	8.22	7.11	8.11	6.24	4.87	5.36	6.50	5.19	4.85	8.31	6.37	6.12	5.76
Total number of band	8	12	13	13	17	17	17	16	17	17	17	14	17	17	17

References

1. Abd El-Wahab, M.A. (2006): The Efficiency of Using Saline and Fresh Water Irrigation as Alternating Methods of Irrigation on the Productivity of Foeniculum vulgare Mill Subsp. Vulgare Var. Vulgare under North Sinai Conditions. Res. J. of Agric. and Biol. Sci., 2(6): 571-577.
2. Abd El-Haleem, A. K.; Kandil, S. A. And Kortam, M. A. (1995): Growth and yield of six wheat cultivars as affected by different levels of chloride salinization. J. of Agic. Sci., Mans. Univ. 20, 117.
3. Abdel-Hamid, A. Khaedr, Mohammad, A. Abbas, Amal, A. Abdel-Wahid, W. Paul Quick and Gaber, M. Abogadallah, 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adoption of Pancratium maritimum L. to salt stress. J. of Experimental Botany, 54(392): 2553-2562.
4. Alam, M., Stuchbury, Z. T., Naylor, R.E.L. and Rashid, M.A. R. (2004): Effect of salinity on growth of some modern rice cultivar. J. Agron., 3:1-10.
5. Al-Rawahy, S.A., J.L. Stroehlein and M. Passarakli, 1992. Dry matter yield and nitrogen. 15, Na+, Cl- and K+ content of tomatoes under sodium chloride stress. J. Plant Nutr., 15: 341-358.
6. Amany A. Abd El–Monem (2007): Polyamines as modulators of wheat growth, metabolism and reproductive development under high temperature stress. Ph.D. Thesis, Ain Shamas Univ., Cairo, Egypt.
7. Amany, A. Bahr (2002): Effect of bio and organic fertilizer on the yield of some mung bean varieties. Egypt. J. Appli. Sci. 17(7), 117.
8. Asik, B. B., Turan, M. A., Celik, H. and Katkat, A. V. (2009): Effects of Humic Substances on Plant Growth and Mineral Nutrients Uptake of Wheat (Triticum durum cv. Salihli) Under Conditions of Salinity Asian Journal of Crop Science
   Research Article 1 (2): 87-95.
9. Bar, Y., A. Apelbaum, U. Kafkafi and R. Goren, 1997. Relationship between chloride and nitrate and its effect on growth and mineral composition of avocado and citrus plants. J. Plant Nutr., 20: 715-731.
10. Barrett-Lennard, E.G., 2003. Interaction between waterlogging and salinity in higher plants: causes, consequences and implications. Plant and Soil, 253: 35-54.
11. Bates L. S., Waldren R. P. and Tear I. D. (1973): Rapid determination of free proline for water – stress studies. Plant and Soil, 39: 205 – 207.
12. Bouchereau A., Aziz A., Larher F. and Murting-Tanguy J. (1999): Polyamines and development challenges recent development. Plant Sci., 140: 103-125.
13. Chapman H. O. and Pratt P. E. (1978): Methods of Analysis for Soils, Plants and Water. Univ. of California Agric. Sci. Priced Publication. 4034. p.50.
14. De Lacerda, C.F., J. Cambraia, M.A. Oliva, H.A. Ruiz and J.T. Prisco, 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ. Exp. Bot., 49: 107-120.
15. Dubois M., Gilles K. A., Hamilton J. K. and Robers P. A. (1956): Colourimetric method for determination of sugars and related substances. Anal. Chem. 28: 350 – 356.
16. Eker, S., Comertpay, G., Konuskan, O., Ulger, A. C., Ozturk, L. and Cakmak, I. (2006): Effect of salinity stress on dry matter production and ion accumulation in hybrid maize varieties. Turk J. Agric. For. 30: 365-373.
17. El- Bassiouny H. M. S. and Bekheta M. A. (2001): Role of putrescine on growth, regulation of stomatal aperture, ionic contents and yield by two wheat cultivars under salinity stress. Egyptian J. Physiol. Sci, 2-3: 235-258.
18. El–Bassiouny, H. M. S.; Mostafa, H. A.; El–Khawas, S. A.; Hassanein, R. A.; Khalil, S. I.; Abd El–Monem, A. A. (2008): Physiological responses of wheat plant to foliar treatments with arginine or putrescine. Austr. J. of Basic and Applied Sci., 2(4): 1390-1403.
19. El-Hindi, K.M. and A.M. El-Ghamry, 2005. Ameliorating the injurious effects of salinity on cherrygold plants using some micro-elements spray. J. Agric. Sci. Mansoura Univ., 30: 4135-4148.
20. El-Karamany, M.F., 2001. Agronomic studies on some exoic mungbean genotypes under Egyptian conditions. Egypt. J. Agron., 23(1): 1-4.
21. El-Karamany, M.F., A.A. Bahr and A.M. Gomaa, 2001. Response of a local and some
22. El-Karamany, M.F., Zeidan, M. S., and Gobarh, M. E. (2003): A comparative study on productivity of some mung bean varieties grown in sandy soil. Egypt. J. Agron. 25 59-67.
23. El-Khimsawy, K. A.; Younis, T. M.; Mohamed, F. A. and Al-Alfy, M. A. (1998): A study on mung bean as a non-traditional feed stuff for broilers. Al-Azhar J. Agric. Res. 28, 101-115.
24. Evans, T.P. and Malmberg, R.L. (1989): Do polyamines have roles in plant development? Ann. Rev. Plant Physiol. Plant Mol. Biol. 40, 235.

25. exotic mungbean varieties to bio - and mineral fertilization. Acta Agronomica Hungarica 49 (3): 251259.
26. Francois, L. E. (1996): Salinity effects on four sunflower hybrids. Agron. J. 88:215-219.
27. Galston, A.W. and Kaur-Sahney (1990): Polyamines in plant physiology. Plant Physiol. 94, 406.
28. Ghoulam, C., A. Foursy and K. Fares, 2002. Effect of saltstress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in fuie sugar beet cultivars. Environ. Exp. Bot., 47: 39-50.
29. Grattan, S.R. and C.M. Grieve, 1999. Salinitymineral nutrient relations in horticultural crops. Scientia Horticulturae, 78: 127-157.
30. Handel E. V. (1968): Direct microdetermination of sucrose. Anal. Biochem. 22, 280.
31. Hassanein R. A. (1977): Effect of certain growth regulators on plant growth and development. Ph.D. Thesis, Ain Shamas Univ., Cairo, Egypt.
32. Hassanein, R. A.; Khalil, S. I.; El–Bassiouny, H. M. S.; Mostafa, H. A. M.; El – Khawas, S. A.; Abd El – Monem, A. A. (2008): Protective role of exogenous arginine or putrescine treatments on heat shocked wheat plant. 1st. International Conference on Biological and Environmental Sciences, Hurghada, Egypt, March 13-16, 2008.
33. Khalil, S. I.; El – Bassiouny, H. M. S.; Hassanein, R. A.; Mostafa, H. A. M.; El – Khawas, S. A. and Abd El – Monem, A. A. (2009): Antioxidant defense system in heat shocked wheat plants previously treated with arginine or putrescine. Austr. J. of Basic and Applied Sci., 1517-1526.
34. Khan, M. A., Qayym, A. and Noor, E. (2007): Assessment of wheat genotypes for salinity tolerance proceedings of 8th. African Crop Sci. Conf. Octo. 27-31., El Minia, Egypt, pp: 75-78.
35. Krishnamurthy R. (1991): Amelioration of salinity effect in salt tolerance rice Oryza sativa L. by foliar application of putrescine., Plant Cell Physiol., 32: 699-703.
36. Lawn, R.J., and C.S. Ahn. 1985. Mungbean(Vigna radiata (L.)Wilczek/Vigna mungo (L.) Hepper). In:R.J. Summerfield and E.H. Roberts (eds.). GrainLegume Crops. Collins, London, England. pp. 584-623.
37. Le Rudulier, D. (2005): Osmoregulation in rhizobia: The key role of compatible solutes. Grain Leg., 42: 18-19.
38. Lea-Cox, J.D. and J.P. Syvertsen, 1993. Salinity reduces water use and nitrate-N use efficiency of citrus. Ann. Bot., 72: 47-54.
39. Mansour M. M. F. and Al – Mutawa M. M. (1999): Stabilization of plasma membrane by polyamines against salt stress. Cytobios 100: 7 – 17.
40. Mass, E. V. and Grieve, C. M. (1990): Spike and leaf development in salt stressed wheat. Crop Sci. 30, 1309.
41. Mo H. and Pua E. C. (2002): Up – regulation of arginine decarboxylase gene expression and accumulation of polyamines in mustard (Brassica juncea) in response to stress. Physiol. Planta. 114: 439 – 449.
42. Mohamedin, A.A.M. Abd El-Kader A.A. and Nadia M. Badran (2006): Response of Sunflower (Helianthus annuus L.) to Plants Salt Stress under Different Water Table Depths Journal of Apllied Sciences Research, 2(12): 1175-1184.
43. MSTAT-C, (1988): MSTAT-C, a microcomputer program for the design, arrangement and analysis of agronomic research. Michigan State University, East Lansing.
44. Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell and Env.* 16, 15-24.
45. Munns, R. (2002): Comparative physiology of salt and water stress. Plant Cell Environ. 25:239-250.
46. Munns, R. (2005): Genes and salt tplerance: bringing them together. New Phytol. 167: 645-663.
47. Munns, R., James, R. A. and Lauchli, A. (2006): Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57: 1025-1043.
48. Muting D. and Kaiser E. (1963): Spectrophotometric method of determining of amino-N in biological materials by means of the ninhydrin reaction. Seyler's Zschr. Physiol. Chem. 332:276.
49. Nassar A. H., El – Tarabily K. A. and Sivasithamparam K. (2003): Growth promotion of bean (Phaseolus vulgaris L.) by a polyamine –producing isolate of Streptomyces griseoluteus. Plant Growth Regul. Kluwer Academic Publishers, Dordrecht, Netherlands 40: (2) 97 – 106.
50. Nou, X., R.A. Bressan, P.M. Hasegawa and J.P. Pardo, 1995. Iron homeostasis in NaCl stress environments. Plant Physiol., 109: 735-742.
51. Nuran Cice and Hűsnű Cakirlar, 2002. The effect of salinity on some physiological parameters in two maize cultivars. Bulg. J. Plant Physiol., 28 (1-2), 66-74.
52. Pessarakli, M., 1991. Dry matter yield, nitrogen-15 absorption and water uptake by green bean under sodium chloride stress. Crop Sci., 31: 1633-1640.
53. Pirie F. G. (1955): Proteins. In Modern Methods of Plant Analysis. edited by (Peach K. and Tracey, M. V.). IV: 23-68 Springer Verlag, Berlin.

54. Reuveni R,, Shimoni M., Karchi Z. and Kuc J. (1992): Peroxidase activity as a biochemical marker for resistance of muskmelon on (Cucumis meb) to Pseudoperonospora cubensis. Phyto Pathol. 82: 749 – 753.
55. Santa – Cruz A., Acosta M., Perez – Alfocea F. and Bolarin M. C. (1997): Changes in free polyamine levels induced by salt stress in leaves of cultivated and wild tomato species. Physiol. Plant. 101: 341 – 346.
56. Sarwat, M.I. and El-Sherif M. H. (2007): Inducing salt tolerance in some rice varieties (Oryza sativa L.) by using some growth regulators. J. Biochem. Environ. Sci., 2: 189-215.
57. Sharma M., Kumar B. and Pandey D. M. (1997): Effect of pre – flowering foliar application of putrescine on ion composition of seeds of chick pea (Cicer arietinum L. cv. H – 82 – 2) raised under saline conditions. Ann. Agri. Bio. Res. 2 (2): 111 – 113.
58. Sharma, P. C. and Gill, K. S. (1994): Salinity induced effect on biomass, yield, yield attributing characters and ionic contents in genotypes of Indian mustard (Brassica juncea). Indian J. Agric. Sci. 64, 785.
59. Shi, D. and Y. Sheng, 2005. Effect of various salt alkaline mixed stern conditions on sunflower seedlings and analysis of their stress factors. Environmental and Experimental Botany, 54: 8-21.
60. Stroganov, B.P. (1962): Physiological basis of the salt tolerance of plants (under different types of soil salinization) Izd. Akad. Nauk. USSR. Moscow.
61. Taffouo V. D.; Kouamou J. K.; Ngalangue L.M.T.; Ndjeudji B.A.N. and Akoa A. (2009): Effects of salinity stress on growth, Ion partitioning and yield of some cowpea (*Vigna unguiculata* L. Walp.) cultivars. Inter. J. of Botany, 5(2): 135-143.
62. Taffouo, V. D., Kenne, M., Fokam Tasse, R., Fotsoe, W.O., Fonkou, T., Vondo, Z. And Amougou, A. (2004): Salt stress variation response in five leguminous plants. Agron. Afr., 16:33-44.
63. Turan, M. A., Kathat, V. and Taban, S. (2007 b): Variations in proline , chlorophyll and mineral elements contents of wheat plant grown under salinity stress. J. Agron., 6:137-141.
64. Turan, M. A., Turkmen, N. and Taban, N. (2007 a): Effect of NaCl on stomatal resistance and proline , chlorophyll, Na, Cl and K concentrations of lentil plants. J. Agron. 6:378-381.
65. Velikova V., Yordanov I. and Edreva A. (2000): Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. Plant Sci., 5: 59 - 66.
66. Walters, D. R. (2000): Polyamines in plant-microbe interactions. Physiol. Mol. Plant Pathol. 57: 137-146.
67. Xu, Y.C., Wang, J., Shan, L., Dong, X. and Li, M. M. (2001): Effect of exogenous polyamines on glycolate oxidase activity and active oxygen species accumulation in wheat seedlings under osmotic stress. Israel J. Plant Sci. 49:173-178.
68. Yemm E. W. and Willis A. J. (1954): The respiration of barley plants.IX. The metabolism of roots during assimilation of nitrogen. New Phytol, 55: 229 – 234.
69. Zadeh H.M. and Naeini, M.B. (2007): Effects of salinity stress on the morphology and yield of two cultivars of canola (*Brassica napus* L.). J. Agron., 6: 409-414.
70. Zeidan, M. S., M.F. El-Karamany and A.A. Bahr, 2001. Response of mungbean varieties to different row spacing under new reclaimed sandy soil. Egypt. J. Agron., 23: 99-110.
71. Zhu, J. K. (2001): Plant salt tolerance. Trends Plant Sci. 6:66-71.
72. Zhu, J. K. (2002): Salt and drought stress signal transduction in plants. Ann. J. Plant Biol., 53: 247-273.

5/1/2010

Emerging trend of urban green space research and the implications for safeguarding biodiversity: a viewpoint

Gairola, S. and M. S. Noresah

School of Distance Education, Universiti Sains Malaysia
11800, MINDEN, Penang, Malaysia
drsanjaygairola@gmail.com, noreshah@usm.my

Abstract: It is now abundantly clear that rapid urbanization is greatly transforming the spatial pattern of urban land use worldwide. Consequently, the resulting losses of urban green space at local to global level are continuously altering urban ecosystems. Recent research on this aspect has stressed on the importance of urban green spaces as well as their losses due to rapid urban growth. The importance of ecosystem services provided by urban green spaces for human well-being is gaining recognition and has been highlighted by most of the recent studies. In this short communication, we discuss the emerging trend of urban green space research and its implications for safeguarding biodiversity in line with the declaration by the United Nations that 2010 to be the International Year of Biodiversity. [Nature and Science 2010;8(7):43-49]. (ISSN: 1545-0740).

Keywords: Urban Green Space, Biodiversity, Ecosystem Services, Urban Dwellers, Sustainable Development

1. Introduction

Biodiversity of urban green spaces is well recognized for the provision of variety of ecosystem services to humanity. Hence green spaces are recognized as one of the most popular resources of the urban ecosystems today. The increasing urbanization and human population growth during recent decades have resulted significant loss of habitats in the urban landscape (Mckinney, 2002) and accompanied by many environmental problems, such as a reduction of green spaces and ecosystem deterioration (Lee *et al*., 2005). In urban areas, the importance of urban green spaces is well highlighted as remnant habitats with high value for biodiversity and because some of rare and endangered species with significant conservation value may present in these habitats. Urban green space includes everything in cities that has the vegetation. Collectively it is sometimes referred to as 'Green Infrastructure', encompassing the entire working landscape in cities that serve roles such as improving air quality, flood protection and pollution control (Girling and Kellett, 2005). Some of the many benefits of urban green spaces are; air and water purification, mitigation of the impact of environmental pollution, carbon sequestration, regulation of microclimate, habitat for urban wildlife, recreational, spiritual and therapeutic value as well as social integration (Miller, 1997; Milton, 2002; Hague and Siegel, 2002). Hence, green space improves the environmental quality of life, urban tourism, active and passive recreations and many other urban ecological functions (Kaplan and Kaplan, 1989; Randall *et al*., 2003). At present, several studies have pointed to urban green spaces as a resource in promoting public health and providing valuable ecosystem services to urban dwellers (Maas *et al*., 2006; Alver, 2006; Jim and Chen, 2008; Rafiee *et al*., 2009; James *et al*., 2009). Monitoring land use changes in the urban environment is an important issue in planning and management and remote sensing and geographic information systems are considered as the most efficient techniques for this type of studies. Using these modern tools, a range of recent studies have focuses on monitoring urban growth pattern, land use and land cover changes, urban green space patterns, and biodiversity conservation in urban areas (for e.g. Herold *et al*., 2003; Jim and Chen, 2003; Bauer *et al*., 2003; Yuan *et al*., 2005; Jim and Chen, 2006a, b; Jensen and Im, 2007; Johari, 2007; Kazmierczak and James, 2008; Faryadi and Taheri, 2009; Rafiee *et al*., 2009; James *et al*., 2009; Chen *et al*., 2009). Considering the fact that urbanization is having enormous impact on the environment at local, regional and global scale (Turner et al., 1990) the trend of studying different aspects of urban green spaces is gaining momentum among urban researchers. Conservation of biological diversity and ecosystem services in urban environment necessitate valuable ecological information which could be incorporated into urban green space planning and management.

2. Urban Green Spaces & Sustainable Development

Presently, urbanization is rapid worldwide and is expected to continue in the coming decades, especially in the developing world where the United Nations Population Fund (UNPF-2007) anticipates 80% of the world's urban communities will be found by 2030 (Beardsley *et al*., 2009). Thus, in order to respond to the idea of sustainability, urban areas have to maintain an internal equilibrium balance between socio-economic and environmental conditions in such a way that the urban system and its dynamics evolve in harmony, internally limiting, and as much as possible low impacts on the natural environment (Barredo and Demicheli, 2003). As far as the roles of urban green spaces in urban environment are considered, they are recognized as key ecological service providers to urban dwellers with multiple functions and also an important pillar of sustainable development. Some authors (e.g. Yli-Pelkonen and Niemelia, 2005; Sandstrom *et al*., 2006) pointed out that the multiple functions of urban green spaces are reasonably well developed, but these are not well integrated into the urban planning, design and management process. Furthermore, reliable and robust approaches to the valuation of urban green spaces that effectively support decision making are often absent (Tyrvainen, 2001; Neilan, 2008). However, under the 'Greenkeys- urban green as a key for sustainable cities' (www.greenkeys-project.net) project, some efforts have been made recently to address the issues of improvement of urban green space systems as a step towards more sustainable cities by developing a 'Pool for Green Space strategies'. Also, a manual on 'Greenkeys @ your city- a guide for urban green quality' was developed under the project which contains suggestions for the green space development in European cities and provide guidance on the preparation and implementation of an urban green space strategy. Subsequently, the key role of urban green space plays for improving the quality of life in urban areas was discussed in the International Conference on Urban Green Spaces (held in Sofia, Bulgaria, 2008). Therefore, there is a continuing need to promote such initiatives for sustainable development of urban landscapes. Gill *et al*. (2007) emphasized that the urban green spaces can play a central role in both climate-proofing cities and in reducing the impacts of cities on climate. Presently, as rapid urbanization is causing losses of even more urban green space across the globe, this may have therefore important implications for future changes in the Earth's climate. Hence, urban green spaces need to be preserved and promoted for future generation as they provide key ecological services.

3. Urban Green Space Research in Malaysia

At present, with the increase in the proportion of global urban population, cities are also expanding spatially and resulting in loss of urban green spaces. Particularly the expansion of residential and commercial land uses towards the periphery of urban areas has been recognized as the main factor in influencing the urban ecosystems (Yuan *et al*., 2005). The negative environmental impacts of urban growth have been demonstrated in developed and developing countries (Chiesura, 2004; Colding, 2007; Theobald *et al*., 2000; Tzoulas *et al*., 2007). Therefore the need of establishing sustainable equilibrium between ecological, social and economic functions of the urban ecosystems has been debated in different forum and continues towards achieving sustainable urban landscapes. In Malaysia, urban population in 2000 was more than 57% of the total population and is projected to reach 70% of the total by 2020 (Department of Statistics, Malaysia 2000, Salleh, 2000). The trends of rapid urbanization are evident in cities such as Kuala Lumpur and Georgetown, Penang with high population density. This increase in urban population in due course will result in a transformation of the physical appearance of many cities in Malaysia (Ghazali, 1999). The need of careful planning and monitoring of urban growth by local and federal authorities of Malaysia has also highlighted by Samat (2006), who pointed out that the changes in land use can generate local, regional or global impact on biodiversity, landscape or living environment. In general some comprehensive studies addresses the issues of urban development, sustainable urban landscapes and urban planning and conservation in Malaysia (e.g. Goh, 1991, 2002; Ghani, 2000; Salleh, 2000; Jenkins and King, 2003; Omar, 2003; Abdullah, 2003; Omar, 2003; Tahir and Roe, 2006; Lee *et al*., 2008). In order to guide economists and urban planners in making a decision pertaining to urban development, Osman *et al*. (2008) developed a framework of understanding urban sprawl and its financial cost in Malaysia. Other researchers applied different approaches to analyze the relationship between urban growth and the impacts on urban environment. Some studies focuses on application of geospatial tools to develop decision support framework for urban environment (Rainis and Noresah, 2004; Noresah, 2006; Samat, 2007; Noresah and Rainis, 2009). And more recently, Tan *et al*. (2009) used an integrated approach of remote sensing to evaluate urban expansion and determine land use changes. Results of this study illustrated that Landsat multi-temporal image could provide an accurate map and detailed descriptions of land cover changes and these findings can be efficiently used for decision-making in land management and policy making. However, studies on urban green spaces have not received much

attention so far. Few studies have been carried out in this area (e.g. Johari, 2007; Hussein, 2006; Mazlina and Ismail, 2007, 2008), however comprehensive studies are still to be conducted in order to achieve sustainable urban landscapes. According to Salleh and Ishak (2002), rapid economic growth and widespread urbanization is deteriorating the Malaysian cities and air and noise pollutions are becoming serious problems in many urban areas. Thus, urban green areas have a vital role to play in the sustainability of towns and cities as they are considered as important parts of natural life support system. The authors also suggested that government must make urban greening mandatory in all development projects and advocated for using local agenda 21 to incorporate sustainable development into local town planning, including tree planting. Urban green space studies have great significance in the near future in accommodating the increasing urban population. Therefore, more studies should be focused on urban green spaces of Malaysian cities. Also getting green space information quickly and accurately can provide a foundation for urban environment, green space system planning and all-level of decision-making. Considering the fact that the understanding of importance of urban green space for obtaining social and ecological sustainability in Malaysian's urban landscapes is essential; such studies therefore would have implications to improve urban environmental quality for people by supporting biodiversity conservation and preserving urban green space resources.

4. Implications for safeguarding biodiversity

The biological diversity or biodiversity was introduced at Rio de Janeiro Earth Summit in 1992 as a major objective in world-wide conservation strategies to ensure conservation and sustainable use of biodiversity. The United Nations General Assembly declared 2010 as the International Year of Biodiversity (IYB), to safeguard the biodiversity and to bring awareness about the significance of biodiversity. Through this, the world is invited to take action in 2010 to safeguard the variety of life on earth: biodiversity (www.cbd.int). This clearly accentuates more and more understanding of biodiversity value and strategic approach to conservation prioritization under global change scenario. The recent biodiversity research revealed that not only natural and semi-natural landscapes can be highly diverse in flora, fauna, and habitats, but that urban and industrial areas also display a wide variety of habitats, communities and organisms (Sukopp, 1998). Hence promoting and preserving biodiversity within urban green spaces is one way to decelerate the rapid rate of biodiversity loss (Alvey, 2006). In urban environment, the urban green spaces offer important harbors for remnant biodiversity (Kong *et al*., 2009). Some authors (Miller, 1988; Duhme and Pauleit, 1998) pointed out that urban biodiversity conservation should receive more attention, as urban areas may contain a rich flora that contributes significantly to biodiversity. Fordham and Brook (2010) suggested that policy makers and resource managers must be armed with the most ecologically 'realistic' projective artillery if the impending biodiversity crisis is to be averted. At present, close to half of the world's population lives in urban centers, and the proportion is likely to grow as an increasing amount of the world's economic activities concentrate in urban centers (Satterthwaite, 2002). The proportion of the world's population living in cities is expected to surpass 65% by 2025 (Schell and Ulijaszek, 1999), and dramatic population increases have been accompanied by intensified urban development. Under these scenarios, there is no doubt that the significance of urban green spaces will increase tremendously with increasing urban population. Among others, the maintenance of biodiversity (i.e. preserving diversity; within species, of species, of ecosystems, and of landscape types in the surrounding countryside) and environmental quality of the urban area (i.e. improvement of the local climate, air quality and decrease of environmental noise) are the main functions of green spaces in cities (Boverket's, 1992), which clearly demand safeguarding biodiversity in urban environment. For conserving urban biodiversity, there must be a feasible and replicable action plan for urban environment as per the geographical condition of the area. Likewise, the urban development authorities should include plantation of multipurpose and high conservation value plant species as a routine activity in their urban action plan. For instance, multipurpose tree species which traps pollutant, having long rotation period, evergreen, and provide shelter to wildlife needs to be promoted. In fact in urban areas, plantation of a multipurpose tree in front or backyard of the house should be mandatory for every household and urban development authorities must maintain a green park in the vicinity of every multistory building/ residential area. This will facilitate in creating new habitats for urban wildlife and increasing opportunities for urban biodiversity, restoring cities degraded areas, regulation of microclimate, improve the environmental quality of life, and eventually achieving sustainable urban landscapes. McKinney (2002) advised that fostering a well informed public may be the most important application of urban ecology. We also suggest that formulating strategy for maintenance and development of urban biodiversity values with improved people's awareness will certainly help in achieving the IYB's

major objective of safeguarding biodiversity and hence safeguarding the life on earth.

Acknowledgement:
The authors would like to thank Universiti Sains Malaysia for all the support and facilities.

Correspondence to:
Sanjay Gairola
Postdoctoral Fellow
School of Distance Education
Universiti Sains Malaysia
11800, MINDEN, Penang, Malaysia
Telephone: +60-142413409
Email: drsanjaygairola@gmail.com

References

[1] Abdullah J. The suburbanization of the Kuala Lumpur Metropolitan Region. Journal of the Malaysian Institute of Planners 2003; 1: 119-126.

[2] Alvey AA. Promoting and preserving biodiversity in the urban forest. Urban Forestry and Urban Greening 2006; 5 (4): 195-201.

[3] Barredo JI, Demicheli L. Urban sustainability in developing countries megacities: modeling and predicting future urban growth in Lagos. Cities 2003; 20(5): 297-310.

[4] Bauer M, Yuan F, Saway K. Multi-Temporal Landsat Image Classification and Change Analysis of Land cover in the Twin Cities (Minnesota) Metropolitan Area. Workshop on the Analysis of multi-temporal remote sensing images, Italy. 2003.

[5] Beardsley K, Thorne JH, Roth NE, Gao S, McCoy MC. Assessing the influence of rapid urban growth and regional policies on biological resources. Landscape and Urban Planning 2009; 93: 172-183.

[6] Boverket. Storstadsuppdraget. En förstudie om storstädernas miljö (A Preliminary Study of the Environment in Big Cities). Karlskrona, The National Board of Housing, Building and Planning. (In Swedish) 1992.

[7] Chen B, Adimo AO, Zhiyi B. Assessment of aesthetic quality and multiple functions of urban green space from the user's perspective: the case of Hangzhou Flower Garden, China. Landscape and Urban Planning 2009; 93: 76-82.

[8] Chiesura A. The role of urban parks for the sustainable city. Landscape and Urban Planning 2004; 68: 129-138.

[9] Colding J. Ecological land-use complementation for building resilience in urban ecosystems. Landscape and Urban Planning 2007; 81: 46-55.

[10] Department of Statistics, Malaysia. Laporan kiraan permulaan banci penduduk dan perumahan 2000.

[11] Duhme F, Pauleit S. A landscape ecological masterplan for the city of Munich. In: Riley JO, Page SE. eds. Habitat Creation and Wildlife Conservation in Urban and Post-Industrial Environments. Packard Publishing, Chichester. 1998.

[12] Faryadi Sh, Taheri Sh. Interconnections of urban green spaces and environmental quality of Tehran. International Journal of Environmental Research 2009; 3(2): 199-208.

[13] Fordham DA, Brook BW. Why tropical island endemics are acutely susceptible to global change. Biodiversity and Conservation 2010; 19: 329-342.

[14] Ghani S. Urbanization and regional development in Malaysia, Kuala Lumpur. Utusan Publications & Distributors Sdn. Bhd. 2000

[15] Ghazali S. Socio-Economic Changes in the Peri Urban Villages in Penang, Malaysia, Ph.D Thesis, University of Leeds. 1999

[16] Gill SE, Handley JF, Ennos AR, Pauleit S. Adapting cities for climate change: the role of the green infrastructure. Built Environment 2007; 33: 115–133.

[17] Girling C, Kellet R. Skinny Streets and Green Neighborhoods: Design for Environment and Community. Washington, Island Press. 2005

[18] Goh BL. Urban planning in Malaysia: history, assumptions and issues. Tempo Publishing (M) Sdn Bhd. Kuala Lumpur. 1991

[19] Goh BL. Rethinking modernity: state, ethnicity, and class in the forging of a modern urban Malaysia. In: Wee CJWL, ed. Local cultures and the New Asia: the state, culture, and capitalism in Southeast Asia. Singapore. Institute of Southeast Asian Studies. 2002; 184-216.

[20] Hague M, Siegel N. Municipal parks in New York City: Olmsted, Riis, and the transformation of the urban landscape, 1858–1897. In: Backhaus G, Murungi J, eds. Transformations of Urban and Suburban Landscapes: Perspectives from Philosophy, Geography, and Architecture. Lexington Books, Lanham, MD. 2002; 153–191.

[21] Herold M, Goldstein N, Clarke K. The spatiotemporal from of urban growth: measurement, analysis and modeling. Remote Sensing of Environment 2003; 86: 286-302.

[22] Hussein H. Barrier-free park design for the disabled persons: a case study of the KLCC Park. Journal of Design and the Built Environment 2006; 2(1): 57-67.

[23] James P, Tzoulas K, Adams MD, Barber A, Box J, Breuste J, Elmqvist T, Frith M, Gordon C, Greening KL, Handley J, Haworth S, Kazmierczak AE, Johnston M, Korpela K, Moretti M, Niemelä J, Pauleit S, Roe MH, Sadler JP, Ward Thompson C. Towards an integrated understanding of green space in the European built environment. Urban Forestry and Urban Greening 2009; 8 (2): 65-75.

[24] Jenkins G, King VT. Heritage and development in a Malaysian city: George Town under threat?. Indonesia and Malay World 2003; 31 (89): 44-57.

[25] Jensen J, Im J. Remote sensing change detection in urban environments: Geo-Spatial Technologies in Urban Environment, 2nd edit. 2007; 7-31.

[26] Jim CY, Chen SS. Comprehensive greenspace planning based on landscape ecology principles in compact Nanjing city, China. Landscape and Urban Planning 2003; 65: 95–116.

[27] Jim CY, Chen WY. Perception and attitude of residents towards urban green spaces in Guangzhou (China). Environmental Management 2006a; 38 (3): 338–349.

[28] Jim CY, Chen WY. Recreation-amenity use and contingent valuation of urban green spaces in Guangzhou, China. Landscape and Urban Planning 2006b; 75 (1–2): 81–96.

[29] Jim CY, Chen WY. Pattern and divergence of tree communities in Taipei's main urban green spaces. Landscape and Urban Planning 2008; 84: 312–323.

[30] Johari J. Assessing the ecological quality of urban green spaces: A case study of Kuala Lumpur City. Postgraduate Conference 2007, Institute of Geography University of Edinburgh, 19-20 March, 2007.

[31] Kaplan R, Kaplan S. The experience of nature: A psychological perspective. Cambridge, Cambridge University Press. 1989

[32] Kazmierczak AE, James P. Planning for biodiversity conservation in larger urban areas: the ecological framework for Greater Manchester. In: Breuste J, ed. Ecological Perspectives of Urban Green and Open Spaces. Salzburger Geographische Arbeiten Band 2008; 42: 129-150.

[33] Kong F, Yin H, Nakagoshi N, Zong Y. Urban green space network development for biodiversity conservation: Identification based on graph theory and gravity modeling. Landscape and Urban Planning 2009; 95 (1-2): 16-27.

[34] Lee KJ, Han BH, Choi JW. A study on the characteristics of urban ecosystems and plans for the environment and ecosystem in Gangnam-gu, Seoul, Korea, Landscape and Ecological Engineering 2005; 1: 207-219.

[35] Lee ML, Lim MY, Nor'Aini Y. Strategies for urban conservation: a case example of George Town, Penang. Habitat International 2008; 32: 293-304.

[36] Maas J, Verheij RA, Groenewegen PP, de Vries S, Spreeuwenberg P. Green space, urbanity, and health: how strong is the relation? Journal of Epidemiology and Community Health 2006; 60: 587-592.

[37] Mazlina M, Ismail S. Green infrastructure as network of social spaces for well-being of urban residents: a review. In: Proceedings of International Conference on Built Environment in Developing Countries. Universiti Sains Malaysia. 2007

[38] Mazlina M, Ismail S. Green infrastructure as network of social spaces for well-being of urban residents in Taiping, Malaysia. Jurnal Alam Bina, Jilid 2008; 11(2): 1-18.

[39] Mckinney ML. Urbanization, biodiversity, and conservation. BioScience 2002; 52: 883-890.

[40] Miller RW. Urban Forestry: Planning and Managing Urban Greenspaces. Prentice-Hall, Englewood Cliffs, NJ. 1988; 25.

[41] Miller RW. Urban forestry: Planning and managing urban green spaces. 2nd (Ed.) New Jersey: Prentice Hall, Inc., Upper Saddle River. 1997

[42] Milton K. Loving Nature: Towards an Ecology of Emotion. Routledge, New York. 2002

[43] Neilan C. CAVAT: Capital asset value for amenity trees. Revised edition. London Tree Officers Association. 2008

[44] Noresah MS, Ruslan R. Modelling urban spatial structure using geographically weighted regression. In: Anderssen RS, Braddock RD, Newham LTH, eds. 18th World IMACS Congress and MODSIM09 International Congress on Modelling and Simulation. Modelling and Simulation Society of Australia and New Zealand and International Association for Mathematics and Computers in Simulation. ISBN: 978-0-9758400-7-8, 2009: 1950-1956.

[45] Noresah MS. Modelling Land Use Impacts of New Highways: The Malaysian North-South Expressway. A PhD Dissertation. University of Manchester. United Kingdom. 2006: 246. *(UNPUBLISHED)*

[46] Omar DB. Sustainability and new town development in Peninsular Malaysia. Proceedings of Conference on Sustainable Cities, Skiathos, Greece. 1-3 October, 2003.

[47] Omar D. Urbanization, urban fringe development and the quality of life: evidence from new town development of Peninsular Malaysia. Paper presented at Adelaide Planning Congress, 2003.

[48] Osman SB, Nawawi AH, Abdullah J. Urban sprawl and its financial cost: a conceptual framework. Asian Social Science Journal 2008; 4(10): 39-50.

[49] Rafiee R, Mahiny AS, Khorasani N. Assessment of changes in urban green spaces of Mashad city using satellite data. International Journal of Applied Earth Observation and Geoinformation 2009; 11: 431–438.

[50] Ruslan R, and Noresah M.S, Modelling urban land use dynamics using GIS and Markov Chain Model. Geoinformation Science Journal 2004; 4(1): 72-81

[51] Randall TA, Churchhill CJ, Baetz BW. A GIS - based decision support system for neighborhood greening. Environment and Planning B: Planning and Design 2003; 30: 541-563.

[52] Salleh G. Urbanization and regional development in Malaysia. Kuala Lumpur, Utusan Publications & Distributors Sdn. Bhd. 2000

[53] Salleh MN, Ishak MY. Greening Our Cities: Challenges and Opportunities. Paper presented at the 18th EAROPH World Planning Congress, Kuala Lumpur, Malaysia. 07-10 October, 2002

[54] Samat N. Characterizing the scale sensitivity of the cellular automata simulated urban growth: a case study of the Seberang Perai region, Penang state, Malaysia. Computers, Environment and Urban Systems 2006; 30(6): 905-920.

[55] Samat N. Integrating GIS and cellular automata spatial model in evaluating urban growth: prospective and challenges. Jurnal Alam Bina Jilid 2007; 9 (1): 79-93.

[56] Sandstrom UG, Angelstam P, Khakee A. Urban comprehensive planning: identifying barriers for the maintenance of functional habitat networks. Landscape and Urban Planning 2006; 75: 43-57.

[57] Satterthwaite D. Coping with Rapid Urban Growth. Royal Institution of Chartered Surveyors, London. 2002

[58] Schell LM, Ulijaszek SJ. Urbanism, Health and Human Biology in Industrialized Countries. Cambridge University Press, Cambridge. 1999

[59] Sukopp H. Urban ecology—Scientific and practical aspects. In Breuste J, Feldmann H, Uhlmann O, eds. Urban Ecology Berlin, Springer. 1998; 3–16.

[60] Tahir OM, Roe MH. Sustainable urban landscapes: making the case for the development of an improved management system. International Journal on Sustainable Tropical Design Research and Practice 2006; 1 (1): 17-24.

[61] Tan KC, Lim SH, MatJafri MZ, Abdullah K. Landsat data to evaluate urban expansion and determine land use/land cover changes in Penang Island, Malaysia. Environ Earth Sci, DOI 10.1007/s12665-009-0286-z. 2009

[62] Theobald DM, Hobbs NT, Bearly T, Zack JA, Shenk T, Riebsame WE. Incorporating biological information in local land-use decision making; designing a system for conservation planning. Landscape Ecology 2000; 15: 35-45.

[63] Tyrvainen L. Economic valuation of urban forest benefits in Finland. Journal of Environmental Management 2001; 62: 75-92.

[64] Tzoulas K, Korpela K, Venn S, Yli-Pelkonen V, Kazmierszak A, Niemela J. Promoting ecosystem and human health in urban areas using green infrastructure: a literature review. Landscape and Urban Planning 2007; 81: 167-178.

[65] Turner II BL, Clark WC, Kates RW, Richards JF, Mathews JT, Meyer WB. The Earth as Transformed by Human Action: Global and Regional Changes in the Biosphere over the Past 300 Years. Cambridge University Press with Clark University, Cambridge. 1990

[66] United Nations Population Fund (UNPF) State of world population 2007: unleashing the potential of urban growth. From http://www.unfpa.org/swp/.2007

[67] Yli-Pelkonen V, Niemela J. Linking ecological and social systems in cities: urban planning in Finland as a case. Biodiversity and Conservation 2005; 14: 1947-1967.

[68] Yuan F, Sawaya KE, Leoffelholz CB, Bauer ME. Land cover classification and change analysis of the twin cities (Minnesota) metropolitan area by multitemporal Landsat remote sensing. Remote Sensing of Environment 2005; 98: 317– 328.

[69] www.cbd.int (Accessed 02.02.2010).

[70] www.greenkeys-project.net (Accessed 03.02.2010).

04/26/2010

Effect of location and growth season on the productivity and quality of some range plants in Wadi Halazien in the North Western Coast in Egypt

El-Zanaty, R. I. A.⋆, A. A. Abdel-Hafez⋆, k. I. Abdel-Gawad⋆, M. H. M. El-Morsy** and H. M. A. Abusaief⋆

⋆Faculty Agriculture, Cairo University, Egypt
**Desert Research Center, Cairo, Egypt

Abstract: This study was conducted during spring and autumn seasons of 2007 and 2008 in wadi Halazien, at the North West Coast region of Matruh governorate in Egypt. The aim of this investigation was to survey and classify natural plants species and to study the effect of location and seasonal changes on range productivity and quality of pasture species. Seventy-two plant species belong to 29 families (41.67 % annuals, 1.39 % biennials and 56.94 % perennials) were found. *Polygonum equisetiforme* in top, *Deverra tortuosa* in ridge, *Chiliadenus candicans* in bed 1 and *Carduncellus eriocephalus* in bed 2 gave the highest abundance in spring 2007. Whereas, *Gymnocarpos decandrus* in top, *Leopoldia comosa* in ridge and *Carduncellus eriocephalus* in bed 1 and 2 gave the highest one in spring 2008. Total abundance in bed 2 significantly surpassed other sites in both years. Species richness and Simpson's index of diversity in spring increased than in autumn in all sites of both years. *Lycium shawii* in top, *Euphorbia dendroides* in ridge, and *Thymelaea hirsuta* in bed 1 gave the highest importance value in spring 2007. Generally, plant species of family *Caryophyllaceae* in top, *Euphorbiaceae* in ridge, *Thymelaeaceae* in bed 1 and *Apiaceae* in bed 2 gave the highest fresh and dry yields in spring 2007, while, *Thymelaeaceae* in top, ridge and bed 2 and *Cistaceae* in bed 1 gave the highest ones in spring 2008. *Gymnocarpos decandrus* in top, *Euphorbia dendroides* in ridge, *Thymelaea hirsuta* in bed 1 and *Atriplex nummularia* in bed 2 gave the highest fresh and dry yields in spring 2007. While, *Thymelaea hirsuta* in top, *Gymnocarpos decandrus* in ridge, *Fumana thymifolia* in bed 1 and *Carduncellus eriocephalus* in bed 2 had the highest ones in spring 2008. Seasonal fresh and dry yields in bed 2 significantly exceeded other sites in both years. *Fabaceae* gave the highest CP % and DCP % at all sites, except in ridge *Brassicaceae* gave the highest ones in spring 2008. *Erodium crassifolium* in top, *Achillea santolina* in ridge, *Lotus polyphyllus* in bed 1 and 2 gave the highest CP % and DCP % in spring 2008. Crude protein % and DCP % in spring significantly exceeded it in autumn at all sites in both years. Abundance, fresh and dry yields had a positive correlation with precipitation, and a negative correlation with temperature. [Nature and Science 2010;8(7):50-70]. (ISSN: 1545-0740).

Key words: The productivity; Wadi Halazien; North Western Coast.

Introduction

The Northern coast region of Egypt extends around 1000 km along the Mediterranean sea and 30 km inland. This region is characterized with an arid Mediterranean climate that has a limited rainfall. Average annual rainfall over 10 years from 1999 to 2008 was 115 mm/year of meteorological authority in Matruh. The natural range considered the basic source of animal feed in the Egyptian deserts. Due to poor management and environmental impacts, the native ranges are deteriorated and are seriously depleted. Range production depends on various factors as climate, soil, vegetation structure, type and intensity of management.

The vegetation survey of an area is of great importance for any type of agricultural development plan. Plant collection and identification are the starting point for any range ecology investigation. Studies of the individual plant species include the evaluation of native range plants since species are essential for possible use in range improvement. The wadi is typical for agricultural use (rain-fed farming), in addition to rangeland plants for grazing animals (sheep, goats and camels) in the range sector of the wadi (agro-pastoral system) and water harvesting practices by stone dams across the stream (Gab Allah, 2006). In Egypt, the general differences of species richness and diversity communities affected mainly by the differences of soil texture in wadi El-Arousia in Sinai (El-Khouly and Fakhry, 1999). In wadi El-Washka, Kulaib (2008) found the highest abundance in spring than autumn and in wadi bed than the others sites in wadi El-Washka. Gab Allah (2006) and Abdel-Gawad *et al.* (2009) found that plant species of families *Poaceae* and *Asteraceae* had the highest importance value (IV) in both years in wadi El-Ramla and El-Washka, respectively. Abdel-Gawad *et al.* (2009) found that *Thymelaea hirsuta* gave the highest fresh and dry yields in spring and autumn in both years in wadi El-Washka. El-Morsy (2002) and Abdel-Gawad *et al.* (2009) concluded that crude

protein of plants grown in wadi bed exceeded it in ridge in all growing seasons in wadi Mehgun, Magid and El-Washka, respectively. The aim of this study was to survey, classify and study the natural vegetation in two locations during two seasons.

2. Materials and Methods

This study was conducted at the North West Coast of Egypt (Figure 1) in Matruh governorate. Vegetation characteristics were taken in spring and autumn of 2007 and 2008.

The aim of this investigation was to survey, classify natural plant species and study the effect of seasonal changes on range productivity, as well as determination of quality of surveyed plant species during different seasons to assist in the evaluation of a suitable vegetation community.

Wadi Halazien is a rocky wadi located about 40 km west of Matruh city at latitudes of 31° 25' 21" N and longitudes of 26° 51' 43" E. Four sites (wadi top, wadi ridge, wadi bed one and bed two) were studied. Sites altitudes were about 80, 50, 30 and 10 m respectively, by apparatus GPS12xL.

During this study, the monthly variations of different climatic factors were recorded (Table 1). Climatologically, this area is classified as arid with mild winter and warm summer (UNESCO, 1977). The distribution of main annual rainfall in Egypt shows a maximum rate over the Mediterranean coast with a rapid decrease toward the south. The total amount percentage of rainfall was 75.8 and 95 mm before cutting in April 2007 and 2008, and 1 and 13 mm in autumn before cutting in October and November in 2007 & 2008 respectively. The monthly mean values of relative humidity were relatively high in summer months. The maximum values of relative humidity were 75 and 66.53 % in August of 2007 & 2008, respectively, but the minimum values were 60.33 and 53.03 % in March and April of 2007 and 2008, respectively.

Fig. 1. Map of the Western Mediterranean sea coastal region of Egypt indicating the location of study area.

Table 1. Monthly averages of climatic factors recorded in Marsa Matruh city during 2007 and 2008*.

Periods / Month	2007						2008					
	Air temperature (°C)			WS** ms^{-1}	RH %	P mm	Air temperature (°C)			WS ms^{-1}	RH %	P mm
	Max.	Min.	Mean				Max.	Min.	Mean			
January	18.17	10.50	13.80	4.17	69.67	18.1	17.06	10.12	13.59	4.53	63.72	53
February	18.47	10.57	14.43	5.43	69.67	31.5	17.20	9.57	13.08	4.91	61.85	24
March	20.93	12.03	16.30	5.07	60.33	5.3	22.98	12.40	17.58	4.91	57.74	2

April	20.80	13.70	17.30	4.90	68.67	2	24.33	14.00	18.83	4.73	53.03	1
May	25.80	17.50	21.45	4.80	70.00	0	25.51	16.19	20.77	3.90	56.35	0
June	27.97	20.13	24.23	5.03	70.67	0	29.13	19.97	24.63	4.32	62.33	0
July	30.07	21.37	26.03	4.83	73.00	0	29.52	22.44	25.78	5.07	63.99	0
August	30.40	22.23	26.57	4.07	75.00	0	30.26	22.01	26.06	3.90	66.53	0
September	28.87	20.97	25.20	3.23	67.00	1	29.93	21.33	25.43	4.78	54.93	0
October	27.73	17.80	22.97	3.03	67.67	0	26.55	17.42	21.97	4.07	57.23	13
November	24.63	13.90	18.73	3.43	63.33	11	23.97	14.27	19.03	3.41	56.40	0
December	20.17	10.70	15.23	4.00	63.00	7.9	20.12	11.76	15.79	3.22	61.32	15
Annual	24.50	15.95	20.19	4.33	68.17	76.8	24.71	15.96	20.21	4.31	59.62	108

***Source: Meteorological Authority, Cairo.**
****WS: Wind speed, RH: Relative humidity, P: Precipitation.**

Generally, soil texture in all the sites of wadi Halazien was sandy loam, except wadi ridge was sandy. The highest percentage of silt was found in wadi bed 1. However, the highest percentage of clay was found in wadi bed 2. Soil samples were collected (0-30 cm) from the studied range area of wadi Halazien. Mechanical analysis was conducted using the international pipette method (Table 2). Chemical determinations of the soil saturated extract were done according to Chapman and Pratt (1961) (Table 3).

Table 2. Mechanical analysis of soil recorded in studied area.

Studied area		Particle-size distribution (%)				Soil texture
		Coarse Sand	Fine Sand	Silt	Clay	
Wadi Halazien	**Top**	28.70	37.85	22.34	11.11	Sandy loam
	Ridge	29.10	63.00	0.80	3.90	Sandy
	Bed one	15.20	20.10	50.00	12.00	Sandy loam
	Bed two	13.00	22.10	48.00	15.00	Sandy loam

The chemical characteristics of the studied soil samples in different sites are shown in Tables (3a) and (3b). The pH value varied from 7.6 to 7.9 in wadi Halazien. The lowest percentage of $CaCO_3$ formed in wadi bed 2 and low salinity.

Table 3a. Some chemical properties of soil recorded in studied area.

Studied area		pH	EC Mmhos cm^{-1}	Organic matter %	Saturation %	Organic carbon %
Wadi Halazien	**Top**	7.9	--	--	--	--
	Ridge	7.6	0.49	0.06	35	0.02
	Bed 1	7.9	3.40	0.12	45	1.22
	Bed 2	7.8	1.30	0.90	45	0.52

Table 3b. Chemical properties of soil recorded in studied area.

Studied area		Cations mequivalent L^{-1}					Anions mequivalent L^{-1}			$CaCO_3$ %
		P^{+++}	K^{+}	Na^{+}	Mg^{++}	Ca^{++}	So_4^{--}	Cl^{-}	HCO_3^{-}	
Wadi Halazien	**Top**	--	0.78	5.26	15.33	2.86	0.32	9.16	--	33.73
	Ridge	2.30	0.31	2.30	2.60	0.90	2.40	3.12	1.16	20.1
	Bed 1	3.60	0.39	7.48	10.98	15.15	18.72	14.73	0.55	17.97
	Bed 2	2.50	0.44	3.42	2.59	6.60	7.83	4.40	0.82	13.36

The species were identified and classified according to family. Species identified primarily in the field for the known plants species, whereas the unknown plants were pressed, mounted and labeled professionally, and were taken to herbarium of Faculty of Science at Cairo University of Egypt for accurate identification. The identification was done according to Boulos (1999, 2000, 2002 & 2005). List and clipping quadrate (1m x 1m) (Ibrahim, 1995) and Reiad *et al.* (1996 b) with 48 replicates (12 replicates in each site, *i.e.* top, ridge, bed 1 and bed 2) were used randomly in wadi Halazien, during each season over the two years. Plant frequency, density and coverage were estimated. Shoot parts of annual plants and new growths of perennials for each species

were clipped for fresh and dry foliage yields (Fresh and dry weight in g m^{-2} were estimated). Vegetation measurements calculated according to Ambshat (1982) as follows:

Abundance the total number of individuals of each species in the total area sampled.

Species diversity, which sometime called species heterogeneity, is a characteristic unique to the community level of the biological organization. It is an expression of community structure. It estimated as the following (Simpson, 1949):

Species richness the number of species in studied area.

$$\text{Diversity} = \frac{\sum_{i=1}^{M} n_i (n_i - 1)}{N(N - 1)}$$ M = species, which we will label as species 1, 2, …, M.

n_i = individuals in species i. or the total number of organisms of a particular species.

N = the total number of organisms of all species.

Simpson's index of diversity = 1 - D

Iindex of diversity increases diversity decreases.

The importance value was determined according to Ludwig and Reynolds (1988) as follows:

Thus: Importance value = R.F. + R.D. + R.C.

$$\text{Frequency (\%)} = \frac{\text{Number of sampled in which species occurs}}{\text{Total number of sampled}} \text{ X } 100$$

$$\text{Density } (m^{-2}) = \frac{\text{Number of individual species}}{\text{Total area (in units)}}$$

$$\text{Coverage (\%)} = \frac{\text{The area occupied by the species (in unit)}}{\text{The whole studied area (in unit)}} \text{ X } 100$$

$$\text{Relative frequency (R.F. \%)} = \frac{\text{Frequency for a species}}{\text{Total frequency for all species}} \text{ X } 100$$

$$\text{Relative density (R.D. \%)} = \frac{\text{Density for a species}}{\text{Total density for all species}} \text{ X } 100$$

$$\text{Relative coverage (R.C.\%)} = \frac{\text{Coverage for a species}}{\text{Total coverage for all species}} \text{ X } 100$$

Shoot parts of annuals and new growths of perennials for each species clipped and weighed for each plant species in each quadrates to calculate fresh foliage yield (g m^{-2}). Each species cleaned and dried in an oven at 65 °C to a constant weight to calculate dry forage yield (g m^{-2}). Crude protein percentage (CP %) was analyzed based on dry yield using modified Micro-Kjeldahl method according to A.O.A.C. (2005). Digestible crude protein percentage (DCP %) was estimated according to Demarquilly's equation, DCP =0.93 CP - 3.52, mentioned by De Ridder *et al.* (1982). This equation is only valid in the case of CP > 3.81 %.

Data were analyzed by Fully Nested analysis (Hierarchical classification) according to Steel *et al.* (1997), using SAS 9 program (1988) and MINITAB 14 program at probability 5 %. Before analysis, transformation for data and then normality distribution in each trait were checked out by Jarque-Bera test using program PAST 1.8 (Hammer *et al.* 2001). Treatment means compared at 5 % level of significance by LSD test. Correlation coefficient between temperature, precipitation and relative humidity and vegetative traits were estimated.

3. Results and Discussion

Botanical composition

Figure (2) and Table (4) show the floristic analysis of the recorded survey and classification included the individual scientific names of species, vernacular name, life duration for each species at different seasons of wadi Halazien in 2007 and 2008. Also, Fig. 3 show the sites of wadi. Natural vegetation contained 72 plant species belong to 29 families. Out of the existing identified 15 species of *Asteraceae*, 14 species of *Poaceae*, 6 species of *Fabaceae*, 4 species of each *Brassicaceae* and *Chenopodiaceae*, 3 species of each *Caryophyllaceae* and *Cistaceae*, 2 species *Lamiaceae*, 1 species for each the other families. Shahba (1994) recorded that species of *Chenopodiaceae* had the highest contribution of the total flora followed by *Asteraceae*, *Poaceae* and *Fabaceae* in wadi Habis. While, in wadis EL-Ramla and El-Washka, *Asteraceae* and *Fabaceae* families were the most important followed by *Poaceae*, *Brassicaceae*, *Chenopodiaceae* and *Caryophyllaceae* (Gab Allah, 2006 and Abdel-Gawad *et al.* 2009).

Fig. (2) Shows that species of *Asteraceae* 20.83 %, species of *Poaceae* 19.44 %, species of *Fabaceae* 8.33 %, *Brassicaceae* or *Chenopodiaceae* 5.56 % and *Caryophyllaceae* or *Cistaceae* 4.17 %.

Life duration of plant species recorded in wadi Halazien 41.67 % of species was annuals, 1.39 % was biennial and 56.94 % was perennials. El-Morsy (2002) and Abdel-Gawad *et al.* (2009) found similar trend in Mehgun and El-Washka wadis, respectively. Whereas, Shahba (1994) and Gab Allah (2006) showed that the annual was more than perennial in wadis Habis and El-Ramla, respectively.

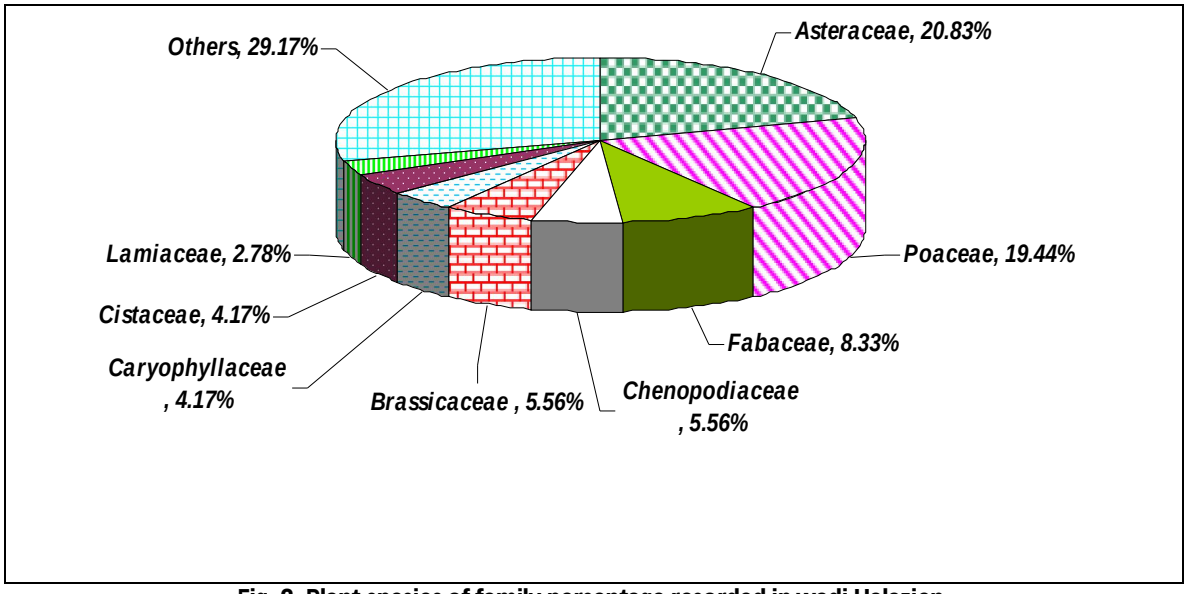


Fig. 2. Plant species of family percentage recorded in wadi Halazien.

Table 4. Botanical composition of plant species recorded in wadi Halazien during spring and autumn seasons in 2007 and 2008.

Family name	Scientific name	Vernacular name	Life duration
Alliaceae	*Allium roseum*	Toam	Per.*
Amaranthaceae	*Amaranthus viridis*	Urf El- Deek	Ann.
Apiaceae (*Umbelliferae*)	*Deverra tortuosa*	Qozzaah	Per.
Araceae	*Arisarum vulgare*	Reinish	Per.
Asparagaceae	*Asparagus stipularis*	Aqool El-Gabal	Per.
Asphodelaceae	*Asphodelus aestivus*	Basal El–Onsal	Per.
Asteraceae (*Compositae*)	*Achillea santolina*	Bisherrn	Per.
	Anacyclus monanthos	Sorret El-Kabsh	Ann.
	Carduncellus eriocephalus	Kharshoof	Per.
	Carduus getulus	Shoak	Ann.
	Centaurea calcitrapa	Shook	Ann.
	Chiliadenus candicans	Zater El-Homar	Per.
	Hyoseris radiata ◄	UnKnow	Per.
	Onopordum alexandrinum ◄	Shoak El-Hanash	Per.
	Picris asplenioides	Seraghah	Ann.

	Reichardia tingitana	Libbein	Ann.
	Scorzonera undulate	Dabbah	Per.
	Senecio coronopifolius	Moreir	Ann.
	Silybum marianum	Shoak El–Gamal	Bien.
	Leontodon tuberosus	Houdaan	Per.
	Urospermum picroides	Galawein	Ann.
Azollaceae	*Anogramma leptophylla* ◄	UnKnow	Ann.
Brassicaceae (*Cruciferae*)	*Didesmus bipinnatus*	Lislis	Ann.
	Diplotaxis acris	Harra	Ann.
	Matthiola longipetala	Shaqaara	Ann.
	Zilla spinosa	Sill	Per.
Capparaceae	*Capparis spinosa* ◄	Abbar	Per.
Caryophyllaceae	*Gymnocarpos decandrus*	Garad or Tashash	Per.
	Paronychia argentea ◄	Farsh EL-Ard	Per.
	Silene succulenta	Khobezyet El – bahr	Per.
Chenopodiaceae	*Atriplex nummularia*	Qataf	Per.
	Haloxylon salicornicum	Rimth	Per.
	Noaea mucronata	ShoakEl-Hanash	Per.
	Salsola villosa ◄	Salsola	Per.
Cistaceae	*Fumana thymifolia*	Unknown	Per.
	Helianthemum lippii	Qoseib or El-oad	Per.
	Helianthemum vesicarium	Raal	Per.
Cyperaceae	*Cyperus rotundus*	Se'd	Per.
Euphorbiaceae	*Euphorbia dendroides*	Omm El-Laben	Per.
Fabaceae (*Leguminosae*)	*Hippocrepis cyclocarpa*	Umm dawara	Ann.
	Lotus polyphyllus	Nafal Haf El-Teir	Per.
	Medicago Arabica ◄	Berseem shogairi	Ann.
	Medicago polymorpha	Kert	Ann.
	Melilotus siculus	Handaqooq helow	Ann.
	Retama raetam	Raetam	Per.
Geraniaceae	*Erodium crassifolium*	Timmeir	Per.
Hyacynthaceae	*Leopoldia comosa*	Bosseil	Per.
Lamiaceae (*Labiatae*)	*Phlomis floccose*	Zeheira	Per.
	Salvia aegyptiaca	Zaeta	Per.
Peganaceae	*Peganum harmala*	Harmel	Per.
Poaceae (*Gramineae*)	*Aegilops kotschyi*	Shaer El faar	Ann.
	Avena fatua	Zammeyr	Ann.
	Bromus madritensis ◄	Khafoor	Ann.
	Bromus rubens	Deil El talab	Ann.
	Cutandia dichotoma	Samma	Ann.
	Cynodon dactylon	Nigeel	Per.
	Hordeum marinum	Shaeer barri	Ann.
	Lamarckia aurea	Unknown	Ann.
	Lygeum spartum	Halfa	Per.

(Continued)

Table 4. (Continued)

Family name	Scientific name	Vernacular name	Life duration
	Lolium perenne ◄	Sammah	Per.
	Lophochloa cristata	Deal elcoat	Ann.
	Panicum turgidum ◄	Thomaam	Per.
	Phalaris minor	Shaer El- faar	Ann.
	Poa annua	Qamh El-Asafeer	Ann.
Polygonaceae	*Polygonum equisetiforme*	Qordaab	Per.
Primulaceae	*Anagallis arvensis* ◄	Ain El qott	Ann.
Ranunculaceae	*Adonis dentata*	Naab EL-gamal	Ann.
Resedaceae	*Reseda decursiva*	Rigl El-ghraab	Ann.
Solanaceae	*Lycium shawii*	Awsage	Per.
Thymelaeaceae	*Thymelaea hirsuta*	Methanan	Per.
Urticaceae	*Urtica urens*	Harraqa	Ann.
Zygophyllaceae	*Fagonia scabra*	Shokaa	Per.

***Ann.=Annual, Per.=Perennial, Bien.=Biennial. ◄ These species cannot found in list and clipping quadrate.**

Fig. 3. Wadi Halazien

Abundance

Results in Table (5) show the abundance of plant species in wadi Halazien varied from plant species to another. *Poaceae* in top and bed 1, *Apiaceae* in ridge and *Asteraceae* in bed 2 gave the highest abundance in spring 2007. Average of the highest abundance in spring 2008 of plant species was recorded for *Poaceae* in top, *Brassicaceae* and *Hyacynthaceae* in ridge, *Polygonaceae* in bed 1 and *Hyacynthaceae* in bed 2. While, *Apiaceae* and *Caryophyllaceae* in top, *Thymelaeaceae* and *Caryophyllaceae* in ridge, *Poaceae* and *Asteraceae* in bed 1 and *Asteraceae* and *Thymelaeaceae* in bed 2 gave the highest abundance in autumn 2007 and 2008, respectively.

The highest abundance was attained among perennial species such as *Polygonum equisetiforme* (Fig. 4) and *Gymnocarpos decandrus* (Fig. 5) in top, *Deverra tortuosa* (Fig. 6) and *Leopoldia comosa* in ridge, *Chiliadenus candicans* (Fig. 7) and *Carduncellus eriocephalus* (Fig. 8) in bed 1, during spring of 2007 and 2008, respectively. However, *Carduncellus eriocephalus* had the highest ones in bed 2 in spring of both years. In addition, *Leopoldia comosa* had the same abundance of *Carduncellus eriocephalus* in bed 1 of spring 2008. *Deverra tortuosa* and *Gymnocarpos decandrus* gave the highest abundance in top, *Thymelaea hirsuta* and *Gymnocarpos decandrus* in ridge, *Cynodon dactylon* and *Chiliadenus candicans* in bed 1, *Chiliadenus candicans* and *Thymelaea hirsuta* in bed 2 in autumn of 2007 and 2008, respectively. Among annual species such as *Hordeum marinum* and *Lophochloa cristata* in top, *Adonis dentata* and *Phalaris minor* in ridge, *Hordeum marinum* and *Phalaris minor* in bed 1, *Avena fatua* and *Poa annua* in bed 2, the highest abundance in spring of 2007 and 2008, respectively.

Significant difference was observed between total abundance of different sites in wadi Halazien in both years. The total abundance of species in spring surpassed than it in autumn. The total abundance of species in bed 2 significantly surpassed the others sites in both years. The total abundance of species in bed 2 in spring 2008 significantly increased than it in spring 2007 by 64 plants. This was happen because of the highest precipitation in 2008 that increased than 2007 by 31.2 mm (Table 1). In addition, environmental conditions in spring were better than in autumn. This trend was observed by Kulaib (2008) who found the highest abundance in spring than autumn and in wadi bed than the others sites in wadi El-Washka.

Species richness

Species richness in spring increased than in autumn in all sites of both years. Except, species richness of bed 2 in autumn increased than in spring of 2007. This

reflects of the richness in wadi bed for fertility and other growing factors when compared to ridge and top of the wadi. In addition, ridge and top soil was not capable to keep available water for long time, which not assists in spreading plants. This may be due to the differences among the three sites in soil physical and chemical properties as shown in Tables (2, 3a and 3b).

Simpson's index of diversity in spring of all sites increased than in autumn of both years. Simpson's index of diversity in both seasons of all sites increased in 2008 than 2007, except, it in autumn of top and ridge in 2007 surpassed 2008.

Importance value (IV)

Results in Table (6) represent the effect of season, site and year on importance value (IV) of the plant species in wadi Halazien in both years. *Solanaceae* in top, *Apiaceae* in ridge and bed 2 and *Thymelaeaceae* in bed 1 gave the highest IV in spring 2007, while, in spring 2008 *Thymelaeaceae* in top, *Caryophyllaceae* in ridge, *Cistaceae* in bed 1 and *Hyacynthaceae* in bed 2 gave the highest IV. The highest IV was *Apiaceae* in top, *Thymelaeaceae* in ridge, *Poaceae* in bed 1 and *Asteraceae* in bed 2 in autumn 2007. Whereas, in autumn of 2008 *Caryophyllaceae* in top and ridge, *Asteraceae* in bed 1 and *Thymelaeaceae* in bed 2 gave the highest IV. Gab Allah (2006) and Abdel-Gawad *et al.* (2009) found that plant species of families *Poaceae* and *Asteraceae* had the highest IV in both years in wadi El-Ramla and El-Washka.

Among perennials species, the highest IV in spring 2007 was for *Lycium shawii* (Fig. 9) in top, *Euphorbia dendroides* (Fig. 10) in ridge, *Thymelaea hirsuta* in bed 1 and *Carduncellus eriocephalus* in bed 2, while, in spring of 2008 were *Thymelaea hirsuta* in top, *Gymnocarpos decandrus* in ridge, *Fumana thymifolia* in bed 1 and *Carduncellus eriocephalus* in bed 2. However, the highest IV was *Deverra tortuosa* in top, *Thymelaea hirsuta* in ridge, *Cynodon dactylon* in bed 1 and *Chiliadenus candicans* in bed 2 in autumn of 2007, whereas, in autumn 2008 were *Gymnocarpos decandrus* in top and ridge, *Chiliadenus candicans* in bed 1 and *Thymelaea hirsuta* in bed 2.

However, among annuals species the highest IV plant species were *Hordeum marinum* in top and bed 1, *Adonis dentata* in ridge and *Avena fatua* in bed 2 in spring 2007, whereas, in spring 2008 were *Lophochloa cristata* in top, *Phalaris minor* in ridge and bed 1 and *Poa annua* in bed 2. While, the highest IV plant species were *Urospermum picroides* in top, *Senecio coronopifolius* in bed 1 and *Medicago polymorpha* in bed 2 in autumn 2007, whereas, in autumn 2008 *Urospermum picroides* gave the highest IV in bed 1.

All plant species belong to *Poaceae* and *Asteraceae* had the highest IV related to the highest relative density. Generally, all plant species belong to *Apiaceae*, *Caryophyllaceae* or *Thymelaeaceae* had the highest IV related to the highest relative density or relative coverage. These because the morphology of plant species of *Poaceae* and *Asteraceae* was erect and had narrow leaves. However, the morphology of plant species of *Caryophyllaceae* or *Thymelaeaceae* was prostrate and had broad leaves which coverage more area. Gab Allah (2006) found the highest IV of perennial species was *Lycium shawii*, *Thymelaea hirsuta* and *Gymnocarpos decandrus*.

Table 5. Abundance of plant species recorded in wadi Halazien during spring and autumn of 2007 and 2008.

Scientific name	2007								2008							
	Top		Ridge		Bed 1		Bed 2		Top		Ridge		Bed 1		Bed 2	
	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au
Allium roseum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2	--
Amaranthus viridis *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--
Deverra tortuosa	4	15	12	11	3	5	9	3	6	2	--	2	15	13	4	1
Arisarum vulgare	--	--	--	--	--	--	--	4	--	--	--	--	--	--	--	--
Asparagus stipularis	--	--	--	--	--	--	--	--	--	2	--	--	--	--	--	--
Asphodelus aestivus	2	--	--	--	--	--	--	--	3	--	--	--	--	--	--	--
Achillea santolina	--	--	--	--	--	--	--	--	--	--	2	--	1	--	--	--
Anacyclus monanthos *	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Carduncellus eriocephalus	2	--	8	--	8	--	42	4	--	--	3	12	51	27	38	10
Carduus getulus *	--	--	--	--	--	--	--	--	4	--	--	--	--	--	--	--
Centaurea calcitrapa *	--	--	--	--	--	--	--	--	--	--	2	--	--	--	--	--
Chiliadenus candicans	--	4	--	--	9	3	27	39	--	--	--	--	--	33	--	--
Picris asplenioides *	--	--	--	--	--	--	--	--	8	--	5	--	2	--	9	--

Reichardia tingitana *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	12	--
Scorzonera undulata	--	--	--	--	--	--	--	--	--	--	5	--	--	--	--	--
Senecio coronopifolius *	2	--	--	--	--	2	--	2	--	--	--	--	--	--	--	--
Silybum marianum **	--	--	--	--	--	4	--	2	--	--	2	--	7	--	2	--
Leontodon tuberosus	--	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Urospermum picroides *	--	1	--	--	--	--	--	--	--	--	--	--	--	4	1	--
Didesmus bipinnatus *	--	--	--	--	9	--	--	--	--	--	--	--	--	--	--	--
Diplotaxis acris *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	12	--
Matthiola longipetala *	--	--	--	--	--	--	--	--	--	--	14	--	2	--	--	--
Zilla spinosa	--	1	--	5	--	--	--	--	--	--	--	--	--	--	--	--
Gymnocarpos decandrus	3	5	4	3	--	--	--	--	8	30	6	44	--	--	--	16
Silene succulenta	--	--	--	--	--	--	--	--	--	--	3	--	--	--	--	--
Atriplex nummularia	--	--	--	--	--	--	10	--	--	--	--	--	--	--	3	3
Haloxylon salicornicum	2	--	--	--	--	7	4	--	--	4	--	--	--	2	7	16
Noaea mucronata	--	--	--	--	--	--	--	--	--	--	--	--	4	2	--	--
Fumana thymifolia	--	--	--	--	--	--	--	--	--	--	--	--	22	--	--	--
Helianthemum lippii	--	--	--	--	--	--	3	--	--	--	2	--	--	--	--	--
Helianthemum vesicarium	--	--	--	--	4	--	--	--	--	--	--	--	--	--	--	--
Cyperus rotundus	--	--	--	--	--	--	--	--	6	--	12	--	--	--	--	--
Euphorbia dendroides	--	--	5	--	--	--	4	--	--	--	--	--	--	--	2	--

(Cont.)

Table 5. (Cont.)

Hippocrepis cyclocarpa *	3	--	--	--	2	--	--	--	--	--	--	--	--	--	--	--
Lotus polyphyllus	2	--	--	--	1	--	--	--	--	--	--	--	2	--	2	--
Medicago polymorpha *	--	--	--	--	--	--	--	2	--	--	--	--	--	--	2	--
Melilotus siculus *	--	--	--	--	--	--	--	--	6	--	--	--	--	--	--	--
Retama raetam	--	--	--	--	--	--	--	2	--	--	--	--	--	--	--	--
Erodium crassifolium	--	--	--	--	--	--	--	--	2	--	--	--	--	--	--	--
Leopoldia comosa	--	--	--	--	--	--	--	--	--	--	14	--	--	--	38	--
Phlomis floccosa	--	1	8	--	--	--	--	--	--	--	--	--	2	--	2	--
Salvia aegyptiaca	--	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--
harmala Peganum	--	--	--	--	--	1	12	--	--	--	--	--	--	--	1	7
Aegilops kotschyi *	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--
Avena fatua *	--	--	--	--	--	--	40	--	--	--	--	--	--	--	10	--
Bromus rubens *	--	--	--	--	--	--	--	--	10	--	4	--	4	--	4	--
Cutandia dichotoma *	--	--	--	--	--	--	--	--	2	--	--	--	2	--	1	--
Cynodon dactylon	--	--	--	--	--	25	--	4	--	--	--	--	--	--	--	--
Hordeum marinum *	8	--	--	--	28	--	--	--	--	--	--	--	--	--	10	--
Lamarckia aurea *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	13	--
Lygeum spartum	--	--	--	--	--	--	5	--	--	--	2	--	--	--	--	--
Lophochloa cristata *	--	--	--	--	--	--	--	--	16	--	3	--	--	--	--	--

Phalaris minor *	--	--	--	--	--	--	--	--	15	--	21	--	14	--	13	--
Poa annua *	--	--	--	--	--	--	--	--	3	--	4	--	--	--	22	--
Polygonum equisetiforme	6	4	--	--	--	--	--	--	5	--	12	--	33	4	2	--
Adonis dentata *	--	--	4	--	--	--	--	--	--	--	--	--	--	--	--	--
Reseda decursiva *	--	--	--	--	--	--	--	--	2	--	--	--	--	--	1	--
Lycium shawii	5	2	--	2	--	3	--	--	--	--	--	2	2	--	--	--
Thymelaea hirsute	--	6	--	13	5	--	--	2	6	--	2	10	2	5	6	61
Urtica urens *	--	--	--	--	--	--	--	2	--	--	--	--	--	--	--	--
Fagonia scabra	--	--	--	--	4	--	--	--	--	--	4	--	--	--	--	--
Total	42 l	41 m	41 m	34 o	73 h	50 k	156 c	66 j	103 f	38 n	122 d	70 i	166 b	90 g	220 a	114 e
Species richness	12	10	6	5	10	8	10	11	17	4	20	5	17	8	27	7
Diversity (D)	0.08	0.18	0.18	0.26	0.19	0.28	0.18	0.36	0.08	0.63	0.08	0.44	0.17	0.24	0.09	0.33
Simpson's index of diversity	0.92	0.82	0.82	0.74	0.81	0.72	0.82	0.64	0.92	0.37	0.92	0.56	0.83	0.76	0.91	0.67

In this table and the following tables; Sp.= spring, Au.= autumn. *= Annual, **= Biennial and others species = Perennial.

Fig. 4. *Polygonum equisetiforme*

Fig. 5. *Gymnocarpos decandrus*

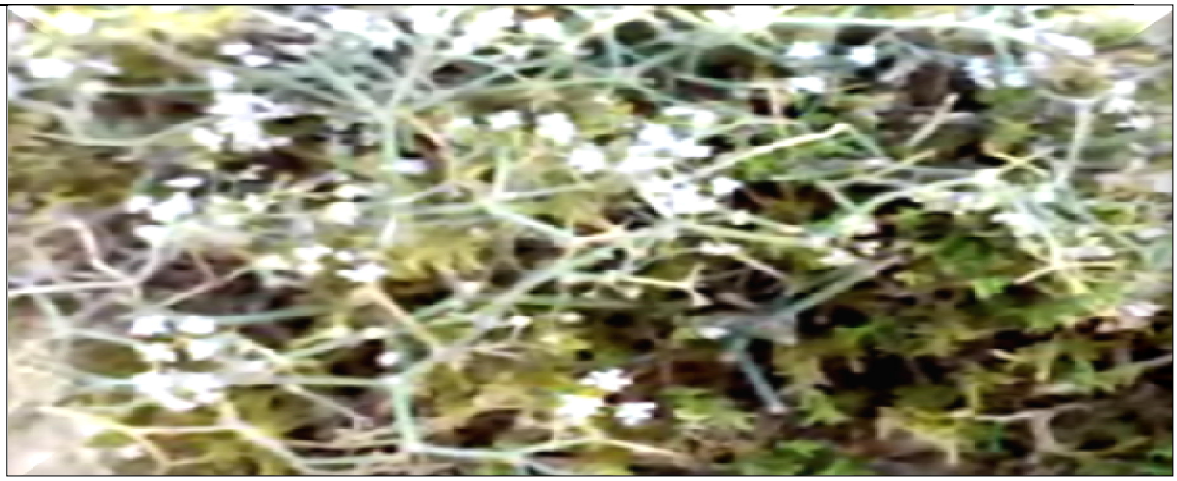


Fig. 6. ***Deverra tortuosa***

Fig. 7. ***Chiliadenus candicans***

Fig. 8. Carduncellus eriocephalus

Fig. 9. Lycium shawii

Fig. 10. Euphorbia dendroides

Fig. 11. *Peganum harmala*

Table 6. Importance value (IV) of plant species recorded in wadi Halazien during spring and autumn of 2007 and 2008

	2007								2008							
	Top		Ridge		Bed 1		Bed 2		Top		Ridge		Bed 1		Bed 2	
Scientific name	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au
Allium roseum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2.9	--
Amaranthus viridis *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2.6	--

Deverra tortuosa	48.7	93.6	89.9	80.7	17.9	75.7	33.1	32.5	33.9	16.7	--	14.5	23.5	67.4	18.8	4.9
Arisarum vulgare	--	--	--	--	--	--	--	10.7	--	--	--	--	--	--	--	--
Asparagus stipularis	--	--	--	--	--	--	--	--	--	31.2	--	--	--	--	--	--
Asphodelus aestivus	9.9	--	--	--	--	--	--	--	18.2	--	--	--	--	--	--	--
Achillea santolina	--	--	--	--	--	--	--	--	--	--	5.1	--	3.4	--	--	--
Anacyclus monanthos *	11.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Carduncellus eriocephalus	14.8	--	38.5	--	34.2	--	61.8	20.8	--	--	8.6	50.4	59.0	73.1	60.4	27.4
Carduus getulus *	--	--	--	--	--	--	--	--	12.6	--	--	--	--	--	--	--
Centaurea calcitrapa *	--	--	--	--	--	--	--	--	--	--	6.7	--	--	--	--	--
Chiliadenus candicans	--	28.6	--	--	35.3	23.2	47.8	146.4	--	--	--	--	--	73.7	--	--
Picris asplenioides *	--	--	--	--	--	--	--	--	20.2	--	12.0	--	3.9	--	6.8	--
Reichardia tingitana *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	10.4	--
Scorzonera undulata	--	--	--	--	--	--	--	--	--	--	9.8	--	--	--	--	--
Senecio coronopifolius *	9.4	--	--	--	--	15.3	--	7.1	--	--	--	--	--	--	--	--
Silybum marianum **	--	--	--	--	--	23.9	--	10.4	--	--	6.9	--	11.7	--	4.8	--
Leontodon tuberosus	--	14.4	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Urospermum picroides *	--	7.9	--	--	--	--	--	--	--	--	--	--	--	10.8	2.6	--
Didesmus bipinnatus *	--	--	--	--	43.0	--	--	--	--	--	--	--	--	--	--	--
Diplotaxis acris *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	10.3	--
Matthiola longipetala *	--	--	--	--	--	--	--	--	--	--	19.7	--	7.0	--	--	--
Zilla spinosa	--	10.0	--	37.7	--	--	--	--	--	--	--	--	--	--	--	--
Gymnocarpos decandrus	35.1	40.8	25.3	55.9	--	--	--	--	34.6	219	64.0	162.2	--	--	--	85.2
Silene succulenta	--	--	--	--	--	--	--	--	--	--	9.1	--	--	--	--	--
Atriplex nummularia	--	--	--	--	--	--	35.3	--	--	--	--	--	--	--	11.2	15.2
Haloxylon salicornicum	10.8	--	--	--	--	49.4	12.9	--	--	33.1	--	--	--	6.2	18.3	34.3
Noaea mucronata	--	--	--	--	--	--	--	--	--	--	--	--	9.0	10.4	--	--

(Cont.)

Table 6. (Cont.)

Fumana thymifolia	--	--	--	--	--	--	--	--	--	--	--	--	66.6	--	--	--
Helianthemum lippii	--	--	--	--	--	--	8.8	--	--	--	4.9	--	--	--	--	--
Helianthemum vesicarium	--	--	--	--	11.4	--	--	--	--	--	--	--	--	--	--	--
Cyperus rotundus	--	--	--	--	--	--	--	--	13.4	--	16.8	--	--	--	--	--
Euphorbia dendroides	--	--	77.6	--	--	--	16.7	--	--	--	--	--	--	--	5.1	--
Hippocrepis cyclocarpa *	15.5	--	--	--	7.1	--	--	--	--	--	--	--	--	--	--	--
Lotus polyphyllus	13.6	--	--	--	5.9	--	--	--	--	--	--	--	4.4	--	3.0	--
Medicago polymorpha *	--	--	--	--	--	--	--	12.0	--	--	--	--	--	--	2.9	--

Melilotus siculus *	--	--	--	--	--	--	--	--	12.4	--	--	--	--	--	--	--
Retama raetam	--	--	--	--	--	--	--	11.5	--	--	--	--	--	--	--	--
Erodium crassifolium	--	--	--	--	--	--	--	--	4.3	--	--	--	--	--	--	--
Leopoldia comosa	--	--	--	--	--	--	--	--	--	--	23.9	--	--	--	27.7	--
Phlomis floccosa	--	7.6	41.0	--	--	--	--	--	--	--	--	--	10.7	--	11.8	--
Salvia aegyptiaca	--	--	--	--	--	--	--	--	4.5	--	--	--	--	--	--	--
Peganum harmala	--	--	--	--	--	9.5	38.7	--	--	--	--	--	--	--	5.9	31.6
Aegilops kotschyi *	--	--	--	--	--	--	--	--	--	--	--	--	4.4	--	--	--
Avena fatua *	--	--	--	--	--	--	35.1	--	--	--	--	--	--	--	6.6	--
Bromus rubens *	--	--	--	--	--	--	--	--	17.0	--	8.6	--	5.0	--	3.9	--
Cutandia dichotoma *	--	--	--	--	--	--	--	--	4.3	--	--	--	4.2	--	2.6	--
Cynodon dactylon	--	--	--	--	--	84.0	--	14.7	--	--	--	--	--	--	--	--
Hordeum marinum *	31.6	--	--	--	54.4	--	--	--	--	--	--	--	--	--	9.0	--
Lamarckia aurea *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	10.1	--
Lygeum spartum	--	--	--	--	--	--	10.1	--	--	--	4.6	--	--	--	--	--
Lophochloa cristata *	--	--	--	--	--	--	--	--	27.4	--	9.4	--	--	--	--	--
Phalaris minor *	--	--	--	--	--	--	--	--	21.9	--	25.3	--	11.0	--	10.0	--
Poa annua *	--	--	--	--	--	--	--	--	13.2	--	10.0	--	--	--	21.3	--
Polygonum equisetiforme	30.5	21.8	--	--	--	--	--	--	17.4	--	29.4	--	35.4	12.0	4.8	--
Adonis dentata *	--	--	27.7	--	--	--	--	--	--	--	--	--	--	--	--	--
Reseda decursiva *	--	--	--	--	--	--	--	--	5.9	--	--	--	--	--	3.0	--
Lycium shawii	68.6	39.5	--	18.4	--	19.0	--	--	--	--	--	15.2	20.6	--	--	--
Thymelaea hirsuta	--	35.9	--	107.4	68.2	--	--	23.2	38.5	--	19.1	57.9	20.1	46.2	22.9	101.3
Urtica urens *	--	--	--	--	--	--	--	10.4	--	--	--	--	--	--	--	--
Fagonia scabra	--	--	--	--	22.5	--	--	--	--	--	6.2	--	--	--	--	--
Total	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300

*** = Annual, ** = Biennial and others species = Perennial.**

Foliage productivity

Results in Tables (7) and (8) represent the effect of season, site and year on fresh and dry foliage yields (g m^{-2}) of the plant species in wadi Halazien. *Caryophyllaceae* in top, *Euphorbiaceae* in ridge, *Thymelaeaceae* in bed 1 and *Apiaceae* in bed 2 gave the highest fresh and dry yields in spring 2007. While, in spring 2008 *Thymelaeaceae* in top, ridge and bed 2 and *Cistaceae* in bed 1 gave the highest fresh and dry yields, except for dry yield of *Apiaceae* in bed 2. *Apiaceae* in top, *Thymelaeaceae* in ridge and bed 2 and *Chenopodiaceae* in bed 1 gave the highest fresh and dry yields in autumn 2007, except for dry yield of *Asteraceae* in bed 2, whereas, in autumn 2008 *Thymelaeaceae* gave the highest fresh and dry yields at all sites, except *Caryophyllaceae* in top. El-Morsy (2002) mentioned that *Chenopodiaceae* and *Thymelaeaceae* plant species gave the highest fresh and dry yields in both years in wadi Magid. Abdel-Gawad *et al.* (2009) found plant species belong to famiy *Chenopodiaceae* gave the highest fresh and dry yields in spring and autumn of both years in wadi El-Washka.

Among perennial *Gymnocarpos decandrus* in top, *Euphorbia dendroides* in ridge, *Thymelaea hirsuta* bed 1 and *Atriplex nummularia* in bed 2 gave the highest fresh and dry yields in spring 2007. While, in spring 2008 *Thymelaea hirsuta* in top, *Gymnocarpos decandrus* in ridge, *Fumana thymifolia* in bed 1 and *Carduncellus eriocephalus* in bed 2 gave the highest fresh and dry yields, except for dry yield of *Gymnocarpos decandrus* in top and *Deverra tortuosa* in bed 2. However, in autumn 2007 *Deverra tortuosa* in top, *Thymelaea hirsuta* in ridge, *Chiliadenus candicans* bed 1 and 2 gave the highest fresh and dry yields. However, in autumn 2008, *Thymelaea hirsuta* gave the highest fresh and dry yields at all sites, except *Gymnocarpos decandrus* gave the highest yields in top. Abdel-Gawad *et al.* (2009) found *Thymelaea hirsuta* gave the highest fresh and dry yields in spring and autumn in both years in wadi El-Washka.

Among annual species *Senecio coronopifolius* in top, *Adonis dentata* in ridge, *Hippocrepis cyclocarpa* in bed 1 and *Avena fatua* in bed 2 gave the highest fresh and dry yields in spring 2007, except for dry yield of *Hordeum marinum* in top. However, in spring 2008 *Carduus getulus* in top, *Phalaris minor* in ridge, *Matthiola longipetala* in bed 1 and *Avena fatua* in bed 2 gave the highest fresh and dry yields. While, in autumn 2007 *Urospermum picroides* in top, *Senecio coronopifolius* in bed 1 and *Medicago polymorpha* in bed 2 gave the highest fresh and dry yields, whereas, in autumn 2008 only one species (*Urospermum picroides*) appeared in bed 1. *Senecio coronopifolius* gave the same fresh yield as *Medicago polymorpha* in bed 2 in autumn 2007.

Fresh and dry yields significantly increased in spring compared with autumn at all sites in both years. Reiad *et al.* (1996 b) recommended that fresh and dry yields obtained during spring season outyielded that of winter, autumn and summer, respectively in Sidi Barrani and El-Negaila.

Fresh and dry yields in wadi bed 2 significantly exceeded the other sites in both years. The increments of fresh yield in bed 2 than top, ridge and bed 1 were 25.60, 19.49 and 13.46 g m^{-2}, respectively in spring 2007. But, in spring 2008 were 53.21, 46.37 and 25.24 g m^{-2} in the same respective order. In autumn 2007 increments were 10.36, 7.93 and 5.77 g m^{-2} and in autumn 2008 were 14.74, 8.53 and 3.22 g m^{-2}. Dry yield in bed 2 surpassed it in top, ridge and bed 1 by 12.09, 9.64 and 3.91 g m^{-2}, respectively in spring 2007. While in spring 2008 increments were 22.02, 17.44 and 6.68 g m^{-2} in the same respective order. The increase of dry yield in autumn 2007 was 5.07, 2.96 and 2.42 g m^{-2}, while, in autumn 2008 it was 6.66, 3.04 and 1.25 g m^{-2}. Abdel-Gawad *et al.* (2009) mentioned that the highest fresh and dry yields were obtained in wadi bed followed by wadi ridge and top in wadi El-Washka. This may be due to mild wind speed and more humidity in wadi bed compared with other studied sites of wadi and more edaphic conditions that are suitable. In other words, the increased productivity in wadi bed may be due to the presence of water quantities because this area considered as rain-fed harvest. While the ridge and the top of wadi faced soil surface erosion due to precipitation and wind speed that formed the surface layer of seed bed resulted.

Table 7. Fresh forage yield of plant species (g m^{-2}) recorded in wadi Halazien during spring and autumn of 2007 and 2008

Scientific name	2007								2008							
	Top		Ridge		Bed 1		Bed 2		Top		Ridge		Bed 1		Bed 2	
	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au
Allium roseum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.333	--
Amaranthus viridis *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.750	--
Deverra tortuosa	1.454	2.354	1.602	0.583	0.141	1.583	7.501	1.942	1.410	0.105	--	1.138	2.715	4.070	7.524	0.038
Arisarum vulgare	--	--	--	--	--	--	--	0.444	--	--	--	--	--	--	--	--
Asparagus stipularis	--	--	--	--	--	--	--	--	--	1.388	--	--	--	--	--	--
Asphodelus aestivus	0.029	--	--	--	--	--	--	--	0.194	--	--	--	--	--	--	--
Achillea santolina	--	--	--	--	--	--	--	--	--	--	0.333	--	0.167	--	--	--
Anacyclus monanthos *	0.261	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Carduncellus eriocephalus	0.553	--	0.401	--	2.842	--	5.129	0.034	--	--	0.389	0.114	3.219	0.217	14.011	0.143
Carduus getulus *	--	--	--	--	--	--	--	--	0.552	--	--	--	--	--	--	--
Centaurea calcitrapa *	--	--	--	--	--	--	--	--	--	--	0.181	--	--	--	--	--
Chiliadenus candicans	--	0.156	--	--	1.531	1.945	0.916	8.681	--	--	--	--	--	2.872	--	--
Picris asplenioides *	--	--	--	--	--	--	--	--	0.449	--	0.115	--	0.094	--	0.167	--
Reichardia tingitana *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.917	--
Scorzonera undulata	--	--	--	--	--	--	--	--	--	--	0.433	--	--	--	--	--
Senecio coronopifolius *	0.281	--	--	--	--	0.500	--	0.222	--	--	--	--	--	--	--	--
Silybum marianum **	--	--	--	--	--	0.333	--	0.111	--	--	0.361	--	0.177	--	1.100	--
Leontodon	--	0.063	--	--	--	--	--	--	--	--	--	--	--	--	--	--

tuberosus																
Urospermum picroides *	--	0.083	--	--	--	--	--	--	--	--	--	--	--	0.008	0.083	--
Didesmus bipinnatus *	--	--	--	--	0.250	--	--	--	--	--	--	--	--	--	--	--
Diplotaxis acris *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.375	--
Matthiola longipetala *	--	--	--	--	--	--	--	--	--	--	0.274	--	0.500	--	--	--
Zilla spinosa		0.833	--	0.410	--	--	--	--	--	--	--	--	--	--	--	--
Gymnocarpos decandrus	2.021	0.434	0.275	0.033	--	--	--	--	1.729	2.584	4.758	2.187	--	--	--	1.166
Silene succulenta	--	--	--	--	--	--	--	--	--	--	0.092	--	--	--	--	--
Atriplex nummularia	--	--	--	--	--	--	8.752	--	--	--	--	--	--	--	4.630	0.410
Haloxylon salicornicum	0.300	--	--	--	--	1.667	3.667	--	--	0.252	--	--	--	0.385	4.889	0.835
Noaea mucronata	--	--	--	--	--	--	--	--	--	--	--	--	0.271	0.389	--	--
Fumana thymifolia	--	--	--	--	--	--	--	--	--	--	--	--	10.391	--	--	--

Table 7. (Cont.) (Cont.)

Helianthemum lippii	--	--	--	--	--	--	0.375	--	--	--	0.333	--	--	--	--	--
Helianthemum vesicarium	--	--	--	--	0.183	--	--	--	--	--	--	--	--	--	--	--
Cyperus rotundus	--	--	--	--	--	--	--	--	0.107	--	0.214	--	--	--	--	--
Euphorbia dendroides	--	--	9.844	--	--	--	1.781	--	--	--	--	--	--	--	1.759	--
Hippocrepis cyclocarpa *	0.219	--	--	--	0.471	--	--	--	--	--	--	--	--	--	--	--
Lotus polyphyllus	0.844	--	--	--	0.017	--	--	--	--	--	--	--	0.333	--	0.333	--
Medicago polymorpha *	--	--	--	--	--	--	--	0.222	--	--	--	--	--	--	0.021	--
Melilotus siculus *	--	--	--	--	--	--	--	--	0.035	--	--	--	--	--	--	--
Retama raetam	--	--	--	--	--	--	--	0.083	--	--	--	--	--	--	--	--
Erodium crassifolium	--	--	--	--	--	--	--	--	0.014	--	--	--	--	--	--	--
Leopoldia comosa	--	--	--	--	--	--	--	--	--	--	1.465	--	--	--	2.944	--
Phlomis floccosa	--	0.115	1.677	--	--	--	--	--	--	--	--	--	5.868	--	6.167	--
Salvia aegyptiaca	--	--	--	--	--	--	--	--	0.028	--	--	--	--	--	--	--
Peganum harmala	--	--	--	--	--	0.583	3.625	--	--	--	--	--	--	--	2.658	2.578
Aegilops kotschyi *	--	--	--	--	--	--	--	--	--	--	--	--	0.333	--	--	--
Avena fatua *	--	--	--	--	--	--	1.500	--	--	--	--	--	--	--	1.650	--
Bromus rubens *	--	--	--	--	--	--	--	--	0.073	--	0.070	--	0.042	--	0.125	--
Cutandia dichotoma *	--	--	--	--	--	--	--	--	0.083	--	--	--	0.083	--	0.167	--
Cynodon dactylon	--	--	--	--	--	1.167	--	0.222	--	--	--	--	--	--	--	--
Hordeum marinum *	0.101	--	--	--	0.212	--	--	--	--	--	--	--	--	--	0.103	--
Lamarckia aurea *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.063	--
Lygeum spartum	--	--	--	--	--	--	0.104	--	--	--	0.083	--	--	--	--	--
Lophochloa cristata *	--	--	--	--	--	--	--	--	0.222	--	0.067	--	--	--	--	--
Phalaris minor *	--	--	--	--	--	--	--	--	0.158	--	0.472	--	0.083	--	0.208	--

Poa annua *	--	--	--	--	--	--	--	--	0.281	--	0.140	--	--	--	0.500	--
Polygonum equisetiforme	0.240	0.043	--	--	--	--	--	--	0.903	--	1.688	--	1.260	0.271	1.296	--
Adonis dentata *	--	--	0.057	--	--	--	--	--	--	--	--	--	--	--	--	--
Reseda decursiva *	--	--	--	--	--	--	--	--	0.047	--	--	--	--	--	0.250	--
Lycium shawii	1.453	0.458	--	0.167	--	1.528	--	--	--	--	--	1.194	3.000	--	--	--
Thymelaea hirsuta	--	0.177	--	5.950	14.044	--	--	3.000	2.083	--	3.160	5.911	7.809	7.643	7.558	13.899
Urtica urens *	--	--	--	--	--	--	--	0.111	--	--	--	--	--	--	--	--
Fagonia scabra	--	--	--	--	0.200	--	--	--	--	--	0.583	--	--	--	--	--
Seasonal yield	7.755 l	4.717n	13.857 h	7.143 m	19.889 d	9.306 j	33.351 c	15.073 g	8.368k	4.328 o	15.211 g	10.544 i	36.346 b	15.854 f	61.582 a	19.069 e
	Spring = 18.713 ns						Autumn = 9.060 ns		Spring = 30.377 ns						Autumn = 12.449 ns	

Table 8. Dry forage yield of plant species (g m^{-2}) recorded in wadi Halazien during spring and autumn of 2007 and 2008

Scientific name	2007								2008							
	Top		Ridge		Bed 1		Bed 2		Top		Ridge		Bed 1		Bed 2	
	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au
Allium roseum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.283	--
Amaranthus viridis *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.358	--
Deverra tortuosa	0.688	1.098	0.846	0.321	0.044	0.985	4.231	1.188	0.833	0.017	--	0.500	1.635	2.303	4.244	0.023
Arisarum vulgare	--	--	--	--	--	--	--	0.042	--	--	--	--	--	--	--	--
Asparagus stipularis	--	--	--	--	--	--	--	--	--	0.638	--	--	--	--	--	--
Asphodelus aestivus	0.021	--	--	--	--	--	--	--	0.035	--	--	--	--	--	--	--
Achillea santolina	--	--	--	--	--	--	--	--	--	--	0.158	--	0.050	--	--	--
Anacyclus monanthos *	0.052	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Carduncellus eriocephalus	0.204	--	0.144	--	1.733	--	1.500	0.014	--	--	0.185	0.051	1.455	0.088	3.634	0.058
Carduus getulus *	--	--	--	--	--	--	--	--	0.346	--	--	--	--	--	--	--
Centaurea calcitrapa *	--	--	--	--	--	--	--	--	--	--	0.099	--	--	--	--	--
Chiliadenus candicans	--	0.059	--	--	0.625	1.464	0.446	4.433	--	--	--	--	--	1.133	--	--
Picris asplenioides *	--	--	--	--	--	--	--	--	0.037	--	0.047	--	0.066	--	0.050	--
Reichardia tingitana *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.376	--
Scorzonera undulata	--	--	--	--	--	--	--	--	--	--	0.210	--	--	--	--	--
Senecio coronopifolius *	0.057	--	--	--	--	0.102	--	0.033	--	--	--	--	--	--	--	--
Silybum marianum **	--	--	--	--	--	0.233	--	0.078	--	--	0.298	--	0.443	--	0.532	--
Leontodon tuberosus	--	0.042	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Urospermum picroides *	--	0.022	--	--	--	--	--	--	--	--	--	--	--	0.002	0.033	--
Didesmus bipinnatus *	--	--	--	--	0.126	--	--	--	--	--	--	--	--	--	--	--
Diplotaxis acris *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.486	--
Matthiola longipetala *	--	--	--	--	--	--	--	--	--	--	0.135	--	0.250	--	--	--
Zilla spinosa	--	0.220	--	0.350	--	--	--	--	--	--	--	--	--	--	--	--
Gymnocarpos decandrus	0.820	0.404	0.070	0.003	--	--	--	--	0.997	1.767	2.461	1.232	--	--	--	0.432
Silene succulenta	--	--	--	--	--	--	--	--	--	--	0.449	--	--	--	--	--
Atriplex nummularia	--	--	--	--	--	--	5.360	--	--	--	--	--	--	--	2.563	0.200
Haloxylon	0.155	--	--	--	--	1.010	1.700	--	--	0.120	--	--	--	0.092	2.267	0.750

salicornicum																	
Noaea mucronata	--	--	--	--	--	--	--	--	--	--	--	--	0.093	0.133	--	--	
Fumana thymifolia	--	--	--	--	--	--	--	--	--	--	--	--	6.084	--	--	--	

Table 8. (Cont.) (Cont.)

Helianthemum lippii	--	--	--	--	--	--	0.263	--	--	--	0.233	--	--	--	--	--	
Helianthemum vesicarium	--	--	--	--	0.099	--	--	--	--	--	--	--	--	--	--	--	
Cyperus rotundus	--	--	--	--	--	--	--	--	0.085	--	0.126	--	--	--	--	--	
Euphorbia dendroides	--	--	3.449	--	--	--	0.178	--	--	--	--	--	--	--	0.185	--	
Hippocrepis cyclocarpa *	0.045	--	--	--	0.166	--	--	--	--	--	--	--	--	--	--	--	
Lotus polyphyllus	0.380	--	--	--	0.108	--	--	--	--	--	--	--	0.217	--	0.217	--	
Medicago polymorpha *	--	--	--	--	--	--	--	0.051	--	--	--	--	--	--	0.008	--	
Melilotus siculus *	--	--	--	--	--	--	--	--	0.014	--	--	--	--	--	--	--	
Retama raetam	--	--	--	--	--	--	--	0.042	--	--	--	--	--	--	--	--	
Erodium crassifolium	--	--	--	--	--	--	--	--	0.004	--	--	--	--	--	--	--	
Leopoldia comosa	--	--	--	--	--	--	--	--	--	--	0.761	--	--	--	1.528	--	
Phlomis floccose	--	0.095	1.168	--	--	--	--	--	--	--	--	--	2.835	--	2.800	--	
Salvia aegyptiaca	--	--	--	--	--	--	--	--	0.008	--	--	--	--	--	--	--	
Peganum harmala	--	--	--	--	--	0.143	0.775	--	--	--	--	--	--	--	0.568	0.438	
Aegilops kotschyi *	--	--	--	--	--	--	--	--	--	--	--	--	0.242	--	--	--	
Avena fatua *	--	--	--	--	--	--	0.833	--	--	--	--	--	--	--	0.917	--	
Bromus rubens *	--	--	--	--	--	--	--	--	0.016	--	0.042	--	0.025	--	0.063	--	
Cutandia dichotoma *	--	--	--	--	--	---	--	--	0.075	--	--	--	0.071	--	0.142	--	
Cynodon dactylon	--	--	--	--	--	0.353	--	0.066	--	--	--	--	--	--	--	--	
Hordeum marinum *	0.067	--	--	--	0.137	--	--	--	--	--	--	--	--	--	0.063	--	
Lamarckia aurea *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.042	--	
Lygeum spartum	--	--	--	--	--	--	0.077	--	--	--	0.083	--	--	--	--	--	
Lophochloa cristata *	--	--	--	--	--	--	--	--	0.079	--	0.013	--	--	--	--	--	
Phalaris minor *	--	--	--	--	--	--	--	--	0.032	--	0.375	--	0.100	--	0.033	--	
Poa annua *	--	--	--	--	--	--	--	--	0.178	--	0.075	--	--	--	0.325	--	
Polygonum equisetiforme	0.127	0.029	--	--	--	--	--	--	0.549	--	1.042	--	0.990	0.025	0.963	--	
Adonis dentata *	--	--	0.046	--	--	--	--	--	--	--	--	--	--	--	--	--	
Reseda decursiva *	--	--	--	--	--	--	--	--	0.023	--	--	--	--	--	0.113	--	
Lycium shawii	0.655	0.306	--	0.015	--	0.743	--	--	--	--	--	0.778	1.683	--	--	--	
Thymelaea hirsuta	--	0.108	--	3.798	8.366	--	--	1.500	0.984	--	1.733	3.601	3.395	4.181	3.519	7.302	
Urtica urens *	--	--	--	--	--	--	--	0.001	--	--	--	--	--	--	--	--	
Fagonia scabra	--	--	--	--	0.050	--	--	--	--	--	0.342	--	--	--	--	--	
Seasonal yield	3.271 n	2.382 p	5.723 j	4.487 l	11.454 d	5.033 k	15.363 c	7.448 h	4.295 m	2.541 o	8.867 f	6.162 i	19.631 b	7.957 g	26.311 a	9.203 e	
Average seasonal	Spring = 8.953 ns			= 4.838 ns				Autumn		Spring = 14.776 ns					Autumn = 6.466 ns		

*** = Annual, ** = Biennial and others species = Perennial.**

Crude protein (CP) and digestible crude protein (DCP)

Data of crude protein percentage (CP %) and digestible crude protein (DCP %) of plant species as influenced by site, season and year in wadi Halazien in both years presented in Tables 9 and 10.

Chenopodiaceae in top, *Euphorbiaceae* in ridge, *Zygophyllaceae* in bed 1 and *Peganaceae* in bed 2 gave

the highest CP % and DCP % in spring 2007. While, in spring 2008 *Fabaceae* gave the highest CP % and DCP % in all sites, except *Brassicaceae* in ridge and DCP % in top *Caryophyllaceae*. *Asteraceae* in top and bed 1, *Apiaceae* in ridge and *Fabaceae* in bed 2 gave the highest CP % and DCP % in autumn 2007, whereas, in autumn 2008 *Asparagaceae* in top, *Caryophyllaceae* in ridge and bed 2 and *Asteraceae* in bed 1 gave the highest CP % and DCP %. El-Morsy (2002) and Abdel-Gawad *et al.* (2009) mentioned that family *Fabaceae* appeared higher of CP % than other families in wadi Magid and El-Washka, respectively. Among perennial species such as *Haloxylon salicornicum* (Fig. 7) in top, *Euphorbia dendroides* in ridge, *Lotus polyphyllus* in bed 1 and *Peganum harmala* in bed 2 gave the highest CP % and DCP % in spring 2007.

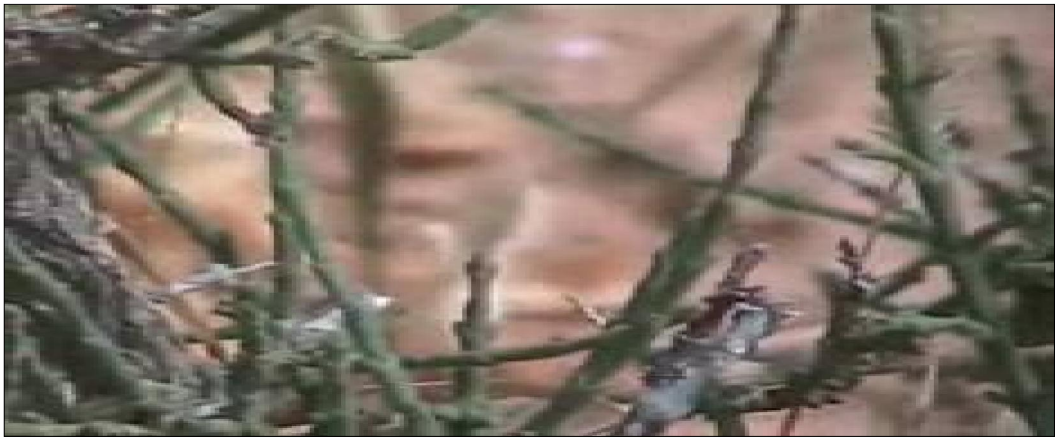


Fig. 7. *Haloxylon salicornicum*

In spring 2008 *Erodium crassifolium* in top, *Achillea santolina* in ridge, *Lotus polyphyllus* in bed 1 and bed 2 gave the highest CP % and DCP %. However, *Chiliadenus candicans* in top and bed 1, *Deverra tortuosa* in ridge, and *Retama raetam* in bed 2 gave the highest CP % and DCP % in autumn 2007. While, in autumn 2008 *Asparagus stipularis* in top, *Gymnocarpos decandrus* in ridge and bed 2, *Chiliadenus candicans* in bed 1 gave the highest CP % and DCP %. Among annual species *Anacyclus monanthos* in top, *Adonis dentata* in ridge, *Hordeum marinum* in bed 1 and *Avena fatua* in bed 2 gave the highest CP % and DCP % in spring 2007. While, in spring 2008 *Melilotus siculus* in top, *Matthiola longipetala* in ridge and bed 1, and *Medicago polymorpha* in bed 2 gave the highest CP % and DCP %, while, in autumn 2007 *Urospermum picroides* in top, *Senecio coronopifolius* in bed 1 and *Medicago polymorpha* in bed 2 gave the highest ones. Crude protein % and DCP % in spring exceeded it in autumn at all sites in both years. CP % and DCP % in spring significantly increased than it in autumn 2008 by 2.33 % and 1.86 %, respectively. Because the average of temperature was low (16.32 ^{o}C) and total precipitation was high (95 mm) in Novmber to April 2008 (Table 1). Plant species in spring 2007 in bed 2 significantly surpassed the top, ridge and bed 1 by 1.46, 1.36 for and 1.11 for CP % and by 1.35, 1.26 and 1.02 for DCP %, respectively. El-Morsy (2002) and Abdel-Gawad *et al.* (2009) concluded that crude protein of plants grown in wadi bed exceeded it.

Table 9. Crude protein (CP %) of plant species recorded in wadi Halazien during spring and autumn of 2007 and 2008

Scientific name	2007								2008							
	Top		Ridge		Bed 1		Bed 2		Top		Ridge		Bed 1		Bed 2	
	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au
Allium roseum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	8.10	--
Amaranthus viridis *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	6.30	--
Deverra tortuosa	6.35	3.97	6.03	6.65	4.21	4.18	7.41	4.83	6.38	3.01	--	3.90	6.80	4.66	6.58	6.60
Arisarum vulgare	--	--	--	--	--	--	--	8.17	--	--	--	--	--	--	--	--
Asparagus stipularis	--	--	--	--	--	--	--	--	--	5.79	--	--	--	--	--	--
Asphodelus aestivus	6.75	--	--	--	--	--	--	--	7.90	--	--	--	--	--	--	--
Achillea santolina	--	--	--	--	--	--	--	--	--	--	10.80	--	11.80	--	--	--
Anacyclus monanthos *	7.90	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Carduncellus eriocephalus	7.24	--	5.28	--	6.36	--	6.16	6.14	--	--	7.39	4.10	6.95	6.28	7.85	7.09
Carduus getulus *	--	--	--	--	--	--	--	--	5.23	--	--	--	--	--	--	--
Centaurea calcitrapa *	--	--	--	--	--	--	--	--	--	--	9.26	--	--	--	--	--
Chiliadenus candicans	--	6.90	--	--	8.94	8.13	8.52	7.68	--	--	--	--	--	8.00	--	--
Picris asplenioides *	--	--	--	--	--	--	--	--	6.47	--	8.18	--	8.25	--	8.10	--
Reichardia tingitana *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	10.23	--
Scorzonera undulata	--	--	--	--	--	--	--	--	--	--	6.80	--	--	--	--	--

Senecio coronopifolius *	5.46	--	--	--	--	7.65	--	7.10	--	--	--	--	--	--	--	--
Silybum marianum **	--	--	--	--	--	6.04	--	6.73	--	--	9.00	--	8.23	--	9.45	--
Leontodon tuberosus	--	6.26	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Urospermum picroides *	--	8.68	--	--	--	--	--	--	--	--	--	--	--	--*	14.23	--
Didesmus bipinnatus *	--	--	--	--	7.44	--	--	--	--	--	--	--	--	--	--	--
Diplotaxis acris *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	9.00	--
Matthiola longipetala *	--	--	--	--	--	--	--	--	--	--	10.85	--	13.25	--	--	--
Zilla spinosa	--	5.07	--	5.70	--	--	--	--	--	--	--	--	--	--	--	--
Gymnocarpos decandrus	6.54	6.26	7.25	6.43	--	--	--	--	9.49	5.33	9.60	8.88	--	--	--	12.49
Silene succulenta	--	--	--	--	--	--	--	--	--	--	8.27	--	--	--	--	--
Atriplex nummularia	--	--	--	--	--	--	8.52	--	--	--	--	--	--	--	7.87	4.48
Haloxylon salicornicum	7.43	--	--	--	--	6.28	11.90	--	--	5.36	--	--	--	5.60	4.30	9.59
Noaea mucronata	--	--	--	--	--	--	--	--	--	--	--	--	7.00	4.79	--	--
Fumana thymifolia	--	--	--	--	--	--	--	--	--	--	--	--	6.92	--	--	--
Helianthemum lippii	--	--	--	--	--	--	5.47	--	--	--	7.93	--	--	--	--	--

Table 9. (Cont.) (Cont.)

Helianthemum vesicarium	--	--	--	--	4.56	--	--	--	--	--	--	--	--	--	--	--
Cyperus rotundus	--	--	--	--	--	--	--	--	7.80	--	8.10	--	--	--	--	--
dendroides Euphorbia	--	--	8.76	--	--	--	8.98	--	--	--	--	--	--	--	8.85	--
Hippocrepis cyclocarpa *	4.95	--	5.55	--	5.06	--	--	--	--	--	--	--	--	--	--	--
Lotus polyphyllus	7.13	--	--	--	10.37	--	--	--	--	--	--	--	14.10	--	14.70	--
Medicago polymorpha *	--	--	--	--	--	--	--	16.60	--	--	--	--	--	--	19.50	--
Melilotus siculus *	--	--	--	--	--	--	--	--	15.05	--	--	--	--	--	--	--
Retama raetam	--	--	--	--	--	--	--	13.00	--	--	--	--	--	--	--	--
Erodium crassifolium	--	--	--	--	--	--	--	--	12.00	--	--	--	--	--	--	--
Leopoldia comosa	--	--	--	--	--	--	--	--	--	--	8.06	--	--	--	7.45	--
Phlomis floccosa	--	4.03	5.41	--	--	--	--	--	--	--	--	--	6.20	--	7.35	--
Salvia aegyptiaca	--	--	--	--	--	--	--	--	10.20	--	--	--	--	--	--	--
harmala Peganum	--	--	--	--	--	5.10	12.35	--	--	--	--	--	--	--	9.30	5.58
Aegilops kotschyi *	--	--	--	--	--	--	--	--	--	--	--	--	5.90	--	--	--
Avena fatua *	--	--	--	--	--	--	6.31	--	--	--	--	--	--	--	7.10	--
Bromus rubens *	--	--	--	--	--	--	--	--	7.87	--	8.35	--	7.85	--	8.47	--
Cutandia dichotoma *	--	--	--	--	--	--	--	--	6.90	--	--	--	6.90	--	7.90	--
Cynodon dactylon	--	--	--	--	--	5.08	--	4.20	--	--	--	--	--	--	--	--
Hordeum marinum *	6.38	--	--	--	7.89	--	--	--	--	--	--	--	--	--	7.35	--
Lamarckia aurea *	--	--	--	--	--	--	--	--	--	--	--	--	--	--	5.30	--
Lygeum spartum	--	--	--	--	--	--	4.65	--	--	--	5.10	--	--	--	--	--
Lophochloa cristata *	--	--	--	--	--	--	--	--	4.93	--	5.70	--	--	--	--	--
Phalaris minor *	--	--	--	--	--	--	--	--	7.33	--	6.68	--	8.01	--	7.35	--
Poa annua *	--	--	--	--	--	--	--	--	6.00	--	6.80	--	--	--	7.93	--
Polygonum equisetiforme	6.94	6.58	--	--	--	--	--	--	8.47	--	8.15	--	7.87	6.47	6.80	--
Adonis dentata *	--	--	8.40	--	--	--	--	--	--	--	--	--	--	--	--	--
Reseda decursiva *	--	--	--	--	--	--	--	--	4.24	--	--	--	--	--	5.74	--
Lycium shawii	5.75	4.78	--	5.43	--	6.71	--	--	--	--	--	2.38	4.42	--	--	--
Thymelaea hirsute	--	6.10	--	6.17	5.81	--	--	4.27	7.00	--	8.00	6.32	8.15	5.87	7.33	5.45
Urtica urens *	--	--	--	--	--	--	--	4.10	--	--	--	--	--	--	--	--
Fagonia scabra	--	--	--	--	8.56	--	--	--	--	--	8.50	--	--	--	--	--
Average sites	6.57 def	5.86 fg	6.67 cdef	6.07 ef	6.92 cde	6.15 ef	8.03 ab	7.53 bc	7.84 ab	4.87 h	8.08 ab	5.12 gh	8.15 ab	5.95 fg	8.53 a	7.33 bcd
Average seasons		Spring = 7.048 AB				Autumn = 6.403 AB					Spring = 8.150 A				Autumn = 5.818 B	

This is not analyzed for protein percentage because there was no enough dry weight for analysis. * = Annual, ** = Biennial and others species = Perennial.

*** These figures were not calculated because crude protein percentage less than 3.81%. * = Annual, ** = Biennial and others species = Perennial.**

Correlation coefficient

Tables (11) show all traits had insignificant differences with climatic factors. Shows that abundance, fresh yields, dry yields and chemical compositions had a positive correlation with precipitation, and a negative correlation with temperature and relative humidity

Table 11. The correlation coefficients between some climatic factors and chemical composition, some vegetation measurements, fresh and dry yields recorded in wadi Halazien.

Characteristics / Climatic factor	Vegetation measurements	Foliage yield (g m^{-2})		Chemical composition (%)	
	Abundance	Fresh	Dry	CP	DCP
Precipitation	+ 0.809	+0.933	+0.916	+0.894	+ 0.889
Temperature	- 0.711	-0.867	-0.844	- 0.843	- 0.826
Relative humidity	- 0.739	-0.594	-0.610	- 0.247	- 0.327

References

1. **Abdel-Gawad, K. I.; El-Deak M. H.; El-Morsy M. H. M. and Kulaib G. F. (2009).** Natural vegetation of wadi El- Washka in the North West Coast of Egypt. Assiut Univ. J. Botany, (1): pp 237-262.

2. **Ambshat, R. S. (1982).** Plant Ecology, 6th edition, Students -Friends and Co Lanka, Varanasi, India, 324 p.

3. **O. A. C. (2005).** Association of official analytical chemists, official methods of analysis, International, ed. W. Horwitz, 18th ed., MD, USA, 1 (10): pp 10-17.

4. **Boulos, L. (1999).** Flora of Egypt (Azollaceae-Oxalidaceae). Vol. 1, Al-Hadara Pub., Cairo, Egypt, 419 p.

5. **Boulos, L. (2000).** Flora of Egypt (Geraiaceae-Boraginaceae). Vol. 2, Al- Hadara Pub., Cairo, Egypt, 352 p.

6. **Boulos, L. (2002).** Flora of Egypt (Verbenaceae-Compositae). Vol. 3, Al-Hadara Pub., Cairo, Egypt, 373 p.

7. **Boulos, L. (2005**). Flora of Egypt (Monocotyledons). Vol. 4, Al-Hadara Pub., Cairo, Egypt, 617 p.

8. **Chapman, H. D. and Pratt, P. F. (1961).** Methods of analysis for soils, Plants and Water. Univ. of Calif. USA.

9. **De Ridder, N.**; **Sroosijder, L.; Cisse A. M. and Van Keulen, H. (1982).** A study of the soils, the vegetation and the exploitation of that natural resource. PPS course book, vol. 1, Wageningen: Agric. Univ., 231 p. Cited by Heneidy (2002).

10. **El-Morsy, M. H. (2002).** Studies on range plants in wadi Magid and wadi Mahgen in the North West Coast of Egypt. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt, 206 p.

11. **Gab Allah, M. S. A. (2006).** Ecological and chemical studies on some common rangeland plants in Egypt and Libya. M. Sc. Thesis, Inst. of Africa Res. and Studies, Cairo Univ., Egypt. pp 57-110.

12. **Hammer, Y. V.; Harper, D. A. T. and Ryan, P. D. (2001).** PAST: Palaeontological Statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9 p.

13. **Ibrahim, K. M. A. (1995).** Productivity and nutritive value of some range plants of the North Western Coast. M.Sc. Thesis. Fac. Agric., Ain Shams Univ., Egypt.110 p.

14. **Ludwig, J. A. and Reynolds, J. F. (1988).** Statistical ecology: a Primer on Methods and Computing. John Wiley and Sons Inc., New York, U.S.A, 337 p.

15. **Reiad, M. Sh.; Ashoub, M. A.; Abo-Deya, I. B.; El-Hakeem, M. S.; El-Basiouny, A. Z. and Ahmed, K. M. (1996 b).** Productivity and nutritive value of some range communities at the North-Western Coast of Egypt 1-*Thymelaea hirsuta* (L.) Endl. Egypt J. Appl. Sci., 11(3). pp 146-163.

16. **SAS. (1988).** Inst. SAS/STAT User guide. SAS Institute, Inc., Cary, NC.

17. **Shahba, M. A. M. (1994).** Studies on range ecosystems of the Libyan desert. M.Sc. Thesis, Fac. Sci., Inst. of Africa Res. and Studies, Cairo Univ., Egypt. 126 p.

18. **Simpson, E. H. (1949).** Measurement of diversity. Nature, 163:688. Cited in http://www.wku.edu/smithch/biogeog/SIMP1949.htm.

19. **Steel, R. G. D.; Torrie, J. H. and Dickey, D. A. (1997).** Principles and Procedures of Statistics: A biometrical approach. 3rd ed. McGraw-Hill New York, Book Co.

20. **UNESCO, (1977).** Map of the world distribution of arid regions MAB Technical Notes, 7. Paris

5/1/2010

Morphological and Isozyme diversity in the accessions of two cultivated species of barnyard millet

Deepti prabha[1], Y. K. Negi[2], V. K. Khanna[1]

1. College of Agriculture, G. B. Pant University of Ag. and Tech, U. S. Nagar (Uttarakhand), India;
2. SBS PG Institute of biomedical Sciences and Research, Balawala, Dehradun (Utarakhand), India
deepti_prabha@rediffmail.com; yknegi@rediffmail.com; vkkhanna@rediffmail.com

Abstract: Present study was conducted to access genetic diversity among 33 accessions of two cultivated species of barnyard millet i.e., *Echinocloa crus-galli* (15 accessions) and *E. frumentacea* (18 accessions). Though crop possesses great nutritional value, little attention has been paid for the improvement of this crop. Peroxidase and esterase analysis showed seven loci possessing 23 alleles in all 33 accessions. Esterase was found to be more useful to assess diversity with more polymorphism in comparison to peroxidase. Based on allozyme frequencies, all the accessions of both the species were grouped separately in to two different groups at a linkage distance of 6.2. The data would be important in detailing the level of variation and relationship within and between species to plan future domestication trials and to manage the wild species collection that is available in the gene banks. [Nature and Science 2010;8(7):71-76]. (ISSN: 1545-0740).

Key words: Barnyard millet, genetic diversity, isozyme analysis, peroxidase, esterase

1. Introduction

Barnyard millet (*Echinochloa* sp.) is one of the oldest domesticated millets in the semi-arid tropics of Asia and Africa. The genus *Echinochloa* includes some 20 species that are distributed widely in the warmer parts of the world. Two of the main species, *E. crus-galli* and *E. frumentacea* are grown as cereals. In addition to these two domesticated species, the genus includes about 30 annual and biennial wild species distributed worldwide (Clayton and Renvoize, 1986). These millet species are morphologically very dissimilar. Indian barnyard millet (*E. frumentacea*) can easily be distinguished from Japanese barnyard millet (*E. crus-galli*) by its panicle, thinner texture of the glumes and lower lemma (Yabuno,1971).

The crop is valued for its drought tolerance, good yield and superior nutritional value. It is the fastest growing crop among all millets and can be harvested in a short period of nine weeks. Barnyard millet is an important dual-purpose crop. Its grains contain 6.2 % protein, 9.8 % crude fiber, 65.5 % carbohydrates and are consumed just like rice (Ruiz-santaella *et al*., 2006). Also it is a nutritive fodder for animals. These aspects make barnyard millet a valuable crop.

But, very little attention has however been paid for the genetic improvement of this crop towards augmenting its yield potential. Heterogeneity of environment and exposure to low temperature at higher elevation causes economic loss in both grain and fodder yields of barnyard millet cultivars. Moreover, in absence of wide genetic diversity among local cultivars and released varieties, the selection for adaptation to cold temperature is discouraging (Gullord *et al.,* 1975).

Genetic diversity of common morphological traits is difficult to measure in a natural population since the traits are influenced by environmental factors to a large degree. On the other hand, their expression is largely governed by different interacting genes. Complementing the use of morphological markers, molecular techniques for evaluating genetic diversity have been improved in the last decades Measurement of genetic diversity with molecular markers is relevant to assessment of ecological conditions because it allows estimation of important population parameters e.g., characterization of the geographic structure or connectivity of populations. Molecular markers such as SDS-PAGE and isozymes have been found more useful to study genotypic diversity in many plant species (Tanksley *et al*., 1989; Paterson *et al*.,1991).

In the present study we evaluated genetic diversity among 33 different accessions of two cultivated species of barnyard millet (*E. crus-galli* and *E. frumentacea*) on the basis of morphological and biochemical markers. Results would be helpful to identify their role in crop improvement by identifying the diverse accessions.

2. Materials and methods

2.1 Plant material

Two sets of different accessions of both the species (*E. crus-galli* and *E. frumentacea*) were examined. 15 accessions of *E. crus-galli* were procured from ICRISAT, Hyderabad (Andhra Pradesh, India) and 18 accessions of *E. frumentacea* were collected from VPKAS, Almora (Uttarakhand, India).

2.2 Morphological characters

All the 33 genotypes were sown in the fields in a Randomized Block Design (RBD) at G.B.P.U.A. & T., Hill Campus, Ranichauri, Tehri Garhwal (Uttarakhand). Crop management was done according to the recommended agronomic practices. Sowing was done in the plots (3m × 1m), plant to plant distance was maintained 10 cm., while row to row distance was kept 22.5 cm. Eight morphological characters, germination percentage, days to 50% flowering, plant height, no. of fertile tillers, length of spikelet, days to maturity, 1000 grain wt and yield per plant were taken to assess genetic variability in accessions.

2.3 Isozyme analysis

Peroxidase and esterase were isolated to assess the genetic variability. For the isolation of both the isozymes, fresh leaves of plants were collected and washed in tap water followed by distilled water. Water droplets were removed with the help of tissue paper. 500 mg leaves were weighed and crushed in minimum amount of extraction buffer (0.1 M tris + 2% β-mercaptoethanol) with the help of mortar and pestle under chilled conditions. The extracts were quickly transferred to pre-cooled eppendorf tubes and kept in a refrigerator. Tubes were centrifuged at 12000 rpm for 30 min at 4^0C in a refrigerated centrifuge. The supernatant was transferred to fresh tubes. 1 part extract: 1 part glycerol: 1 part bromophenol blue (0.05 mg/ml) were mixed and then the samples were stored at -20^0 C for further use.

The experiments were carried out in order to characterize the genetic variability by Native Polyacrylamide Gel Electrophoresis (PAGE). The staining procedure for peroxidase and esterase isozyme was done according to method by Wendel and Weeden (1989) with minor modifications.

2.4 DATA analysis

Field data for morphological characters was evaluated by the analysis of variance (ANOVA) using RBD to calculate the significance by magnitude of F value (P= 0.01) and D^2 statistics as suggested by Rao (1952) using computer software. The calculation of D^2 values involved the steps followed by Murthy and Arunachalam (1996).

For isozyme analysis Pair-wise similarity and cluster analysis were done on the basis of presence and absence of bands. Squared Euclidean distances among the accessions were calculated by computer software (Statistica) to perform the similarity matrix analysis using unweighted pair-group method.

3. Results

3.1 Estimation of genetic diversity based on morphological characters

On the basis of D^2 analysis, all the accessions were grouped into three clusters (Table-1). Cluster-I comprised of accessions of *E. frumentacea,* cluster-II consists of *E. crus-galli*, while cluster-III had 2 accessions of *E. crus-galli* and rest 10 were of *E. frumentacea* (Table-1).

Table 1: D^2 statistics* based clustering pattern and average inter and intra cluster d values of the different accessions of two different species of *Echinochloa*.

Clusters	Accessions grouped in different clusters	d values of Clusters		
		I	II	III
I	VRS-MB-1554, VRS-MB-1839, VRS-MB-886, VRS-MB-846, VRS-MB-1361, VRS-MB-1535, VRS-MB-1377, VRS-MB-871 (n=8)	1.76		
II	IEC-530, IEC-531, IEC-540, IEC-545, IEC-546, IEC-547, IEC-548, IEC-549, IEC-555, IEC-556, IEC-542, IEC-533, PRB-9404 (n=13)	3.40	2.25	
III	IEC-535, IEC-538, VRS-MB-1202, VRS-MB-893, VRS-MB-889, VRS-MB-1506, VRS-MB-882, VRS-MB-1546, VRS-MB-1508, VRS-MB-1543, VRS-MB-858, VRS-MB-1372 (n=12)	2.50	3.41	1.91

* The D^2 analysis was done according to Murthy and Arunachalam, 1996.

Eight different morphological characters (Germination percentage, Days to 50 percent flowering, Plant height, Number of fertile tillers, Panicle length, 1000 seed weight, Days to maturity and Yield per plant) were considered to study genetic diversity.

IEC and PRB: Accession number for the accessions of *E. crus-galli*, VRS-MB: Accession number for the accessions of *E. frumentacea.*

On the basis of D^2 analysis, all the accessions were grouped into three clusters (Table 1). Cluster-I comprised of accessions of *E. frumentacea,* cluster-II consists of *E. crus-galli,* while cluster-III had 2 accessions of *E. crus-galli* and rest 10 were of *E. frumentacea* (Table-1).

The average intra- and inter-cluster genetic distance (d values) shown in Table 1. Inter-cluster centeroids distance ranged from 0.00 to 3.14 and Intra-cluster distance ranged from 1.76 to 1.9. Maximum intra-cluster distance was observed in cluster-II (D=2.25) followed by cluster-III (D=1.91) and cluster-I (D=1.77). Maximum inter cluster centeroids distance was observed in between cluster-III and cluster-II (D=3.41) followed by cluster-I and cluster-II (D=3.40).

The cluster means of various agronomic traits are presented in Table 2. Cluster-III, which consists of 13 accessions, showed maximum average value of germination percentage (92.62 %), followed by cluster-II (91.49 %) and cluster-I (89.83 %). Maximum average value of days to 50 % flowering was shown by cluster-II i.e. 83.65 followed by cluster-III and cluster-I. Maximum average plant height was observed in cluster-III (186.56) followed by cluster-I (173.25) and cluster-II (135.40). Cluster-II showed the maximum mean value of number of fertile tillers (3.43), followed by cluster-III (3.26), while cluster-I showed the least mean value for number of fertile tillers. Maximum average seed weight was shown by cluster-II (4.26), followed by cluster-I (3.51) and cluster-III (2.88).

Table 2: Inter and intra species diversity among *E. frumentacea and E. crus-galli* accession on the basis of isozyme (Esterase and Peroxydase) analysis

Accessions	P	A	A_P	H_T
All	54.50	3.28	3	10
E. frumentacea	22.75	3	0.57	6.2
E. crus-galli	45.45	2	0.57	5.4

P- Percent Polymorphic Loci, **A-** Mean number of alleles per locus, **A_P-** Mean number of alleles per polymorphic loci, **H_T-** Total genetic similarity based on linkage distance. Each of the gel was run three times to confirm best reproducible results.

Cluster-II with 13 accessions exhibited highest mean value of days to maturity (128.62) followed by cluster-I (123.71) and cluster-III (123.71). Least mean value of days to maturity was shown by cluster-III. Mean value of highest Yield/ plant was shown by cluster-II (2.06) followed by cluster-III (1.25) and cluster-I (1.16).

3.2 Genetic diversity based on Isozyme markers

A total of 23 alleles were observed at the seven Isozyme loci in all 33 accessions of barnyard millet. Mean number of alleles per locus was 3.28 (Table 2). All allelic distribution is shown in Figure 1,2 . Both the species of barnyard millet i.e. *E. frumentacea* and *E. crus-galli* were clearly separated on the basis of both the Isozyme systems.

3.2.1 Peroxidase

Three loci were observed and named as Per-1, Per-2 and Per-3. Per-1 was monomorphic among the studied loci (Figure 1). The other two loci were polymorphic. Two alleles (Per-2^a and Per-2^b) were observed at Per-2 locus. Allele Per-2^a was polymorphic and was present only in the accessions of *E. crus-galli.* Four alleles were detected at Per-3 locus (Per-3^a, Per-3^b, Per-3^c and Per-3^d). Allele Per-3^c was common among all the accessions. Allele Per-3^a was present in all the 15 accessions of the species *E. crus-galli,* while Per-3^c and Per-3^d were present among all 18 accessions of *E. frumentacea.*

3.2.2 Esterases

Esterases showed abundant genetic variability among and within the barnyard species. Four loci (Est-1, Est-2, Est-3 and Est-4) were detected (Figure 2). Locus Est-1 was highly diverse. Four alleles were observed at Est-1 locus (Est-1^a, Est-1^b, Est-1^c and Est-1^d). Est-1^a allele was present in the accessions VRS-MB-886, IEC-535, IEC-538, IEC-548, VRS-MB-893, VRS-MB-846, VRS-MB-889, VRS-MB-1508, VRS-MB-1361, VRS-MB-1372, and VRS-MB-871.

Locus Est-2 showed three alleles i.e. Est-2^a, Est-2^b and Est-2^c. Two alleles (Est-2^a and Est-2^c) were present in all the 18 accessions of *E. frumentacea* and were absent in the accessions of *E. crus-galli*. Five alleles were detected at Est-3 locus ((Est-3^a, Est-3^b, Est-3^c, Est-3^d and Est-3^e). Est-3^a and Est-3^c were polymorphic and present in the accessions of *E. frumentacea*. Total three alleles were detected at locus Est-4 (Est-4^a, Est-4^b, Est-4^c and Est-4^d). Only allele Est-4^c was polymorphic and was present in the accessions of *E. crus-galli.*

Dendrogram was prepared on the basis of banding pattern obtained by both the isozyme patterns. Accessions of both the species (*E. frumentacea* and *E crus-galli*) were grouped into two groups at a linkage distance of 10 (Fig- 3). Again within the species of *E. frumentacea*, all the accessions were grouped at a linkage distance of 6.2 and were separated into two groups. Other branch of the tree, which separates accessions of *E crus-galli*, again divided into two subgroups at a 10linkage distance of 5.4.

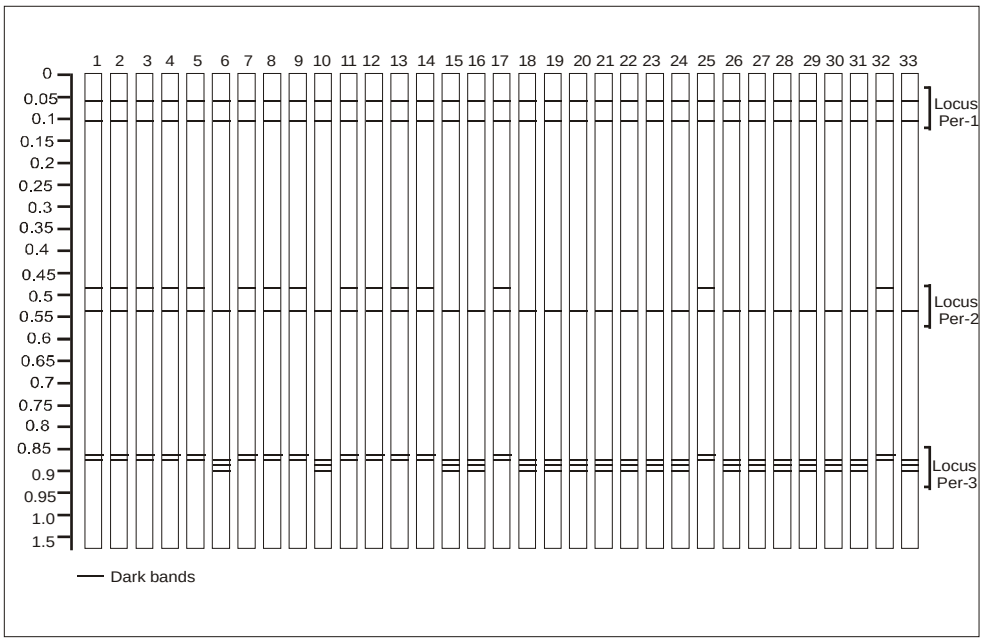


Figure 1: Zymogram of protein profile of thirty-three barnyard millet accessions obtained through peroxidase. (1. IEC-549, 2. IEC-555, 3. IEC-556, 4. IEC-546, 5. IEC-531, 6. VRS-MB-886, 7. IEC-535, 8. IEC-538, 9. IEC-547, 10. VRS-MB-1546, 11. IEC-335, 12. IEC-545, 13. IEC-542, 14. IEC-584, 15. VRS-MB-893, 16. VRS-MB-1377, 17. IEC-530, 18. VRS-MB-1554, 19. VRS-MB-1262, 20. VRS-MB-882, 21. VRS-MB-1506, , 22. VRS-MB-1839, 23. VRS-MB-1535, 24. VRS-MB-1542, 25. IEC-543, 26. VRS-MB-846, 27. VRS-MB-889, 28. VRS-MB-1508, 29. VRS-MB-1361, 30. VRS-MB-1372, 31. VRS-MB-871, 32. IEC-533, 33. VRS-MB-1510).

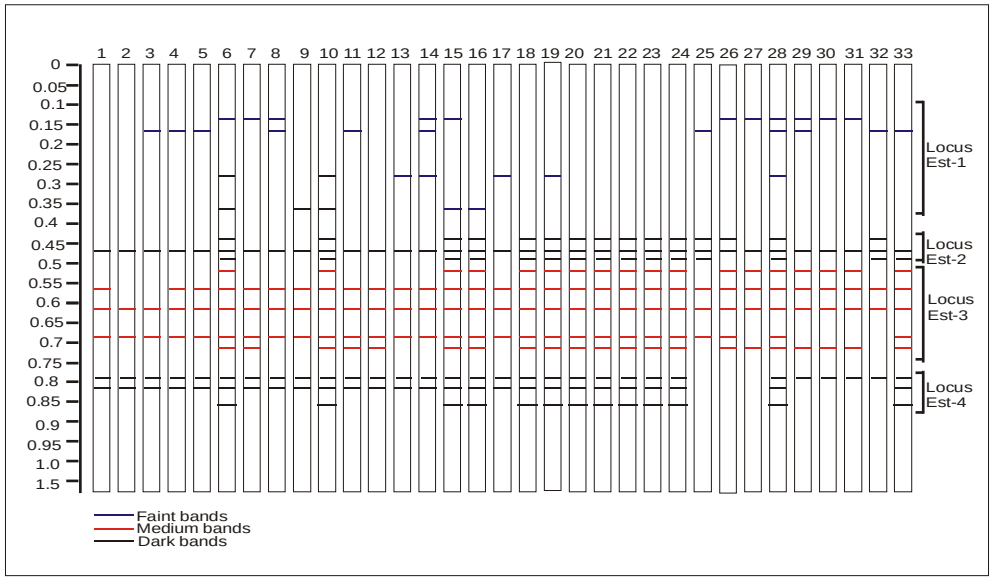


Figure 2: Zymogram of protein profile of thirty-three barnyard millet accessions obtained through esterases. (1. IEC-549, 2. IEC-555, 3. IEC-556, 4. IEC-546, 5. IEC-531, 6. VRS-MB-886, 7. IEC-535, 8. IEC-538, 9. IEC-547, 10. VRS-MB-1546, 11. IEC-335, 12. IEC-545, 13. IEC-542, 14. IEC-584, 15. VRS-MB-893, 16. VRS-MB-1377, 17. IEC-530, 18. VRS-MB-1554, 19. VRS-MB-1262, 20. VRS-MB-882, 21. VRS-MB-1506, , 22. VRS-MB-1839, 23. VRS-MB-1535, 24. VRS-MB-1542, 25. IEC-543, 26. VRS-MB-846, 27. VRS-MB-889, 28. VRS-MB-1508, 29. VRS-MB-1361, 30. VRS-MB-1372, 31. VRS-MB-871, 32. IEC-533, 33. VRS-MB-1510).

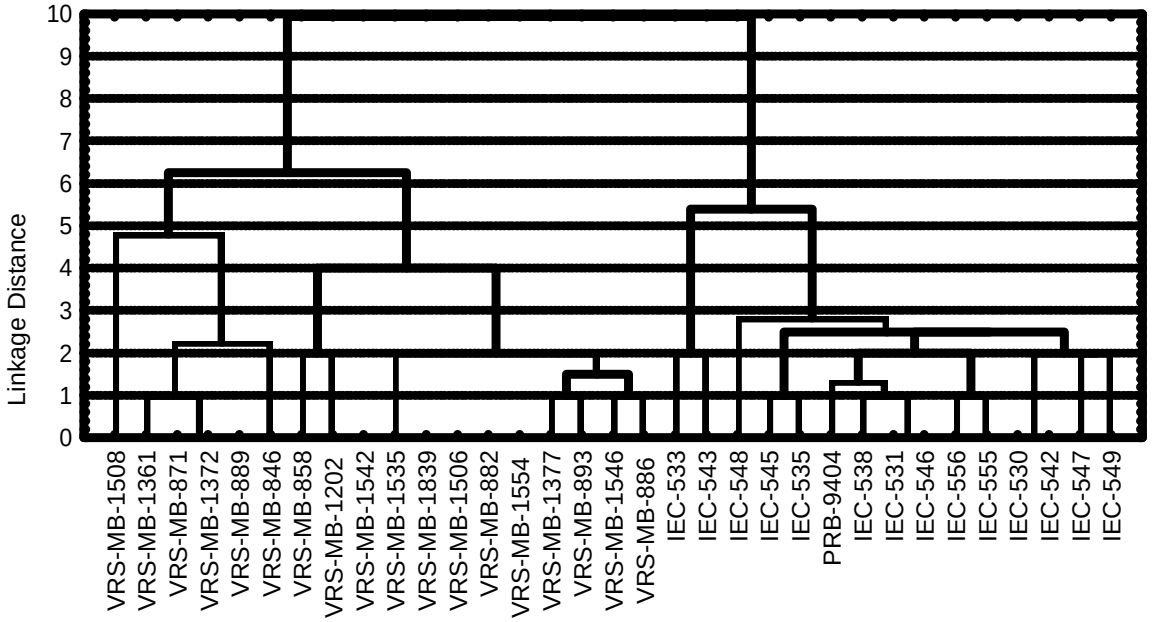


Figure 3: Cluster analysis of 33 accessions of Barnyard millet (belonging to two cultivable species, *Echinochloa crus-galli* and *Echinocloa frumentacea*) on the basis of banding pattern of two isozymes (Peroxidase and Esterase). The unweighted pair-group method, arithmetic mean (UPGMA) algorithm was applied to calculate Squared Euclidean Distance using computer software (Statistica).

4. Discussion

On the basis of D^2 analysis, the 33 accessions were grouped into three clusters (Table-1). Comparison of these morphological clusters revealed that maximum intra-cluster distance (D=2.25) was observed in cluster-II whereas, maximum inter cluster centeroids distance (D=3.41) was observed in between cluster-III and cluster-II (Table 1). It has previously been suggested that genetic drift and selection in different environments can produce greater diversity (Raje and Rao 2001). We observed that accessions of *E. crus-galli* showed genetic superiority to *E. frumentacea* in different agronomic characters. The findings get support by Bandyopadhyay 1998, 1999. He reported that the accessions of *E. crus-galli* were better adapted for Uttarakhand hills and were promising for high grain yield. Average inter-cluster and intra-cluster distance (D) values presented in Table 1 indicated that the maximum variability was present in cluster-II and cluster-III for agronomic characters. So the clusters showing greater genetic diversity can be used in further crop improvement programme. Several workers suggested selection of parents for hybridization from two clusters having wide inter-cluster and intra-cluster distance to get maximum heterosis (Pradhan and Rao 1990; Mehta et al., 2005).

In isozyme analysis, peroxidase was able to differentiate both the species but, it was not able to differentiate accessions of the same species. Among the three loci genetic diversity per locus for peroxidase followed the trend, Per-3> Per-2> Per-1. on the other hand, esterases showed large number of alleles and polymorphism to differentiate the two species as well as accessions of the same species. Maximum diversity was observed in locus Est-1, which differentiated accessions, while the other loci differentiate two species (*E. frumentacea and E crus-galli*).

Variation at isozyme loci revolutionized the research and evolution (Schwartz 1969; Wendel and Weeden 1989; Sachs et al., 1996,). Only the polymorphic bands are actually of use in genetical, physiological or taxonomical studies (Bassiri 1976). Similarity coefficient based on the Isozyme data showed that the linkage distance ranged from 0 to 10. Grouping of accessions near to 0 indicates the close similarity among them, whereas the increase in linkage distance to 9.9/10 tends to differentiate two species into two groups. Group 1 consisted of all the accessions of *E. frumentacea*, whereas accessions of *E. crus-galli* were grouped into group 2. The findings of our study get supporte by Werth et al. (1994). They studied the isozyme variation in finger millet species and reported the distinct allelic composition between two species.

The maximum variability was present in cluster-II and cluster-III for agronomic characters. The accessions from these clusters can be used in further hybridization programme. In isozyme analysis, peroxidase was able to differentiate both the species but, it was not able to differentiate accessions of the same species. Esterases showed large number of alleles and polymorphism to differentiate the two species as well as accessions of the same species.

Selection of better species can be made for species improvement on the basis of percent similarity with other species. Two more similar but possessing distinct characters can be chosen for the purpose.

Acknowledgment: We are thankful to Dr. R. Prasad, Dr. Arun Kumar for providing seed material, Dr. Usha for assistance.

For correspondence: Dr. Deepti Prabha
Deptt. of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Ag. and Tech, U. S. Nagar (Uttarakhand), India
Mob:09720849962
E-mail: deepti_prabha@rediffmail.com

References

1. Bandyopadhyay BB. A study on variability in sink capacity and grain yield of barnyard millet (*Echinochloa frumentacea).* Annals of Agriculture Research 1998; 19:34-8.
2. Bandyopadhyay BB. Genotypic difference in relation to climate adaption of two cultivated barnyard millet species at Garhwal hills. Indian Journal of Genetics and Plant Breeding 1999; 59: 105-8.
3. Bassiri A. Barley cultivar identification by use of Isozyme electrophoretic patterns. Canadian Journal of Plant Science1976; 56: 1-6.
4. Clayton W D, Renvoize S A. Genera Geranium. Kew bull. Add. Ser. 1986; 13: 280-1.
5. Gullord M, Olien CR, Everson EH. Evaluation of freezing hardness in winter wheat. Crop Science 1975;15:153-7.
6. Murthy BR, Arunachalam V. The nature of genetic divergence in relation to breeding system in some crop plants. Indian Journal of Genetics 1996;26: 188-8.
7. Paterson AH, Tanksley SD, Sorrels ME. Marker in plant improvement. *Advances of Agronomy* 1991; 46: 39-90.
8. Rao B, Murty VAR, Subramanya RJ. The amylase and amylopectin content of rice and their influence on cooking quality of cereals. Proc. Indian Academy of Science1952; 36: 70-4.
9. Ruiz-Santaella, Bastida JP, Franco F, De Prado RAR. Morphological and Molecular Characterization of Different *Echinochloa* spp. and *Oryza sativa* Populations. J. Agric. Food Chem 2006;54:1166–72.
10. Schwartz D. An example of gene fixation resulting from selective advantage in suboptimal conditions. American Naturalist 1969; 103:479-81.
11. Tanksley SD, Young ND, Paterson AH, Bonierbale MW. RFLP mapping in plant breeding: new tool for an old science. Biotechnology 1989;7:257-64.
12. Wendel JF, Weeden NF. Visualization and interpretation of plant isozymes. In : Soltis, D. E., Soltis, P. S., (Eds.), Isozymes in plant biology. Discorides, Portland, OR, 1989; pp. 5-45
13. Werth CR, Hilu KW, Langher CA. Isozyme of *Eleusine* (Graminae) and the origin of finger millet. *American Journal of Botany* 1994;18:1186-97.
14. Yabuno T. A note on barnyard millet. *SABURAO News letter*1971; 3:43-5.

5/5/2010

Cyanobacteria of a Tropical Lagoon, Nigeria.

Adesalu, Taofikat Abosede [1], Nwankwo, Dike Ikegwu.[2]

[1]Department of Botany and Microbiology, University of Lagos, Nigeria.
[2]Department of Marine sciences, University of Lagos, Nigeria.
boseadesalu@yahoo.com.

Abstract: Investigations for the first time into the blue green algae of Lekki lagoon were carried out for 24 months (June 2003- May 2005) at monthly intervals using standard plankton net of mesh size 55µm. One hundred and seventy nine species belonging to thirty genera were observed. The filamentous blue green algae *Oscillatoria* formed the most abundant genus making up twenty three species followed by *Phormidium* eighteen species. *Anabaena* and *Chroococcus* recorded thirteen species each while the genera, *Gleocapsa, Merismopedia* and *Microcystis* recorded ten, eight and twelve species respectively. Only one genus each of *Cyanosarcina, Calothrix* and *Scytonema* were encountered. Bloom forming species identified were *Microcystis aeruginosa, M. flos-aquae, M. wesenbergii* and *Anabaena flos-aquae.* In this study, thirty-nine new species were recorded for Lagos lagoon complex in which Lekki lagoon is one of it while *Cyanosarcina hueberliorum* is new record for Nigeria. [Nature and Science 2010;8(7):77-82]. (ISSN: 1545-0740).

Key words: Cyanophytes, tropical, bloom, Lagos lagoon complex

Introduction

The coastline of South Western Nigeria is a meandering network of lagoons and creeks of which Lagos lagoon with an area of 208sqkm is the largest (Nwankwo 1989).The geography and hydrology of various parts of Lagos lagoon complex in which Lekki lagoon is one it have been described by several workers. These include Lekki lagoon (Ikusemiju 1973); Lagos lagoon (Hill and Webb 1958) and harbour (Olaniyan 1957). Checklists of planktonic algae in some parts of Nigeria have been documented by different workers. For instance in the North, Holden and Green (1960) studied the phytoplankton of River Sokoto while Khan and Agugo (1990) studied Kongiri dam, Jos mine lakes was studied by Anadu et al. (1990).

In Southern region, studies include Opute (1990,1991,1992) who studied Warri Forcados estuary phytoplankton, New Calabar river by Nwadiaro and Ezefili (1986). Biswas (1984, 1992) had report for eastern region while western region reports include that of Imevbore (1968) on Eleiyele reservoir, Egborge and Sagey (1979) on Ibadan freshwater ecosystem. Nwankwo (1988) studied the planktonic algae of Lagos lagoon, Nwankwo (1993) reported eight cyanobacteria bloom species of coastal waters in South Western Nigeria excluding Lekki lagoon, Nwankwo (1997) reported dinoflagellates list of Lagos lagoon. Adesalu and Nwankwo (2005, 2009) reported the diatoms of Olero creek and Lekki lagoon respectively, Wujek et al. (2003) studied the chrysophytes of Lekki lagoon while Kadiri (1989, 1993, 1999, 2000) reported the rich flora of *Micrasterias*, desmids, algae composition and euglenoids of Ikpoba reservoir respectively.

Of the entire aforementioned checklist, none specifically reported the cyanobacteria checklist in Nigeria coastal waters. The present study was undertaken to investigate the composition of cyanobacteria species of Lekki lagoon for possible biological monitoring since the lagoon is a source of fish supply for people of South Western states and beyond.

Description of study area

Lekki lagoon (Fig.1) a large expanse of shallow freshwater extends between Lagos and Ogun states. It covers an area of about 247km^2. A greater part of the lagoon is shallow (<3.0 m), while some areas are up to 6.4m deep. It lies between longitudes $4^0 00'$ E and $4^0 12'$ E and latitude $6^0 25'$ N and $6^0 37'$ N. The lagoon is fed by river Oni in the north eastern part, while rivers Osun and Saga flow into the north western part. Two peaks of rainfall are associated with this lagoon, a major peak in July and a lesser peak in September. There are two peaks of sunshine hours which approximately correspond to the equinoxes. The mainstay of communities that live around this environment is artisanal fishing.

Materials and methods

Collection of sample

Biological samples were collected monthly from twelve stations (Table 1) using Hydrobios plankton net of 55µm mesh size. For quantitative analysis 5litres of the water was concentrated. Biological samples were

preserved in 4% unbuffered formalin. Identification was done using Olympus BX51 photomicroscope. Water samples were collected into clean plastic containers for chemical analysis while *in situ* measurements of temperature, transparency, pH and depth were made.

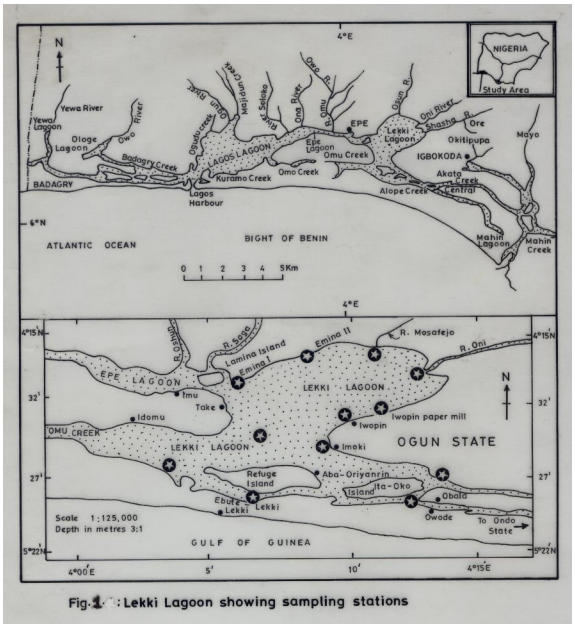


Fig.1 : Lekki Lagoon showing sampling stations

Table 1 : Average Depth (M) And Geographical Position Of Sampling Stations

STATIONS	Average depth	Longitudes	Latitudes
Emina 1	2.77	$4^0$5.080E	$6^0$32.754N
Emina II	1.61	$4^0$7.511E	$6^0$34.07N
Entrance of River Mosafejo	1.51	$4^0$10.239E	$6^0$35.344N
Entrance of River Oni	1.80	$4^0$12.153E	$6^0$35.090N
Iwopin 1	1.80	$4^0$13.153E	$6^0$32.309N
Iwopin 11	2.69	$4^0$9.651E	$6^0$32.137N
Imoki	2.17	$4^0$10.048E	$6^0$31.253N
Ise 1	1.81	$4^0$13.413E	$6^0$26.833N
Ise 11	2.41	$4^0$9.788E	$6^0$26.181N
Ebute lekki	1.29	$4^0$5.353E	$6^0$26.685N
Entrance of Omu creek	1.88	$4^0$7.604E	$6^0$28.867N
Lagoon centre	2.23	$4^0$3.348E	$6^0$28.577N

Physical and chemical analysis of water sample

The methods described by America Public Health Association (APHA 1998) were used for physical and chemical analysis. The air and surface water temperature were measured in situ with a simple mercury thermometer while the transparency was measured using a 20cm diameter Secchi disc. The depth was measured with a calibrated pole and the water pH determined using a Phillips pH meter (Model PW950). The chemical factors determined include Salinity, conductivity, dissolved oxygen (DO) and biological oxygen demand (BOD_5). Salinity was determined using the Silver Nitrate Chromate titration method as described by BARNES (1980) while Dissolved oxygen content was determined using a Griffin oxygen meter. Oxygen saturation was recorded in percentage. Biological Oxygen Demand is the measure of the amount of dissolved oxygen that could be depleted from the water body during natural biological assimilation or degradation of organic compounds by the organisms present especially bacteria. This was done after the dissolved oxygen had been measured using the standard method of biochemical consumption of oxygen in 5 days at 20^0C while conductivity was determined using the HANNA instrument (H18733), a wide range conductivity meter that has salinometer range in µS. Conductivity values were recorded as $mScm^{-1}$ at 25^oC (APHA 1998). The department The Federal Meteorological Department, Oshodi, Lagos kindly provided rainfall and sunshine hours data for the period of investigation (Table 2)

Nutrient determination

For nitrate determination, Hach Cadmium reduction method was used (APHA 1998). Phosphate-phosphorus is known to be important in a number of ways, one being that it facilitates the uptake of nitrogen. It was determined by ascorbic acid method. The values obtained were recorded in milligrams per litre (mgl^{-1}) (APHA 1998) (Table 2)

Results

Water chemistry

The physical and chemical characteristics of the study area are presented in Table 2.The mean pH of the water with a range of 7.41-7.46 indicated that the system is highly buffered. Conductivity which is the numerical expression of the ability of a solution to carry an electric current represents the total ions of water ranged between 0.47-0.56 μScm^{-1} the lowest value for phosphate-phosphorus, nitrate-nitrogen and sulphate were 2.42, 2.70 and 0.002 mgL^{-1}. Salinity recorded the least value of 0.40‰ while chloride ion had the highest value of 10.00 mgL^{-1}.

Table 2: Mean physico-chemical values for Lekki lagoon (concentrations in mg L^{-1})

STATIONS	A	B	C	D	E	F	G	H	I	J	K	L
Surface Water tempemperature (^{0}C)	30.54	30.73	30.55	30.61	30.40	30.31	30.35	30.23	30.38	30.63	30.70	30.55
Air tempemperature (^{0}C)	29.68	29.60	29.60	29.71	29.51	29.44	29.55	29.08	29.29	28.90	29.27	29.21
Transparency (cm)	5.74	7.38	11.33	9.25	11.67	10.29	11.08	10.42	14.42	7.30	10.04	12.33
Total suspended solids	9.07	8.56	9.16	8.45	9.28	9.78	11.76	12.71	8.39	11.50	8.87	8.87
pH	7.42	7.43	7.41	7.43	7.44	7.46	7.44	7.46	7.44	7.42	7.37	7.38
Salinity ‰	0.47	0.47	0.45	0.45	0.40	0.55	0.47	0.44	0.45	0.44	0.50	0.47
Phosphate-phosphorus	2.53	2.70	2.42	2.50	2.56	2.61	2.47	2.49	2.96	2.53	2.54	2.44
Nitrate-nitrogen	2.73	2.93	3.42	2.98	2.54	3.85	3.44	3.25	2.75	3.31	2.70	3.97
Chloride	9.23	9.67	9.59	10.00	9.88	9.52	9.40	9.17	9.31	9.31	9.54	9.99
Conductivity (μScm^{-1})	0.56	0.52	0.56	0.47	0.47	0.48	0.49	0.52	0.46	0.53	0.62	0.56
Dissolved Oxygen	4.15	3.46	4.21	4.10	4.16	4.25	4.25	4.19	4.17	4.16	4.18	4.17
Biological Oxygen demand	0.23	0.22	0.26	0.23	0.22	0.25	0.29	0.30	0.31	0.28	0.26	0.24
Chemical oxygen demand	0.36	0.30	0.26	0.25	0.24	0.29	0.34	0.32	0.30	0.31	0.31	0.25
Oil and grease	0.03	0.02	0.02	0.02	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Turbidity (FTU)	8.59	9.25	10.00	7.46	9.57	8.87	8.37	7.99	8.42	10.29	9.15	8.69
Total dissolved solids	9.48	10.29	10.65	8.51	11.29	11.50	9.87	9.95	10.31	10.58	10.81	10.29
Sulphate	0.03	0.02	0.03	0.04	0.03	0.03	0.02	0.02	0.05	0.04	0.06	0.03
Ca	10.46	11.11	11.92	12.07	11.57	10.71	11.04	10.96	10.70	10.21	11.40	11.52
Fe	0.30	0.23	0.24	0.26	0.22	0.25	0.21	0.23	0.25	0.28	0.30	0.55
Pb	0.01	0.08	0.25	0.08	0.08	0.01	0.02	0.01	0.00	0.00	0.04	0.02
Hg	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01
Cu	0.05	0.02	0.02	0.02	0.03	0.06	0.01	0.01	0.01	0.00	0.02	0.04
Ni	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Zn	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01

Cyanobacteria analysis

In this study, cyanobacteria genera are arranged alphabetically within families and the species in alphabetical order within genera (Table 3).

Table 3: Cyanobacteria checklist at Lekki lagoon, Nigeria

Division : Cyanophyta
Class : Cyanophyceae
Order: Chroococcales
Family 1: Chroococcaceae
Chroococcus decorticans
C. dispersus (V.Keiss) Lemm.
C. limnecticus Lemm.
**C. limnecticus var. subsalsus* Lemm.
C. minor
C. minutus (Kutz.) Rabenh.
C. palidus Nageli
C. prescottii Dr. & Daily
C. turgidus (Kutz.) Lemm.
C. turicensis (Nag.) Hangirg
C. varius A. Braun
Chroococcus sp 1
Chroococcus sp 11
**Cyanosarcina huebeliorum* Komarek & Anagnostids
Dactylococcopsis raphidiodes Hansg.
D. smithii Chodat & Chodat
Dactylococcopsis sp.
Family 2: Merismopediaceae
Agmenellum quadriplicatum
Agmenellum sp.
Aphanocapsa delicatissima West & West
A. elaschista West & West
A. elachista var conferta West & West
**A. nubilum* Nygaard
A.pulverea
A. rivularis
Aphanocapsa sp.
**Aphanothece bullosa var major* Geitler
Aphanothece sp.
Merismopedia angularis Geitler
M. convoluta Breb.

M. elegans A.Br.
M. glauca (Ehr.) Nag.
M. major G.M.Smith
M. marsonii Lemm.
M. punctata Meyen.
M. tennuisima Lemm.
Family 3: Chaemasiphonaceae
Clastidium setigerum
Clastidium sp.
Family 4: Microscystaceae
**Gleocapsa alpicola* (Lyng.) Bornet
**G. arenaria* (Hass.) Rabenh.
G. biformis Novacek
G. compacta Kutz.
G. conglomerata Kutz.
**G. decorticans* (A.Br.) P.Richter
G. delicatissima
G. magma (Breb.) Kutz.
G. quarternata (Breb.) Kutz
Gleocapsa sp.
Gleothece heufleri
Gleothece linearis Nag
Gleothece sp.
Microcystis aeruginosa Kutz.
M. aeruginosa var elongata Rao,C.B
**M. aeruginosa var major* (Wittr.) Smith
M. elongata sp.nov.
**M. firma* (Kutz.) Dr. & Daily
M. flos-aquae (Wittr.) Kirchner
M. paludosus
**M. pulverea* (Wood) Forti
**M. ramosa* Bharadwaja
**M. robusta* (Clack) Nygaard
M. viridis (A. Br.) Lemm.
M wesenbergii Kosinskaja
Order 2: Nostocales
Family: Nostocaceae
Anabaena azollae Strasburger
*A. circinalis (*Kutz.) Rabh.
**A. confervoides* Reinsch
A. constricta Lauter b.
A. cycadeae J.Reinsch
A. cylindrica Lemmermann
A. fircinalis
A. flos-aquae (Lyng.) Breb.
A. limnectica G.M.Smith
A. spiroides Lemm.
**A. torulosa* (Carm.) Lagerh.
Anabaena sp.1
Anabaena sp 11
Aphanabaena sp
Calothrix sp
Cylindrospermum catenatum Ralfs
Cylindrospermum majus Kutz.

Cylindrospermum sp 1
Nostoc carneum
Nostoc linkia
N. muscorum Agardh
N. peltigerae Letellier
N. sphaericum Vauch.
Nostoc sp.1
Nostoc sp 11
Order 3: Oscillatoriales
Family 1: Oscillatoriaceae
Lyngbya birgei G.M.Smith
L. contorta Lemm.
**L lagerheimia* (Mobius) Gom.
L limnectica Lemmermann
**L. martensiana* Menegh.
L versicolor (Wattman)Gomont
Lyngbya sp
Oscillatoria acuminata Gomont
O. acutissima Kufferath
O. agardii Gomont.
O. angustissima West & West
O. articulata
O. brevis Kutz.
O curviceps Agardh
O. formosa Bory.
**O formosa f. edaghica* Novickova
O. germinata Meneghini
O lacustris
**O. lemmermanni* Wolosz
O. limnectica Lemm.
O. limosa (Roth) Ag.
O. margaritifera Kutzing (Gomont)
O. minima
O. planctonica Wolosz
**O. rubescens* DC ex Gomont
O. sancta (Kutz.)Gom
**O simplissima* Gomont.
O. subrevis Schmidle
O. tenuis Ag.
Oscillatoria sp.
Family 2:Phormidiaceae
Arthrospira fusiformis Fott & Karim
Arthrospira sp.
Microcoleus codii Fremy
M. subtorulosus
M. willeana
Microcoleus sp.1
Microcoleus sp 11
Phormidium angustissimum West & West
**P. caerulescens* Geitler
**P. chlorinum* Komarek
P. cortianum
P. crouanii Gomont
P. foveolarum (Mont.) Gomont

**P. insigne* Skuja
**P. laetevirens* Skuja
P. luridum (Kutz.) Gomont
**P luteum* Kosinskaja
**P. molle* Palik
P nigro-viride Gomont
**P. papyraceum* (Ag.) Gom.
P. retzii (Ag.) Gomont
*P. tenue (*Menegh.)Gom.
P. tinctorium Kutz.
Phormidium sp 1
Phormidium sp 11
**Plantothrix clavarata* Skuja
**P. cryptovaginata* Skacelova & Komarek
**P. isothrix* Komarek
P. minor
P. planctonica
Plantothrix sp1
Plantothrix sp 11
Trichodesmium laucustre Klebahn
Trichodesmium sp.
Family 3:Pseudanabaenaceae
**Limnothrix planctonica* Geitler
Limnothrix sp.
**Pseudoanabaena curta* Hollerbach
**P. moniliformis* Komarek & Kling
**P thermalis* Anagnostidis
Pseudoanabaena sp
Spirulina filiformis
S. princeps W.et G.S.West
S. major Geitler
S. meneghiniana Anagnostidis
S. tennerima
Spirulina sp 1
Spirulina sp 11
**Leptolyngbya hypolimnectica*
**Leptolyngbya ocridana* Cardo *(Phormidium)*
L. tenuis
Leptolyngbya sp.
**Plantolyngbya brevicellularis* Cronberg &Komarek
**P. minor* Komarek&Cronberg
**P. tallingii* Komarek & Kling
**P. minor*
Planktolyngbya sp.
Family:Schizotrichaceae
Schizothrix pulvinata
S. friesii (Ag.) Gomont
Schizothrix sp.
Scytonema sp.

Discussion

The cyanobacteria checklist reflects the influence of hydrological conditions of this area. The dominance of *Oscillatoria* throughout the season could be a pointer that the hydrology and salinity of the studied area favours its growth. The particular high diversity of blue-green observed in the lagoon could also be that the water chemistry favours growth of cyanophytes. Five bloom forming cyanophytes identified in this study include *Microcystis aeruginos, M. wesenbergii, , Anabaena flos-aquae, A. spiroides* and *Oscillatoria formosa.* The variation in physical and chemical parameters observed during the study period may be as a result of the influence of weather conditions. For instance, the rainy season occurring between June and October, characterized by low transparency and pH; increased total suspended solids, higher turbidity and increased flood water condition which might have initiated stressful environmental condition and these conform with Dart and Stretton (1980) who stated that variations in water temperature could cause alterations in the pH due to changes in ionization and increased solubility or precipitation of bottom deposits. Nwankwo and Onitiri (1992) also pointed out that it is possible that rainfall triggers off flood situations which usually increases total solids, reduces transparency and consequently light penetration and also dislodges attached algal forms. The phytoplankton community and the physio-chemical parameters exhibited seasonal changes closely related to the pattern of rainfall. The presence or absence of any blue-green species may be due to the changing physical environment other than pollution (Nwankwo 1994).

Acknowledgements

Adesalu T.A. is grateful to Fulbright exchange program scholarship, University of Lagos, Nigeria; Central Michigan and Bowling green state Universities U.S.A. Thanks are also due to Dr Akinsoji, O. my co-supervisor.

Corresponding author:
Adesalu, T. A.Department of Botany and Microbiology University of Lagos, Akoka, Nigeria
E-mail: boseadesalu@yahoo.com.

References

1. Adesalu,T.A., Nwankwo, D.I. Studies on the phytoplankton of Olero creek and parts of Benin river, Nigeria. The Ekologia. 2005; 3(2):21-30.
2. Adesalu,T.A., Nwankwo, D. I. A checklist of Lekki lagoon diatoms. International Journal of Botany. 2009; (5) 2: 126-34.
3. American Public Health Association. Standard methods for the examination of water and waste water, 20th ed. American Public Health Association Incorporated, New York. 1998: 1270pp.
4. Anadu, D.I.,Obioma, A., Ejike, C. Water

chemistry and plankton periodicity in two contrasting mine lakes inJos,Nigeria. Hydrobiologia, 1990; 206:17-25

5. Biswas, S.. Phytoplankton periodicity of Ogelube Lakes, Anambra State, Nigeria. During 1979-1980.Hydrobiologia, 1992; 246: 169-172.

6. Biswas, S. Phytoplankton of Opi Lakes, Anambra State, Nigeria.Verht.Int.Verein. Limnology, 1984; 22:1584-1589.
7. Dart,R.K., Stretton, R. J. Microbiological aspects of pollution control and environmental science. 6 Elsevier Scientific Publishers Amsterdam 1980.
8. Egborge, A. B. M., Sagay, E. G.. The distribution of phytoplankton and zooplankton in some Ibadan freshwater cosystems. Polskie Archiwum Hydrobiologii, 1979; 26: 23-335.
9. Hill, M. B., Webb, J. EThe ecology of Lagos lagoon II. The topography and physical
10. features of Lagos harbour and Lagos lagoon. Phil. Trans. Royal Society London, 1958; 241**:** 319–417.
11. Holden, M. J., Green, J. The hydrology and plankton of the River Sokoto. Journal of
12. Animal Ecology, 1960; 29: 65–84.
13. Ikusemiju, K.. A study of catfishes of Lekki lagoon with particular reference to species of *Chrysichthys walkeri* (Bagridae) Ph.D Thesis University of Lagos, Nigeria, 1973.
14. Imevbore, A. M. A. Planktonic algae of Eleiyele reservoir, Nigeria. Journal of Science,
15. 1968; 2: 85–90.
16. Kadiri, M.O. A rich flora of Micrasterias from Ikpoba reservoir, Nigeria. Archiwum Hydrobiologii, 1989; 116(3): 391-399.
17. Kadiri, M.O. Further desmids from Ikpoba reservoir (Nigeria) compared with other records from Africa. Algological studies, 1993; 71:23-35.
18. Kadiri, M.O..A comprehensive preliminary checklist of the algae of the Ikpoba reservoir Edo state,Nigeria. Global journal of pure and applied sciences, 1999; 5 (4): 485-491.
19. Kadiri, M.O. Seasonal trend in the chemical limnology of a shallow Nigerian man-made lake. Acta Hydrobio. 2000; l (42): 29-40.
20. Nwankwo, D. I., Onitiri, A.O. Periphyton(Aufwuchs) community on submerged aquatic macrophytes in Epe lagoon, Nigeria. Journal of Agriculture Science and Technology. 1992; 2 (2): 135- 41.
21. Nwankwo, D. I. A checklist of Nigerian marine algae. (Tarkwa Bay). Nigerian Journal of Botany, 1988; 1: 47–50.
22. Nwankwo, D. I. Cyanobacteria bloom species in coastal waters of South-western Nigeria. Archiwum Hydrobiologie Supplement, 1993*;* 90: 553–542.
23. Nwankwo D. I. Hydrochemical properties and bottom-dwelling of aLagos lagoon
24. sewage disposal site. Polskie Archiwum Hydrobiologia.,1994; 41(1): 35-47.
25. Nwankwo, D. I. A first list of dinoflagellates (Pyrrophyta) from Nigerian coastal waters (creeks,estuaries,lagoons).*Polskie Archiwum Hydrobiologia*, 1997; 44(3): 313–321.
26. Olaniyan, C. I. O. The seasonal variation in plankton in Lagos harbour, Nigeria. Ph.D
27. Thesis,University of London, London, 1957; 173pp.
28. Opute, F. I. Phytoplankton flora of the Warri/Forcados estuaries of Southern Nigeria.Hydrobiologia. 1990; 208:101-109.
29. Opute, F.I. A checklist of the freshwater, brackish and marine phytoplankton of the Warri/Forcados estuaries of southwestern Nigeria. Nigerian Journal of Botany.1991; 4: 227-254.
30. Opute, F.I. Contribution to the knowledge of algae of Nigeria 1: Desmids from the Warri/Forcados estuaries, Part 2. The genera Euastrum and Micrasterias. Archiwum Hydrobiologii. Supplement, 1992; 93: 73-92.
31. Wujek, D. E., Adesalu, T. A., Nwankwo D. I. Silica-scaled chrysophyceae and synurophyceae (chrysophyta) from Nigeria II Lake Lekki. Tropical Freshwater Biology. .2003/2004; 12/13:99-103.

9/9/2009

Lipid profile among chronic hepatitis C Egyptian patients and its levels pre and post treatment

Ehab H Nashaat ,MD
Associated professor of internal medicine ,Faculty of medicine ,Ain Shams university.
ehabnashaat@hotmail.com

Abstract: Background: Hepatitis C is a common infection in the Egyptian population, specially genotype 4 .It is well recognized in many studies that hepatitis C chronic infection is associated with hypolipidemia ,so in our study we compare the lipid profile between 150 patients with chronic hepatitis C and 150 normal persons with comparable age, sex and body mass index (BMI). The fasting cholesterol ,low density lipoprotein(LDL),high density lipoprotein(HDL),and triglyceride were compared .Then 36 patients of them received treatment in the form of pegylated interferon and ribavirin and then the patients who achieved viral clearance were reevaluated as regard the lipid profile versus the patients who did not achieve viral clearance and the relpsers. In our study we found that patients with chronic hepatitis C had significant lower LDL, cholesterol, and triglycerides than normal persons with comparable age, sex and BMI .The treated patients with sustained virological response showed increased LDL, cholesterol, and triglycerides from baseline compared to patients without viral clearance and even 2of them had increased LDL more than 130 mg/dl and had increased in cholesterol level more than 200 mg/dl which necessate treatment for dyslipidemia in order to prevent the risk of coronary heart disease. Conclusion : patients with chronic hepatitis C had low levels of LDL, cholesterol, and triglycerides than non infected persons and after viral clearance a significant number of patients showed LDL, cholesterol, and triglycerides rebound even to levels may be associated with increased risk for coronary heart disease, so lipids should be carefully followed up after successful clearance of hepatitis C infection. [Nature and Science 2010;8(7):83-89]. (ISSN: 1545-0740).

Key words: HCV infection,lipids profile ,pre and post treatment.

1. Introduction:

Hepatitis C virus is a major public health problem in Egypt specially genotype 4. Egypt has the highest prevalence of HCV in the world (overall prevalence of HCV antibody is 12% among the general population and reaches 40% in persons 40 years of age and above in rural areas whom at higher risk for coronary heart disease.(Habib et al, 2001) (Medhat et al, 2002). The available therapy uptill now is the combination of pegylated interferon and ribavirin, which result in sustained virological response in only half of patients (Armstrong et al, 2006).

Interactions between chronic hepatitis C virus (HCV) and lipid metabolism are strongly noticed. (Fabris et al, 1997) (Serfaty et al, 2001). Several important lipid –HCV interactions have recently been found .First ,host serum lipid play a role in hepatitis C virion circulating and hepatocyte entery.A proportion of circulating hepatits C viral particles are complexed with host triacyl glycerol-rich lipoproteins, known as lipo-viroparticles. (Diaz et al, 2006). Lipo-viroparticles use LDL receptors on hepatocytes as points of entery and are associated with high rate of infectivity(Andre et al, 2002).Once hepatitis C virions have enter the hepatocytes their replication is again dependant on host lipid interactions . New hepatitis C virion formation requires viral binding to either an endoplasmic reticulum phospholipid membrane or to an endoplasmic reticulum –associated membranous web (Dubuisson et al, 2002).

Also HCV replication may produce effects similar to those observed with HMGR inhibitors. HCV replication could decrease intrahepatic cholesterol synthesis through two possible pathways; first, it may shunt geranylpyrophosphate, out of the mevalonate pathway, decreasing the quantity of this necessary intermediate available for cholesterol synthesis. Second, it may divert cholesterol to the synthesis of intracellular membranes that are necessary for the viral replication complex. The net effect of these diversions is the decrease of available cholesterol for physiologic intracellular processes and for peripheral delivery via VLDL, ultimately resulting in decreased serum cholesterol levels. The decrease in available intracellular cholesterol may also lead to an increase in LDL receptors and intrahepatic LDL. This increase in LDL uptake may account for the decreased serum LDL levels in HCV infection. (Dubuisson et al, 2002).

Also the metabolic processes which is associated with viral replication may be associated with a drop in triglycerides levels. (Perlemuter et al, 2002), (Marzouk et al, 2007).

So chronic HCV infection by interrupting cholesterol synthesis and using host lipids for replication ,decreasing circulating lipids, and clearance of the virus results in rebound increase of lipid levels.

The purpose of this study was to study the effect of chronic HCV infection on the lipids profile among

Egyptian patients and if the lipids values is affected after HCV treatment and comparing lipids values between patients with sustained virological response, nonresponders, and relapsers, and if the post HCV treatment lipid rebound in patients with sustained virological response reached levels that are associated with increased risk of development of coronary heart disease and necessate treatment per the National Cholesterol Education Program Guidelines(NCEP).

Patient and methods:

This study was carried out in the Gasteroenterology and Hepatology clinics at Ain Shams University Hospitals .

The study was conducted on two groups with comparable age, sex and body mass index (BMI).

Group 1: 150 patients with chronic HCV hepatitis who did not receive treatment for HCV before.

Group 2: 150 normal persons as a control group.

All patients and controls in this study were subjected to:

1. Full medical history and medical examination.
2. Liver functions tests (total plasma proteins, serum albumin, SGOT, SGPT, total and direct serum bilirubin, alkaline phosphatase and prothrombin time).
3. Lipid profile: fasting cholesterol, low density lipoprotein(LDL),high density lipoprotein(HDL), and triglycerides.
4. Kidney functions tests (serum creatinine, blood urea nitrogen (BUN) and uric acid).
5. Random blood glucose level.
6. Complete blood count.
7. HCV Antibody for both groups and HCV PCR quantitative for patients only.
8. Thyroid function test for patients only.
9. Body mass index (BMI) (weight in kilogram/height in cubic meter) for both groups.
10. Abdominal Ultrasonography.
11. Liver biopsy for 22 patients from the 36 patients who received treatment.

A written consent was taken from all the sharers in this study.

Exclusion criteria for patients group :

1. Patients with biopsy proved or clinical evidence of cirrhosis (Ishak stage 5-6 fibrosis on biopsy or presence of portal hypertension manifestation in the form of esophageal or gastric varices ,ascites or splenomegaly, or evidence of synthetic dysfunction on laboratory evaluation) .
2. Patients with hepatitis B infection or any other chronic liver diseases.
3. Patients on lipid lowering medications .
4. Recipients of solid organ transplantations .

Exclusion criteria for control group :

1. Patients with HCV, or HBV infection or any other chronic liver diseases.
2. Patients on lipid lowering medications .
3. Recipients of solid organ transplantations.

From the patients group 36 patients received treatment in the form of pegylated interferon and ribavirin, in this group of patients lipid profile is reevaluated after the 24 weeks after stoppage of treatment in the SVR group patients and in non responders group and after 24 weeks after relapse in relapsers group to evaluate and compare the results between patients with sustained virologic response (SVR)(undetectable HCV RNA six months following completion of therapy), non responders (failure to clear HCV RNA during therapy), and relapsers (initial clearance of HCV RNA at the end of treatment but detectable HCV RNA following cessation of treatment),and to detect any change in lipids profile and if any of these patients lipids levels reaches the levels which needs treatment according to National Cholesterol Education Program Adult Treatment Plan Guideline III (NCEP ATP-III) which recommends that patients with coronary heart disease (CHD) or CHD equivalents be treated for a LDL>100 mg/dL (National heart, lung and blood institute, 2004). Patients with two or more major CHD risk factors (including cigarette smoking, hypertension, HDL<40, family history of premature CHD or age greater than 45 in men or 55 in women) should be treated for a LDL>130 mg/dL. Patients without CHD, CHD equivalents or two or more major CHD risk factors require treatment for LDL>160 mg/dL. Total cholesterol level is also a known risk factor for the development of coronary heart disease. (Stamler et al, 1986). Total cholesterol level of 200 mg/dL or less is considered desirable by the NCEP-ATP III and levels above 200 mg/dL carry a 44% increased risk of CHD when compared to levels below 200 mg/dL (National heart, lung and blood institute 2004) (Stamler et al, 1986).

Data Management:

After tabulation, all data were analyzed statistically using SPSS statistical package version 16 & the following tests were done:

1. Mean =X
2. Standard Deviation=SD.
3. Student t test. $p<0.05$ indicated statistical significance.

Results:

This study was carried out on 150 chronic HCV infected patients and 150 normal persons as control group, both groups are comparable as regard age which

was 38.7(±3.15) and 38.1(±3.11) respectively, with no significant difference between both groups, as regard sex men/women were 103/47 in both groups, and as regard BMI there was no significant difference between both groups which was 29.8(±2.43) in patients group and was 30.1(±2.45) among controls (Table 1). Liver biopsy was done in 22 patient of the 150 patients included in this study, the Median Ishak Hepatic Activity Index was 6 (range was 0-11) and Ishak fibrosis stage was from stage 0-2 in 14 patients (63.63%) and from stage 3-4 in 8 patients (36.37%). (Table 1).

Patients in the HCV group had significantly lower total cholesterol levels, (mean 162.44 mg/dl) than the uninfected control group (mean 208.76mg/dl). (P < 0.0001). Patients in the HCV group also had significantly lower total LDL levels when compared to the uninfected control group ,(mean 95.2mg/dL) versus (mean117.9mg/dL); (P < 0.0001). Patients in the HCV group also had significantly lower trigtlycerides levels when compared to the uninfected control group (mean 114.9mg/dL) versus (mean164.3mg/dL); P < 0.0001). (Table 1, fig 1). HDL levels were not statistically significant between the HCV group and uninfected controls. (Table 1, fig 1).

From the patients group 36 patients received treatment in the form of pegylated interferon and ribavirin, 16 patients achieved SVR, 14 were nonresponders and 6 relapsed after an initial response to therapy. Because of the small numbers of relapsers, relapsers and nonresponders were combined into a single group.

In this group of patients lipid profile is reevaluated after the 24 weeks after stoppage of treatment for SVR patients and non responders and after 24 for weeks after relapse in relpsers to evaluate and compare the results between responders ,non responders and relapsers group.

The mean pretreatment cholesterol, LDL, HDL and triglycerides levels did not differ significantly between the responder and nonresponder/relapser groups (Table 2, fig 2). The mean values of cholesterol, LDL and triglycerides are below the recommended levels for treatment with lipid lowering medications based on NCEP guidelines for primary prevention of atherosclerosis. A significant changes were seen in circulating lipid levels post treatment between responders group and nonresponders/relapsers group. Responders had significantly higher mean post treatment cholesterol levels than nonresponders (206.5 versus 156.4, P=<0.001) as well as significantly higher LDL levels (114.37 versus 96.35, P=<0.05), also responders had significantly higher triglycerides levels than nonresponders (148.8 versus 1111.95, P=< 0.001). The mean LDL in patients who achieved sustained virologic response was 114.37 mg/dL. This level of LDL requires lipid lowering therapy in patients with coronary heart disease (CHD) or CHD equivalents. None of our patients had previously diagnosed CAD and non required lipid lowering treatment prior to HCV therapy. However, 2 of the 16 patients (2.5%)who had SVR had LDL increases to greater than 130 mg/dL, one of them was131 mg/dl and the other was 136 mg/dl both were cigarette smokers and one of them was hypertensive which necessate treatment for dyslipidemia.

Moreover the patients who achieved SVR also had a mean post treatment cholesterol of 206.5 mg/dL. The two patients with LDL increases to greater than 130 mg/dL,both of them had total cholesterol levels far above 200, one of them was223 mg/dl and the other was 231 mg/dl, which also necessate treatment for dyslipidemia

Table 1 : General characters and lipids values for HCV group and contol group

Characteristics	HCV group	Control group	P value
Number	150	150	
Sex(men/women)	103/47	103/47	
Mean age(SD)	38.7(±3.15)	38.1(±3.11)	1.66 NS
BMI Mean(SD)	29.8(±2.43)	30.1(±2.45)	1.06 NS
Medain Ishak Hepatic Activity Index(Range)	6(0-11)	_	_
Ishak fibrosis stage Stage 0-2 Stage 3-4	 14(63.63%) 8(36.37%)	_	_
Cholesterol(SD)	162.44(±13.26)	208.76(±17.04)	26.27(<0.0001)
LDL(SD)	95.2(±7.77)	117.9(±9.6)	22.5 (<0.0001)
HDL(SD)	46.93(±3.83)	46.96(±3.83)	0.06 NS
Triglycerides(SD)	114.9(±9.38)	164.3(±13.41)	36.9 (<0.0001)

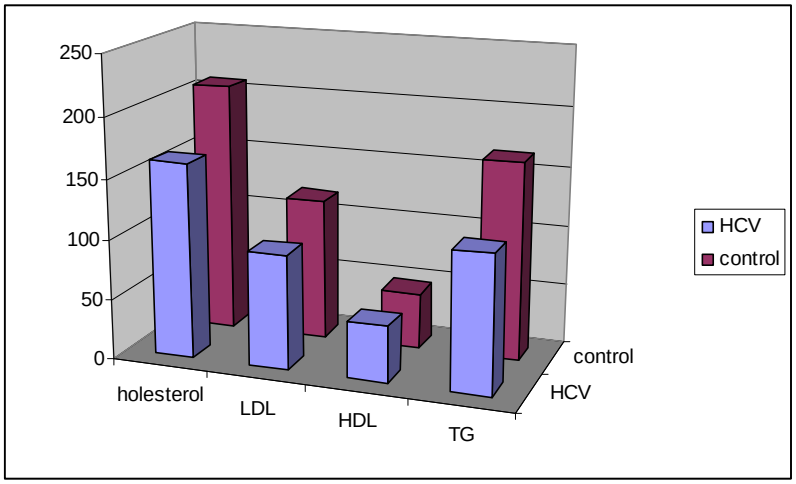


Fig 1: lipids profile among HCV patients versus control group

Table 2. Mean pretreatment and post treatment lipid levels for responders and nonresponder /relapsers

	Sustained virologic response		Norespnder/relapsers		P value	
	Pretreatment	posttreatment	Pretreatment	post treatmen	Pretreatment	Posttreatment
Cholesterol	165.25(±39.06)	206.5(±51.6)	156.3(±34.9)	156.4(±33.8)	0.71 NS	3.35 (<0.001)
LDL	98.8(±24.7)	114.37(±28.4)	97.2(±21.7)	96.35(±21.5)	0.2 NS	2.1 (<0.05)
TG	111.93(±27.9)	148.8(±37.2)	111.1(±24.8)	111.95(±25.1)	0.09 NS	3.39 (< 0.001)
HDL	45.88(±11.46)	46.18(±11.5)	45.91(±11.5)	46.03(±11.45)	0.07 NS	0.04 NS

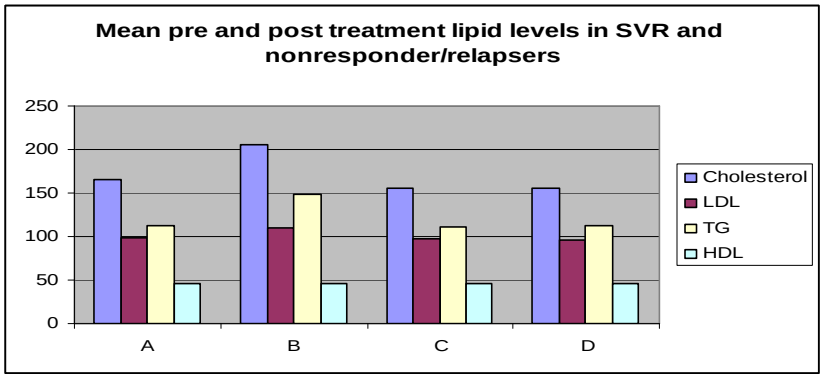


Fig 2 Mean pretreatment and post treatment lipid levels for responders and nonresponder /relapsers
A=SVR Pretreatment B=SVR Post treatment
C=Nonresponders/relapsers pretreatment
D=Nonresponders/relapsers posttreatment

Discussion:

Hepatitis C virus is a major public health problem in Egypt specially genotype 4 . Egypt has the highest prevalence of HCV in the world (overall prevalence of HCV antibody is 12% among the general population and reaches 40% in persons 40 years of age and above in rural areas whom at higher risk for coronary heart disease.(Habib et al,2001),(Medhat et al, 2002).

The origin of the HCV epidemic in Egypt has been attributed to intravenous schistosomiasis treatment in rural areas in the 1960s–70s.(Frank et al, 2000).

Interactions between chronic hepatitis C virus (HCV) infection and lipid metabolism have been

described in some studies, (Thomson et al,1993), (Maggi et al,1996) , (Cicognani et al, 1997), (Fabris et al, 1997),), (Serfaty et al, 2001). Some studies have reported a higher prevalence of hypocholesterolemia and low LDL levels in HCV-infected patients compared to control groups (Maggi et al, 1996), (Fabris et al, 1997) (Serfaty et al, 2001). Other studies showed decrease levels of triglycerides among chronic HCV patients, (marzouk et al, 2007). Although changed serum lipid is commonly found in patients with chronic liver disease of any etiology, the relationship between HCV and lipid metabolism seems to be more specific: binding of HCV particles to human high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) has been described (Thomson et al,1993). Moreover the LDL receptors could permit the entry of HCV in hepatocytes, (Agnello et al, 1999, and(Monazahian et al,1999). Also HCV replication could decrease intrahepatic cholesterol synthesis. The decrease in available intracellular cholesterol may also lead to an increase in LDL receptors and intrahepatic LDL. This increase in LDL uptake may account for the decreased serum LDL levels in HCV infection(Monazahian et al,1999).

The purpose of this study was to study the effect of chronic HCV infection on the lipid profile among Egyptian patients and if the lipids values is affected after HCV treatment and comparing lipids values between patients with sustained virological response, nonresponders, and relapsers, and if the post HCV treatment lipid rebound in patients with sustained virological response reached levels that are associated with increased risk of development of coronary heart disease and necessate treatment per the National Cholesterol Education Program Guidelines(NCEP).

This study was conducted on two groups with comparable age, sex and body mass index (BMI), (table 1) ,group 1 was 150 patients with chronic HCV hepatitis who did not receive treatment for HCV before, group 2 was150 normal persons as a control group, we excluded patients with biopsy proved or clinical evidence of cirrhosis, (Ishak stage 5-6 fibrosis on biopsy or presence of portal hypertension manifestation in the form of esophageal or gastric varices ,ascites or splenomegaly, or evidence of synthetic dysfunction on laboratory evaluation) ,Patients or controls with any other chronic liver diseases (other than chronic HCV in the patients group) ,patients on lipid lowering medications ,and recipients of solid organ transplantations .

Patients in the HCV Group had significantly lower total cholesterol levels than the uninfected control group, ($P < 0.0001$). Patients in the HCV Group also had significantly lower total LDL levels when compared to the uninfected control group (95.2mg/dL versus 117.9mg/dL; $P < 0.0001$). These results agree with (Fabris et al, 1997), (Serfaty et al, 2001), (Floris-Moore et al, 2007), (Marzouk et al, 2007) and (Corey et al, 2009), who observed that frequency of hypocholesterolemia in noncirrhotic HCV-infected patients was five times higher than in their reference population. Also patients in the HCV group had significantly lower trigtlycerides levels when compared to the uninfected control group , ($P < 0.0001$) ,(table 2, fig 2). These results agree with Perlemuter et al, 2002 and Marzouk et al, 2007, which refers this drop to the metabolic processes associated with viral replication .But these results disagree with Corey et al, 2009 who did not found significant difference as regard triglycerides between patients and controls.

HDL levels were not statistically significant between the HCV group and uninfected controls (table 2, fig 2). These results agree with Corey et al,2009 . From the patients group 36 patients received treatment in the form of pegylated interferon and ribavirin , 16 patients achieved SVR, 14 were nonresponders and 6 relapsed after an initial response to therapy. Because of the small numbers of relapsers, relapsers and nonresponders were combined into a single group.

In this group of patients lipids profile is reevaluated after the 24 weeks after stoppage of treatment for SVR patients and non responders and after 24 weeks after relapse in relapsers to evaluate and compare the results between responders ,non responders and relapsers group ,and this was the first time according to my knowledge that follow up lipids profile pre and post treatment in chronic HCV patients was evaluated prospectively, as the other studies were retrospective studies which depend on patients filings.

The mean pretreatment cholesterol, LDL, HDL and triglycerides levels did not differ significantly between the responder and nonresponder/relapser groups (table 2, and fig 2). The mean values of cholesterol, LDL and triglycerides were below the recommended levels for treatment with lipid lowering medications based on NCEP guidelines for primary prevention of atherosclerosis.

A significant changes was seen in circulating lipids levels post treatment between responders and nonresponders/relapsers. Responders had significantly higher mean post treatment cholesterol levels than nonresponders ,as well as significantly higher LDL levels, our findings are consistent with (Corey et al, 2009), also responders had significantly higher triglycerides levels than nonresponders ,and this findings are consistent with (Marzouk et al, 2007)(table 2, fig 2).

The mean LDL in patients who achieved sustained virologic response was 114.37 mg/dL. This level of LDL requires lipid lowering therapy in patients

with coronary heart disease (CHD) or CHD equivalents. None of our patients had previously diagnosed CAD and none required lipid lowering treatment prior to HCV therapy. However, 2 of the 16 patients (2.5%) who achieved SVR had LDL increased to greater than 130 mg/dL, one of them was131 mg/dl and the other was 136 mg/dl, both were cigarette smokers and one of them was hypertensive, which necessate treatment for dyslipidemia. The patients who achieved SVR also had a mean post treatment cholesterol of 206.5 mg/dL. The two patients with LDL increased to greater than 130 mg/dL,both of them had total cholesterol levels far above 200, one of them was 223 mg/dl and the other was 231 mg/dl, which also necessate treatment for dyslipidemia as recommended by the Scandanivian Simvastatin Survival Study (4S) 1994, and NCEP ATP-III 2004, these results were also consistent with (Shepherd et al, 1995) ,and (Corey et al, 2009).

In spite of the small number of these patients but on the bases of the high prevalence of chronic HCV infection in Egypt ,and if we studied older age group it is thought that post treatment elevation of lipids profile will be significant risk for CHD among this group and may warranate proper follow up for lipids profile in treated HCV patients and early recognition and treatment to reduce CHD mortality in this population.

This study has shown several important findings. First, HCV infection is associated with lowering of lipids, providing further evidence of an important interaction between HCV and host lipids, and suggesting a possible novel therapeutic target as some authors suggested that high concentrations of triglycerides at the time of acute infection compete with HCV for binding to the hepatocyte receptors, resulting in lower hepatocyte entry and easier infection clearance among those with high triglyceride levels,(Kenny, 1999), (Wise et al, 2000).

Second, post treatment viral clearance is associated with increased LDL and cholesterol levels, sometimes to levels associated with an increased risk for coronary heart disease, so we suggest that serum lipid levels should be assessed during follow-up among patients undergoing successful antiviral therapy. Further researches are needed to correlate the rise in lipid levels with clinically significant outcomes, such as the development of coronary heart disease.

Correspondence to:
Ehab H Nashaat
Ass. Professor of Internal Medicine
Faculty of Medicine, A in Shams University.
Telephone: 0226717901
Cellular phone: 0101458181
Emails: **ehabnashaat@hotmail.com**

References :

1-Agnello, V, Abel, G, Elfahal, M, et al. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci USA* 1999;**96**:12766–12771.

2-Andre P,Komurian-Pradel F,Deforges S,Perret M,Berland JL,Sodoyer M. Charcterizationof low and very low denisity hepatitis C virus RNA-containing particles.J Virol 2002 ;76:6919-6928.

-3-Armstrong GL, Simard EP, McQuillan GM, Kuhnert WL,Alter MJ.The prevlance of hepatitis C virus infection in the United States,1999 through 2002 ,Ann Int Med 2006;144:705-714.

4- Cicognani, C, Malavolti, M, Morselli-Labate, AM, et al. Serum lipid and lipoprotein patterns in patients with liver cirrhosis and chronic active hepatitis. *Arch Intern Med* 1997;**157**:792–796.

5- Diaz O,Delers F,Maynard M,Dmignot S,Zoulim F,Chambaz j.Preferential association of hepatitis Cvirus with apolipoprotien B48-containing lipoproteins. J GenVirol 2006;87:2983-2991.

6 -Dubuisson J,Penin F,Moradpour D.Interaction of hepatitis C virus proteins with host cell membranes and lipids .Trends Cell Biol 2002;12:517-523.

7- Fabris C,Federico E,Soardo G. Blood lipids of patients with chronic hepatitis :Differences related to viral etiology .Clin Chem Acta 1997;261:159-161.

8-Floris-Moore M, Howard AA, Lo Y, Schoenbaum EE, Arnsten JH, Klein RS. Hepatitis C infection is associated with lower lipids andhigh sensitivity C-reactive protein in HIV infected men . AIDS Patients Care STDS 2007;21:479-491.

9-Frank C, Mohamed MK, Strickland GT, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000;355:887–91.

10- Habib M,Mohamed MK,Abdel-Aziz F.Hepatitis C virus infection in a community in the Nile Delta:risk factors for seropositivty.Hepatology 2001;33:248-53.

11- Kenny-Walsh E for the Irish Hepatology Research Group.Clinical outcomes after hepatitis C infection from contaminated anti-Dimmune globulin.N Engl J Med1999;340:1228-33.

12-Maggi, G, Bottelli, R, Gola, D, et al. Serum

cholesterol and chronic hepatitis C. Ital J Gastroenterol 1996;28:436–440.

13-Marzouk D , Sass J, Bakr I, El Hosseiny M, Abdel-Hamid M, Rekacewicz C, Chaturvedi N, Mohamed M K, Fontanet A. Metabolic and cardiovascular risk profiles and hepatitis Cvirus infection in rural Egypt. Gut 2007 ;65:1105-1110.

14- Medhat A, Shehata M, Magder S, et al. Hepatitis C in a community in Upper Egypt: Risk factors for infection. Am J Trop Med Hyg 2002;66:633–8.

15-Monazahian, M, Bohme, I, Bonk, S, et al. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol 1999;57:223–229.

16-National, Heart, Lung and blood institute . Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Bethesda, MD: National Institutes of Health; 2004.

17-Perlemuter G, Sabile A, Letteron P, et al. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secreation: a model of viral-related steatosis. FASEB J 2002;16:185–94.

18- Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease :the Scandanivian Simvastatin Survival Study (4S). Lancet 1994;344:1383-1389.

19-Serfaty,c, Andreani,t, Giral,P. Hepatitis C virus induced hypobetalipoprotienemia:A possible mechanism for steatosis in chronic hepatitis C.J Hepatol 2001 ;34:428-434.

20-Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR,MacFarlane PW .Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia . West of Scotland Coronary Prevention Study Group.N Engl med 1995;333:1301-1307.

21-Stamler J, Wentworth D, Neaton JD .Is relationship between serum cholesterol and risk of premature death from coronary heart disease cotinous and graded?Finding in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT).JAMA 1986 ;256:2823-2828 .

22-Thomssen, R, Bonk, S, Thiele, A. Density heterogeneities of hepatitis C virus in human sera due to the binding of B-lipoproteins and immunoglobulins. Med Microbiol Immunol 1993;182:329–334.

23-Wiese M ,Berr F, Lafrenz M .Low frequency of cirrhosis in a hepatitis C (genotype 1b)single –source outbreak in germany: a20-year mulyicenter study. Hepatology 2000;32: 91-6.

4/1/2010

Comparative studies on the Indian cultivated *Pleurotus* species by RAPD fingerprinting

Swarnendu Chandra, Kabita Ghosh and Krishnendu Acharya*

Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700 019, West Bengal, India.
krish_paper@yahoo.com

Abstract: The oyster mushrooms (*Pleurotus*) are the second most important cultivated mushroom in the world. The genus consists of a number of species. The genetic divergent of eight Indian species of *Pleurotus* viz. *P. florida* – P1, *P. membranaeceus* – OE128, *P. sajor-caju* – PL1140, *P. djamor* – X375, *P. cystidiosus* – P19, *P. flabelletus* – PL50, *P. sapidus* – PL40 and *P. ostreatus* – PO1803 was determined based on random amplified polymorphic DNA (RAPD) pattern. Result showed that all the species tested could be differentiated by RAPD data and even one individual primer (OPD-07) could also discriminate all tested species. Genetic similarity analysis and grouping derived from RAPD markers reveals a high level of genetic diversity. Therefore the RAPD technique can provide a powerful tool to discriminate the species and the molecular information are useful for the breeding system. [Nature and Science 2010;8(7):90-94]. (ISSN: 1545-0740).

Key words: Oyster mushroom, *Pleurotus* species, RAPD

1. Introduction

Members of the mushroom genus *Pleurotus* (Jacq. Fr.) P. Kumm. (Basidiomycotina, Pleurotaceae) form a heterogeneous group of edible species of high commercial importance. They are characterized by the production of fruit bodies with an eccentric stalk and a wide cap shaped like an oyster shell, with the widest portion of the cap being away from the stalk. They grow over a wide range of temperatures and are able to colonize a wide spectrum of unfermented, natural, lignino-cellulosic wastes. Because of their fast mycelial growth rate, they colonize the substrates rapidly; the yield of fruit bodies is also high. The bifactorial inheritance, observed in many of the species, suggests the likelihood of a high degree of genetic variability, and, hence, considerable breeding potential. Mating compatibility studies have demonstrated the existence of discrete intersterility groups (biological species) in *Pleurotus*, many of which are broadly distributed over one or more continents. Mushrooms recognized as natural and healthy foods originating from an environmentally friendly organic farming system (Moore and Chiu, 2001). To make mushroom cultivation sustainable and highly productive, novel improved strains with improved characteristics are greatly needed. However, mushroom strains are very difficult to discriminate, due to lack of clearly distinguishable characters. This makes strain protection problematic, and impedes strain improvement. Molecular markers of rDNA sequencing, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), microsatellite and mitochondrial genotypes have all been used to discriminate mushroom species and/or strains of *Agaricus* (Castle et al., 1987; Sonnenberg et al., 1991; Khush et al., 1992; Barroso et al., 2000; Calvo-Bado et al., 2000; Moore et al., 2001; Ramirez et al., 2001), *Auricularia* (Yan et al., 1999), *Ganoderma* (Hseu et al., 1996), *Lentinula* (Chiu et al., 1996), *Stropharia rugoso-annulata* (Yan et al., 2003), and *Volvariella* (Chiu et al., 1995). These technologies provide ways to obtain reliable data for mushroom strain identification and protection. RAPD analysis was first developed to detect polymorphism between organisms, despite the absence of sequence information, to produce genetic markers, and to construct genetic maps (Williams et al., 1990). In this work, RAPD was tested in the eight Indian cultivated species of *Pleurotus* for differentiation of individual species.

2. Materials and methods:

2.1 Species studied:

The material used for RAPD analysis consisted of eight *Pleurotus* dikaryotic cultures P. *florida* – P1, *P. membraneceus* – OE128, *P. sajor-caju* – PL1140, *P. djamor* – X375, *P. cystidiosus* – P19, *P. flabelletus* – PL50, *P. sapidus* – PL40 and *P. ostreatus* – 1803 were purchased from National Research Centre for Mushroom, ICAR, Chambaghat, Solan, India. The cultures were maintained in potato dextrose agar (PDA) media for further use.

2.2 Isolation of genomic DNA:

Petri dishes containing PDA were inoculated with each species and incubated at 22ºC for 7 days. Next, 4 discs (7 mm diameter) were cut with a sterile cork borer, from the border of the mycelial colony and placed (using a sterile scalpel) into 250 ml flask

containing 100 ml of PDA broth and incubated at 22ºC for 12 days. When species colonized the surface of the flask, the mycelia were harvested from the liquid by being drained through a funnel containing Whatman No. 1 filterpaper. The mycelia were frozen in liquid nitrogen and ground to power in a mortar (Lewinsohn et al., 2001). DNA isolation used SDS as lysis buffer (3% SDS, 1% 2marcaptoethanol, 50 mM EDTA, 50 mM Tris/HCl pH 7.2) and phenol/chloroform/isoamyl alcohol (25:24:1) as extractant. The DNA was precipitated with 0.1 volume of 3 M sodium acetate and 0.6 volume of isopropanol, washed with 70% ethanol and resuspended in sterile double distilled water (Zervakis et al., 2004). The purity and quality of genomic DNA was determined spectrophotometrically and confirmed by use of 1.2% agarose against a known concentration of unrestricted lambda DNA. Standard 30 µg/ml DNA working solution were formulated for each sample in sterile double distilled water. DNA stock solution was kept in freezer for further test (Lewinsohn et al., 2001).

2.3 RAPD-PCR analysis:

Amplification reactions were performed in a final volume of 25 µl containing 10 ng of genomic DNA. The reaction solution consisted of 200 µM each of dATP, dCTP, dGTP and dTTP (Sigma) 50 µmol oligonucleotide primer (Sigma) and 2 units *Taq* polymerase (Fermentas) in 10 mM Tris pH 8.3, 2 mM $MgCl_2$, 0.001% gelatin, 0.05% Tween 20, 50 mM KCl. Amplification was performed in Applied Biosystems 2720 thermal cycler: one cycle at 94ºC for 5 min, 39.5ºC for 1 min, 72ºC for 1 min, and 44 cycles at 94ºC for 1 min, 39.5ºC for 1min and 72ºC for 1 min. The final synthesis was performed at 72ºC for 5min (Zervakis et al., 2001). Total ten primers were used for the amplification. Positive results obtained against three primers were chosen for the final amplification. The amplified fragments were resolved on a 1.2% agarose gel, run under standardized conditions, and stained by ethidium bromide. A 100 bp ladder DNA marker (Fermentas) was used as a size standard. The size of the obtained DNA was calculated against the ladder.

2.4 Data scoring and analysis:

The gels were scored for presence or absence of reproducible bands. Following Lynch and Milligan (1994), each band was regarded as a locus with two alternative alleles: present (1) or absent (0). The identification of 76 scorable bands led to the construction of 8 isolates x 76 loci data matrix, which was analyzed for diversity between populations (Lewinsohn et al., 2001). A binary matrix combined all the data records for all strains used in this study from all three primers. The matrix was then used as input for the prerelease version 0.9.1.50 of *FreeTree* software package with the similarity coefficient set to Dice. Clustering was performed by bootstrap neighbor joining (NJ) method and the tree was constructed by using the software TreeView (Win 16) 1.4.

3. Results and discussion:

The RAPD-PCR reaction was setup with eight Indian cultivated species of *Pleurotus* using the different ten-mar primers (Table 1). The amplifications were carried out twice to check for reproducibility. Occasionally, the intensity of some bands were reduced or increased slightly, but the total number of bands obtained with a primer remains the same. A negative control without the *Pleurotus* genomic DNA template was kept for amplification along with the *Pleurotus* genomic DNA with its primer. This was to confirm the quality of the primer and to avoid the scoring of bands which may arise due to primer for possible contaminants. Among the 10 primers scanned, 3 were chosen for amplifying genomic DNA of all the eight *Pleurotus* species. Primer OPA-4 amplified the lowest number of scorable bands and primer OPD-07 amplified the highest number of scorable bands. The third primer OPD-11, which also gave almost similar scorable bands like OPD-7. The primer OPA-4 produced bands ranging between 1.2 to 0.18 kb in size except *P. membranaeceus* showed no visible band with this RAPD primer. OPD-7 showed 1-5 bands with size ranging from 0.9 kb to 0.12 kb. *P. sapidus, P. flabelletus, P. sajor-caju* and *P. florida* showed 4 bands but they differed from each other by at least one band position. In case of OPD-11, 3-4 bands appeared in each of the seven *Pleurotus* species (ranging 1.1 kb to 0.14 kb), but only one band (0.9 kb) appeared in case of *P. ostreatus*. In total three primers yielded 76 scorable bands ranging from 1.2 kb to 0.14 kb for all eight *Pleurotus* species. All the three primers showed the specific RAPD profile. Primer OPD-7 and OPD-11 gave the most number of RAPD fingerprints and by using these two primers all the eight *Pleurotus* species can be differentiated individually.

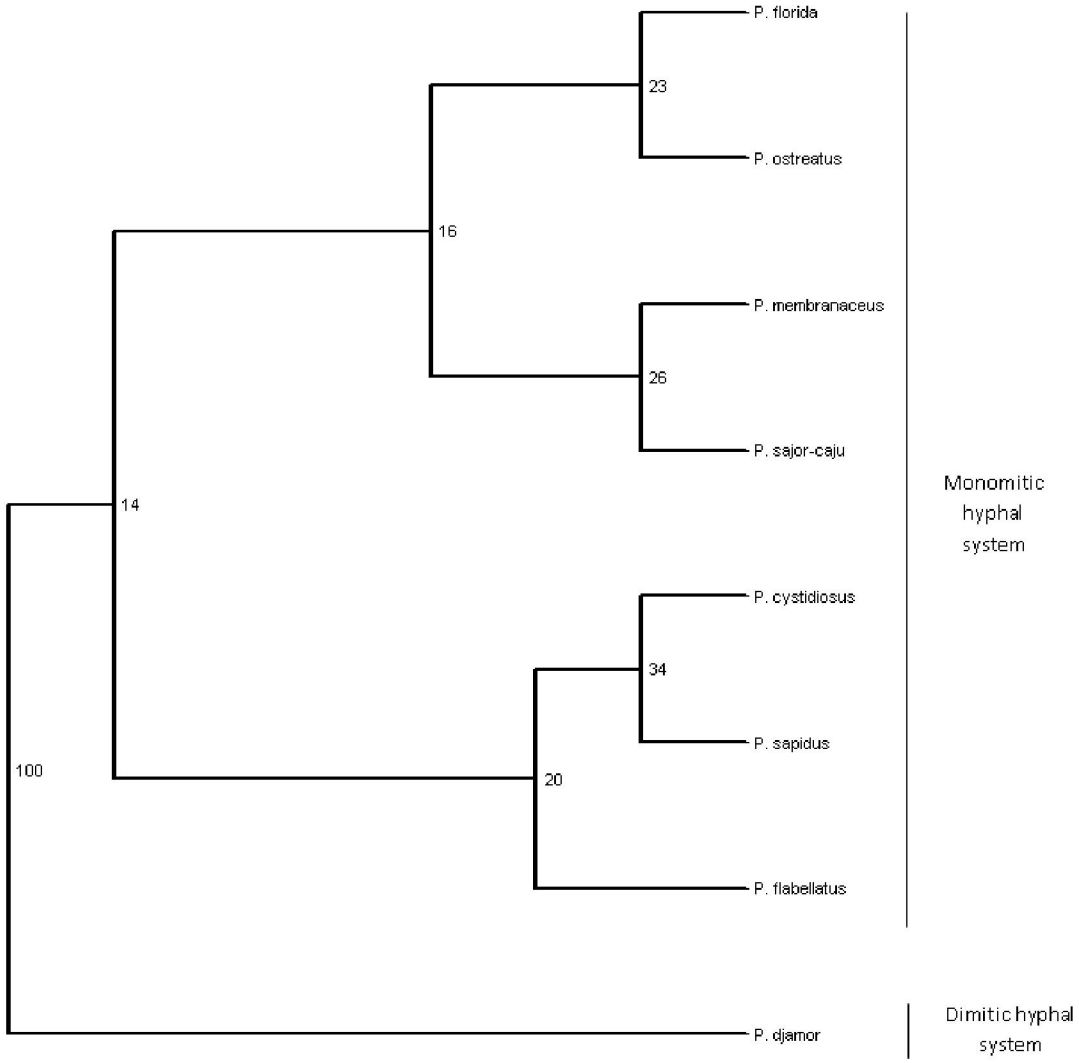


Figure 1. Consensus tree obtained by the bootstrap neighbour-joining method from the RAPD analysis.

Table 1: primer name and sequences:

PRIMERS	SEQUENCES	POLYMORPHISM
OPA-04	5' AATCGGGCTG 3'	+
OPA-16	AGCCAGCGAA	-
OPA-20	GTTGCGATCC	**X**
OPB-7	GGTGACGCAG	**X**
OPB-12	CCTTGACGCA	**X**
OPC-4	CCGCATCTAC	**X**
OPC-15	GACGGATCAG	**X**
OPD-07	TTGGCACGGG	+
OPD-11	AGCGCCATTG	+
OPD-20	ACCCGGTCAC	-

+ With polymorphism; - one or two species showed amplification; **X** without amplification.

Coefficients of genetic similarity were calculated form paired comparison of the eight species, based on normalized identity of each locus in each of species (Nei, M., 1978). The results are given in Table 2. The mean value of similarity coefficient was 0.357 (range 0.111 to 0.727). The highest similarity coefficient was obtained 0.727 between *P. cystidiosus* – P19 and *P. sapidus* – PL40 and lowest similarity coefficient was obtained 0.111 between *P. flabelletus* – PL50 and *P. ostreatus* – 1803. The cladogram produced by 8 RAPD phenotypes was evaluated by Bootstrap method and are presented in figure 1. Phylogenetic study revealed that species of *Pleurotus* having monomitic hyphal system might be originated from the *Pleurotus* having dimitic hyphal system (Figure 1). Previous phylogenetic studies have given rise to various ambiguities in the genus *Pleurotus*. According to the molecular markers used, *P. ostreatus*, *P. colombinus* and *P. cornucopiae*

have been in turn associated in the same clade (Iraçabal et al., 1995) or clearly separated (Zervakis et al., 1994), and the position of *P. sapidus* has remained uncertain (Zervakis and Balis, 1996).

Table 2: Similarity matrix of eight species of *Pleurotus*.

	P. flo	*P. mem*	*P. saj*	*P. dja*	*P. cys*	*P. fla*	*P. sap*	*P. ost*
P. flo	1.000							
P. mem	0.375	1.000						
P. saj	0.273	0.375	1.000					
P. dja	0.300	0.286	0.300	1.000				
P. cys	0.476	0.400	0.476	0.421	1.000			
P. fla	0.500	0.444	0.250	0.364	0.696	1.000		
P. sap	0.435	0.353	0.435	0.476	0.727	0.560	1.000	
P. ost	0.250	0.200	0.125	0.143	0.133	0.111	0.118	1.000

P. flo: *P. florida* – P1; ***P. mem:*** *P. membranaeceus* – OE128; ***P. saj:*** *P. sajor-caju* – PL1140; ***P. dja:*** *P. djamor* – X375; ***P. cys:*** *P. cystidiosus* – P19; ***P. fla:*** *P. flabelletus* – PL50; ***P. sap:*** *P. sapidus* – PL40 *and* ***P. ost:*** *P. ostreatus* – PO1803.

The RAPD technique has also been successfully used to distinguish other genera of cultivated mushrooms, such as for the discrimination of different strains of *Agaricus bisporus* (Khush et al., 1992, Moore and Chiu, 2001), *Ganoderma lucidum* complex (Hseu et al., 1996), *Lentinula edodes* (Chiu et al., 1996), and for the identification and genetic evaluation of single-spore progenies of *Agaricus bisporus* (Calvo-Bado et al., 2000) and *Stropharia rugoso-annulata* (Yan et al., 2003). Therefore, the RAPD genetic variability in *Pleurotus* mushroom will also provide useful information for breeding of commercial strains.

Correspondence to:
Krishnendu Acharya
Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700 019, West Bengal, India.
Cellular phone: 08013167310
E mail: krish_paper@yahoo.com

References

1. Barroso G, Sonnenberg AS, Van Griensven LJ, Labarere J. Molecular cloning of widely distributed microsatellite core sequence from the cultivated mushroom *Agaricus bisporus*. Fungal Genetics and Biology. 2000;31:115-123.
2. Calvo-Bado L, Noble R, Challen M, Dobrovin-Pennington A, Elliott T. Sexuality and genetic identity in the *Agaricus* section *Arvenses*. Applied and Environmental Microbiology. 2000;66:728-734.
3. Castle AJ, Horgen PA, Anderson JB. Restriction fragment length polymorphisms in the mushrooms *Agaricus brunnescens* and *Agaricus bitorquis*. Applied and Environmental Microbiology. 1987;53:816-822.
4. Chiu SW, Chen MJ, Chang ST. Differentiating homothallic *Volvariella* mushrooms by RFLPs and AP-PCR. Mycological Research. 1995;99:33-336.
5. Chiu SW, Ma A, Lin F, Moore D. Genetic homogeneity of cultivated strains of shiitake (*Lentinula edodes*) used in China as revealed by the polymerase chain reaction. Mycological Research. 1996;100:1393-1399.
6. Hseu RS, Wang HH, Wang HF, Moncalvo JM. Differentiation and grouping of isolates of the *Ganoderma lucidum* complex by random amplified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. Applied and Environmental Microbiology. 1996;62:1354-1363.
7. Iraçabal B, Zervakis G, Labarère J. Molecular systematic of the genus *Pleurotus*: analysis of the restriction polymorphism in ribosomal DNA. Microbiology. 1995;141:1479-1490.
8. Khush RS, Becker E, Wach M. DNA amplification polymorphisms of the cultivated mushroom *Agaricus bisporus*. Applied and Environmental Microbiology. 1992;58:2971-2977.
9. Lewinsohn D, Nevo E, Wasser PS, Hadar Y, Beharav A. Genetic diversity in populations of the *Pleurotus eryngii* complex in Israel. Mycological Research. 2001;105(8):941-951.
10. Lynch M, Milligan BG. Analysis of population gene structure with RAPD markers. Molecular Ecology. 1994;3:91-99.
11. Moore AJ, Challen MP, Warner PJ, Elliott TJ. RAPD discrimination of *Agaricus bisporus* mushroom cultivars. Applied and Environmental Microbiology. 2001;55:742-749.
12. Moore D, Chiu SW. Filamentous fungi as food. In: Pointing SB, Hyde D, eds. Exploitation of Filamentous Fungi. Fungal Diversity Press, Hong Kong. 2001.
13. Nei M. Estimation of average heterozygosity and genetic distance from a small sample of individuals. Evolutionary Biology. 1978;17:73-118.
14. Ramirez L, Muez V, Alfonso M, Garcia Barrenechea A, Alfonso L, Pisabarro AG. Use of molecular markers to differentiate between

commercial strains of the button mushroom *Agaricus bisporus*. FEMS Microbiology Letters. 2001;198:45-48.
15. Sonnenberg ASM, Van Loon PCC, van Griensven LJLD. The occurrence of mitochondrial genotypes and inheritance of mitochondria in the cultivated mushroom *Agaricus bisporus*. Mushroom Science. 1991;13:85-92.
16. Williams JGK, Kubelik AR, Lilvak KJ, Rafalski JA, Tingey SV. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nuclic Acids Research. 1990;18:6534-7213.
17. Yan PS, Jiang JH, Li GF, Deng CL. Mating system and DNA polymorphism of monokaryons with different mating type of *Stropharia rugoso-annulata*. World Journal of Microbiology and Biotechnology. 2003;19:737-740.
18. Yan PS, Luo XC, Zhou Q. RFLP analysis of amplified nuclear ribosomal DNA in the genus *Auricularia*. Mycosystema. 1999;18:206-213.
19. Zervakis G, Balis C. A pluralistic approach in the study of *Pleurotus* species with emphasis on compatibility and physiology of the European morphotaxa. Mycological Research. 1996;100:717-731.
20. Zervakis G, Sourdis J, Balis C. Genetic variability and systematics of eleven *Pleurotus* species based on isozyme analysis. Mycological Research. 1994;98:329-341.
21. Zervakis IG, Moncalvo JM, Vilgalys R. Molecular phylogeny, biogeography and speciation of the mushroom species *Pleurotus cystidiosus* and allied taxa. Microbiology. 2004;150:715-726.
22. Zervakis IG, Venturella G, Papadopoulou K. Genetic polymorphism and taxonomic infrastructure of the *Pleurotus eryngii* species-complex as determined by RAPD analysis, isozyme profiles and ecomorphological characters. Microbiology. 2001;147:3183-3194.

4/26/2010

Factors affecting the distribution and abundance of bottom fauna in Lake Nasser, Egypt

Isaac A. Iskaros and M. El Dardir*
*National Institute of Oceanography and Fisheries, Aswan, Egypt
dr_mona_zaki@yahoo.co.uk

Abstract: This study aim to determine which factors regulate the benthic invertebrates in the offshore area of Lake Nasser. The area investigated represents about 80% of the total lake and that is not well utilized. Seven stations along the main channel of the lake and three main khors out of 85 were selected. Transparency, temperature, conductivity, dissolved oxygen, hydrogen ion concentration, some characteristics of bottom sediments, the population density and biomass of bottom fauna were measured. Only 10 species belonging to oligochaetes (3 species), chironomid larve (4 species) and molluscs (3 species) were recorded. The former were the most common group. The highest standing stock of bottom fauna was noticed in the main channel, particularly during spring (avg. 5846 org./m^2 and 29.6 g. f.w./m^2) associated with clay and silt grains representing (40.5 – 54.5%) and (37.0 – 46.0%), respectively; and subsequently high content of organic matter (8.0 – 12.5%). The three khors sustained low densities of bottom fauna and sediments constituted mainly of sand with low organic matter. [Nature and Science 2010;8(7):95-108]. (ISSN: 1545-0740)

Keywords: Lake Nasser, water quality, bottom sediments, bottom fauna, community structure.

1. Introduction:

The purpose of constructing Aswan High Dam during the period 1959 – 1969 was primarily to benefit the downstream side by controlling annual floods. Aswan High Dam Reservoir covers an area of about 6500 km2 at the final storage level of 183 m above mean sea level (m.s.l), of which northern two – third (known as Lake Nasser) is in Egypt and one – third (called Lake Nubia) in Sudan. Surface area of Lake Nasser is about 5248 km^2, water volume is roughly 131 km^3, mean depth 25.2 m at 180 m above m.s.l. The deepest part of the lake (the item of the present work) is the ancient river bed with its bottom elevation between 85 and 150 m above m.s.l. The lake has many embayments locally called khors. The total numbers of important khors reached about 85 khors. Some khors as Kalabsha, El-Allaqi and Tushka are wide, with a sandy bottom and slope gently; others as El-Sabakha, Singari and Korosko are steep, relatively narrow with a rocky bottom. The water level in Lake Nasser depends on the flood season originating from the Ethiopian highlands that occurs from late August to November. It is known by its high turbidity carrying a heavy load of a mixture of sand, silt and clay. The yearly flood of the Nile is the most important factor affecting the conditions of Lake Nasser (Mageed & Heikal, 2006).

The study of bottom fauna in Lake Nasser has received minor interest. Entz (1978) and Latif *et al*., (1979) regarded gradual change in the components of benthos with the development of the lake, particularly molluscs and oligochaetes. Iskaros (1988 & 1993), Fishar (1995 & 2000) and (SECSF, 1996) identified 48 species in the littoral zones related to four major groups: Cnidaria (Coelentrata) (1 class & 1 sp.), Bryozoa (1 class & 1 sp.), Arthropoda (2 classes & 31 spp.), Annelida (2 classes & 5 spp.) and Mollusca (2 classes & 10 species). The aim of the present study is to carry out quantitative and qualitative estimations on bottom fauna in the offshore area of Lake Nasser which was carried out for the first time in relation to the prevailing environmental conditions, particularly the characteristics of bottom sediments.

2. Material and Methods

The bottom fauna was seasonally collected, during spring (May), Summer (July), autumn (November) 2006 and winter (February) 2007. Seven stations were selected in the main stream of Lake Nasser to represent the different habitats. These were El-Ramla, Kalabsha, El-Allaqi, El-Madiq, Korosko, Tushka and Abu-Simbel. Besides, three main khors were also chosen on both western and eastern sides, namely; Khor Kalabsha, Khor Korosko and Khor Tushka (Fig. 1).

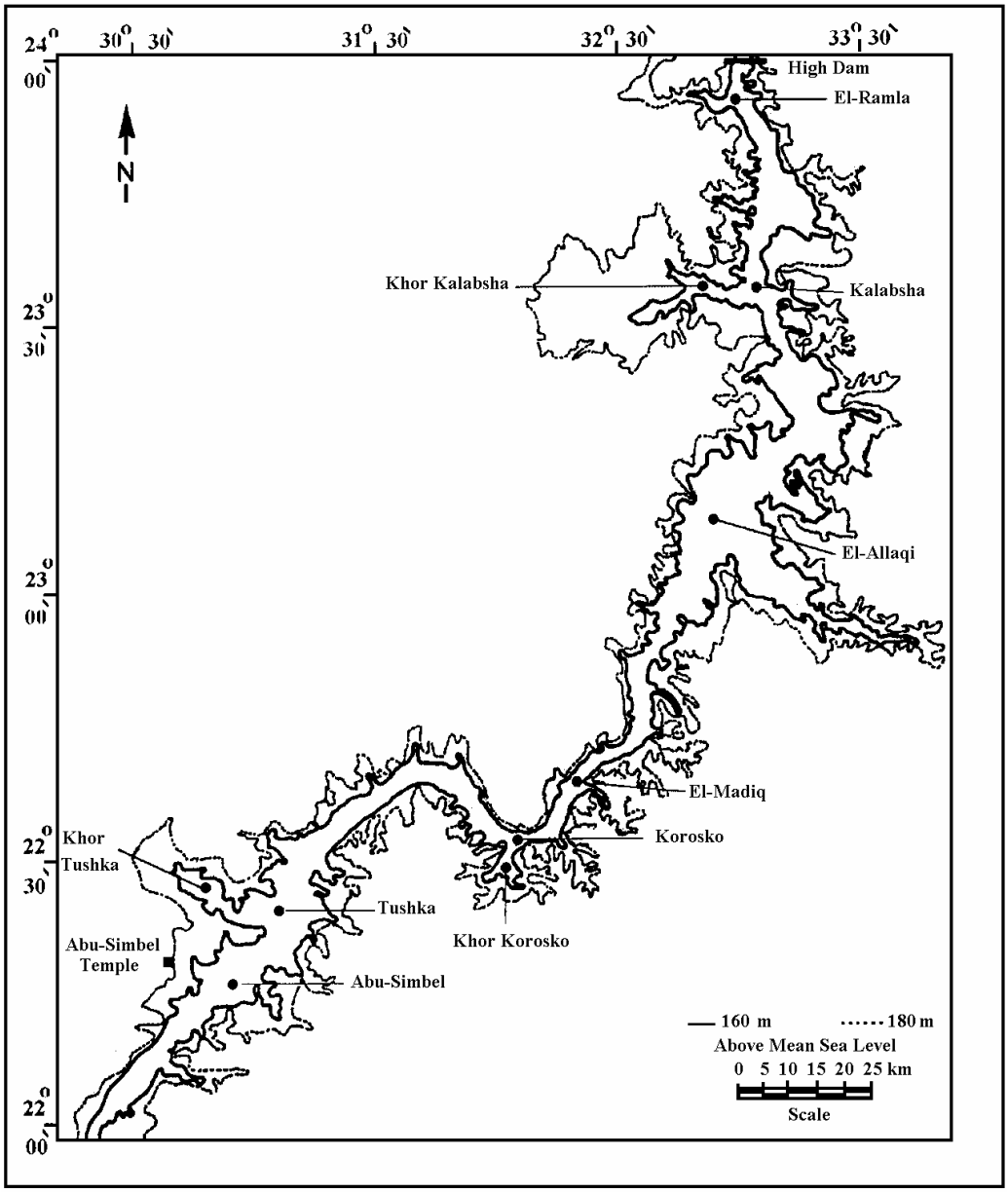


Fig. (1): Map of Lake Nasser showing the stations of study.

Bottom fauna samples were collected using Ponnar bottom grab with an opening of 234 cm^2 (1/43 m^2). Samples were thoroughly washed from muds in a metallic sieve with mesh size of 0.4 mm sorted directly in the field and preserved in 5% formaline solution. In lab, the number of the different species and genera were determined (no. ind./m^2) and their weights (g.f.w/m^2) were also determined. The shells of molluscs were removed for weighing their flesh.

Oligochaetes, were identified according to Brinkhurst & Jamieson (1971) and Pennak (1978) and were further checked by Dr. E.G. Easton from the British Museum. The identification key used by Wirth & Stone (1968) and Hilsenhoff (1975) was adopted for chironomid larvae, while those of Brown (1980) and Brown et al., (1984) for molluscs.

Simultaneous with sample collection, transparency was obtained by a black-white enameled

Secchi disc of 30 cm diameter at the shaded side of the boat. Water samples were collected using Van-dorn bottle at three levels (surface, middle and bottom). Temperature, electrical conductivity, dissolved oxygen and hydrogen ion concentration were analyzed using standard methods (APHA, 1995) in the different sampling stations. Sieving and settling velocity techniques were made for grain size analysis. Organic matter estimation was carried out as described by Hanna (1965). The calcium carbonate was determined using Jackson methodology (1958).

3. Results

3.1. Physicochemical Parameters

3.1.1. Transparency

Transparency fluctuated between 150 cm at Khor Korosko during spring to 450 cm at El-Ramla in winter (Fig. 2). During spring and summer, the flourishing of phytoplankton in Lake Nasser reduces water transparency. The highest values were observed in the north (El-Ramla – El-Allaqi) (170 – 450 cm) and in the middle (El-Madiq – Khor Korosko) (150 – 320 cm) when compared with 160 – 220 cm for the southern ones (Tushka – Abu Simbel) which are affected by the flood.

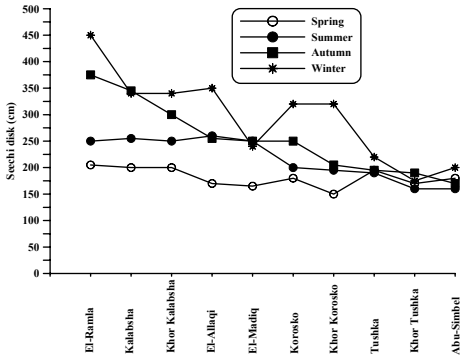


Fig. (2): Secchi disc readings in Lake Nasser during the study.

Table (1): Some water quality parameters measured in Lake Nasser during the study.

Station	Depth	Temperature (T)				Electrical conductivity (µmhos)				Dissolved oxygen (mg/l)				pH values			
		spr.	sum.	aut.	win.	spr.	sum.	aut.	win.	spr.	sum.	aut.	win.	spr.	sum.	aut.	win.
El-Ramla	surface	22.90	29.00	23.50	16.50	236	278	271	230	7.06	7.84	7.98	8.51	8.72	8.76	8.54	8.27
	middle	19.02	19.88	21.97	16.51	231	275	264	225	4.12	0.00	5.46	8.37	8.18	7.69	8.29	8.02
	bottom	18.05	18.72	19.82	16.33	229	264	257	223	2.86	0.00	0.00	8.37	8.17	7.79	7.83	8.00
Kalabsha	surface	23.90	28.80	23.35	17.00	249	253	250	243	7.68	6.84	7.35	8.96	8.67	8.77	8.53	8.12
	middle	18.80	19.93	21.87	16.71	241	245	244	235	2.79	0.00	6.34	8.55	8.07	7.85	8.46	7.67
	bottom	18.23	18.73	19.79	16.39	236	239	240	230	3.06	0.00	0.00	8.54	8.09	7.80	7.83	7.79
Khor Kalabsha	surface	24.34	29.60	23.60	16.90	233	253	245	220	8.03	6.83	8.41	9.13	8.92	8.92	8.56	8.27
	middle	19.90	22.35	22.89	16.69	213	234	225	210	3.02	0.00	6.59	8.90	8.46	7.84	8.50	8.00
	bottom	-	20.05	-	-	195	202	200	188	-	0.00	-	-	-	7.84	-	-
El-Allaqi	surface	24.60	29.55	23.90	17.50	230	248	239	227	8.42	6.77	8.98	8.36	8.77	8.86	8.89	8.48
	middle	18.97	19.91	22.09	17.28	225	243	235	223	3.00	0.00	4.53	8.43	8.23	7.69	8.36	8.36
	bottom	18.45	18.87	-	17.14	221	240	233	218	2.57	0.00	0.00	7.12	8.13	7.68	0.00	8.18
El-Madiq	surface	26.10	29.70	24.80	18.00	239	261	247	235	7.10	6.94	9.20	8.19	8.80	8.97	8.98	8.11
	middle	19.40	20.19	23.07	17.59	229	246	234	225	3.03	0.00	5.21	7.19	8.13	7.64	8.36	8.02
	bottom	-	19.01	-	17.51	221	233	225	220	-	0.00	0.00	7.20	-	7.62	-	8.02
Korosko	surface	25.70	29.99	24.60	18.50	228	239	234	231	7.75	6.88	9.49	8.07	8.99	8.88	8.76	8.37
	middle	20.00	19.77	22.69	17.39	227	237	232	224	2.87	0.27	4.50	7.70	8.39	8.00	8.41	8.24
	bottom	18.38	18.74	-	17.08	210	230	225	205	2.28	0.00	0.00	7.29	8.27	-	-	8.22
Khor Korosko	surface	25.70	31.90	24.90	18.20	243	260	250	230	7.82	6.9	9.24	8.12	8.92	8.91	8.64	8.24
	middle	20.91	24.78	23.60	18.10	240	255	246	226	4.27	1.91	5.50	7.91	8.57	7.22	8.28	8.22
	bottom	-	23.96	-	18.08	235	252	242	223	4.02	1.26	5.39	7.23	8.52	-	8.24	8.26
Tushka	surface	25.70	31.00	23.40	17.50	229	240	235	220	7.97	7.17	6.96	8.99	8.92	8.87	8.33	8.44
	middle	19.71	19.75	22.74	16.95	220	228	225	212	3.56	0.00	6.77	8.54	8.30	7.71	8.26	8.34
	bottom	18.64	18.66	-	16.55	212	220	217	200	3.17	0.00	-	8.38	8.27	7.70	8.20	8.30
Khor Tushka	surface	24.10	30.70	23.40	17.30	228	249	238	225	7.33	7.23	6.91	8.63	8.89	8.84	8.32	8.35
	middle	23.93	25.29	23.22	17.13	227	245	236	223	7.22	0.93	6.98	8.44	8.87	7.88	8.39	8.38
	bottom	-	-	-	-	230	243	233	220	-	0.00	-	8.21	-	-	-	8.38

Table (1): Continue

Station	Depth	Temperature (T)				Electrical conductivity (μmhos)				Dissolved oxygen (mg/l)				pH values			
		spr.	sum.	aut.	win.	spr.	sum.	aut.	win.	spr.	sum.	aut.	win.	spr.	sum.	aut.	win.
Abu-Simbel	surface	24.50	31.90	23.80	17.10	220	267	219	215	7.97	6.56	8.16	9.94	8.93	8.86	8.56	8.30
	middle	19.67	19.83	21.86	16.65	216	265	218	210	3.24	0.23	7.61	-	8.25	7.77	8.33	8.52
	bottom	18.78	19.22	-	-	211	260	215	200	2.50	0.22	-	-	8.17	7.79	-	-

Where spr. = spring, sum. = summer, aut. = autumn & win. = winter

3.1.2. Temperature

The water temperature (Table 1) measured for several depths during the different seasons varied between 18.05 – 26.1, 18.66 – 31.9, 19.79 – 24.9, 16.5 – 18.5 °C in spring, summer, autumn and winter, respectively. The lowest average value of surface water temperature of the lake was 17.4 °C in winter while the highest average value of 30.2 °C recorded in summer. The lake water was vertically homothermal during winter. The increase in air temperature during spring was followed by increase in the surface temperature (avg. 24.75 °C) and the difference in water temperature with depth becomes clear (4.85 – 7.32 °C), indicating progressive development of thermocline which becomes established in summer (7.24 – 12.34 °C). Considerable winds together with the incoming cooling flood water during autumn may be required to mix the whole eplimnion down to the primary thermocline.

3.1.3. Electrical Conductivity

The measured electrical conductivity (E.C.) values (Table 1) for the different depths during the different seasons varied between 195 – 249, 202 – 278, 200 – 271 and 200 – 243 in spring, summer, autumn and winter, respectively. The E.C. was affected by variations of water temperature (Gindy & Dardir, 2008). Thus, the relative increase of E.C. during summer and autumn particularly at the surface (219 – 278) was coincided with the high water temperature which leads the hydrolysis and redissolution of insoluble salts and subsequently their adsorption onto the lake water. Otherwise, the E.C. decreased during spring and winter (188 – 249) with the falling water temperature, may be ascribed to the uptake of dissolved salts by phytoplankton (Awadallah & Moalla, 1996).

3.1.4. Dissolved Oxygen

The values of dissolved oxygen given in Table (1) for the different depths varied between 2.28 – 8.42, 0.0 – 7.84, 0.0 – 9.49 and 7.12 – 9.94 mg/l during spring, summer, autumn and winter, respectively. During winter, dissolved oxygen were more less homogenous at different depths of the lake, reaching its highest concentration for the whole year. During spring, dissolved oxygen showed a gradual decrease towards the bottom which still sustained sufficient amount of dissolved oxygen (2.28 – 4.02 mg/l). During summer, the oxygen depleted layer prevailed at the middle and bottom depths of most stations parallel with the thermal stratification. Oxygenation of the water column was restored again during autumn with the incoming flood water together with the decrease in water temperature where the epilimnion extended to the middle depths at most stations (4.53 – 7.61 mg/l). Iskaros *et al*.(2008) observed that the epilimnion occupied the upper 10 m for most Lake Nasser during summer and there was a sharp drop in dissolved oxygen between 10 & 20 m depth which represents the thermocline.

3.1.5. Hydrogen Ion Concentration

The pH values (Table 1) were always on the alkaline side and varied between 7.22 at the middle depth of Khor Korosko during summer and 8.99 at the surface of Korosko during spring. The highest values of pH were recorded in the surface, particularly during spring and summer (8.67 – 8.99) due to the increased photosynthetic activities of phytoplankton. On the other hand, the middle and bottom water had lower pH values during summer (7.22 – 8.0 & 7.62 – 7.84, respectively), parallel to the development of thermocline while these two layers sustained higher values during the other seasons.

3.1.6. Characteristics of The Bottom Sediments:

a- Grain size analysis

Grain size composition is an important factor that should be taken into consideration to interpret some bottom fauna distribution in the clastic sediments. The mechanical analysis of Lake Nasser sediments are given in Table (2). In the main channel, the silt and clay fractions form the main size of the sediments (37 – 46% and 40.5 – 54.5%), respectively. The distribution of grain size is controlled by depth of sediments where the clay size increased with depth while the silt and sand fractions decreased in the same trend. El-Dardir (1994) concluded that, in the main channel of Lake Nasser, the grain size of sediments decreased from south to north.

This is a reflection of decreasing in the following current competency, but some samples deviate from this pattern. This may be due to the presence of the Nubian sand stone and/or sand sheets on the shores and to geomorphic features of the reservoir. In khors, sand contents are found in high fraction (49.5 – 55.5%) while clay formed low ones. This is attributed to that khors mainly received main detrital sediments from the surrounding sand sheets and rocks drifted by the wind.

Table (2): Distribution of sand, silt and clay of the analysed sediments in Lake Nasser during the study.

station	Km/ H.D	Depth (m)*	Sand (%)	Silt (%)	Clay (%)	Type of sediments
El-Ramla	10	100	8.0	37.5	54.5	Sandy silty clay
Kalabsha	55	90	7.5	39.0	53.5	" " "
Khor Kalabsha	55	35	49.5	38.5	12.0	Clayey silty sand
El-Allaqi	110	85	10.5	37.0	52.5	Sandy silty clay
El-Madiq	140	85	12.5	39.5	48.0	" " "
Korosko	180	80	11.0	40.5	48.5	" " "
Khor Korosko	180	40	56.5	33.5	10.0	Clayey silty sand
Tushka	245	72	12.0	46.0	42.0	Sandy silty clay
Khor Tushka	245	25	55.5	30.5	14.0	Clayey silty sand
Abu-Simbel	280	62	15.5	44.0	40.5	Sandy clayey silt

Km/H.D.: Distance from the High Dam (km); Depth (m): Depth below lake water level.

b- Organic matter

The organic matter content in the bottom sediment of Lake Nasser is represented in Fig. (3). In the main channel, it fluctuated between 8 – 12.5%, 7.5 – 11.5%, 3.5 – 9.5% and 4.0 – 9.5% during spring, summer, autumn and winter, respectively. The organic matter was very high at Kalabsha (12.5 & 11.5%) during spring and summer, respectively while it was low at Abu-Simbel and Tushka representing 3.5% for each during autumn and increased as going from south to north. This is related to organic matter content and grain size distribution. On the other hand, khors sustained low values of organic matter (1.0%) at khors Korosko (spring) and Tushka (winter) and 5.5% at khor Kalabsha during autumn. Hence, the highest values of organic matter in the main channel were explained by high production of bottom fauna (Fig. 4). Besides, the organic matter is used as an indication of the amount and type of food settling to the sediment from the water column.

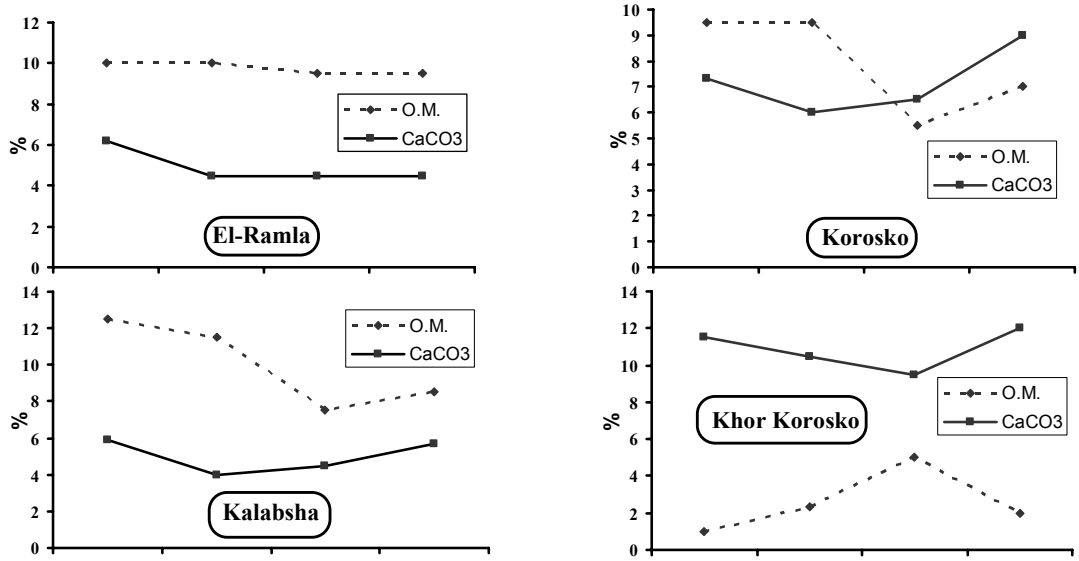


Fig. (3): Distribution of organic matter and Calcium carbonate (%) of the analysed sediments in Lake Nasser.

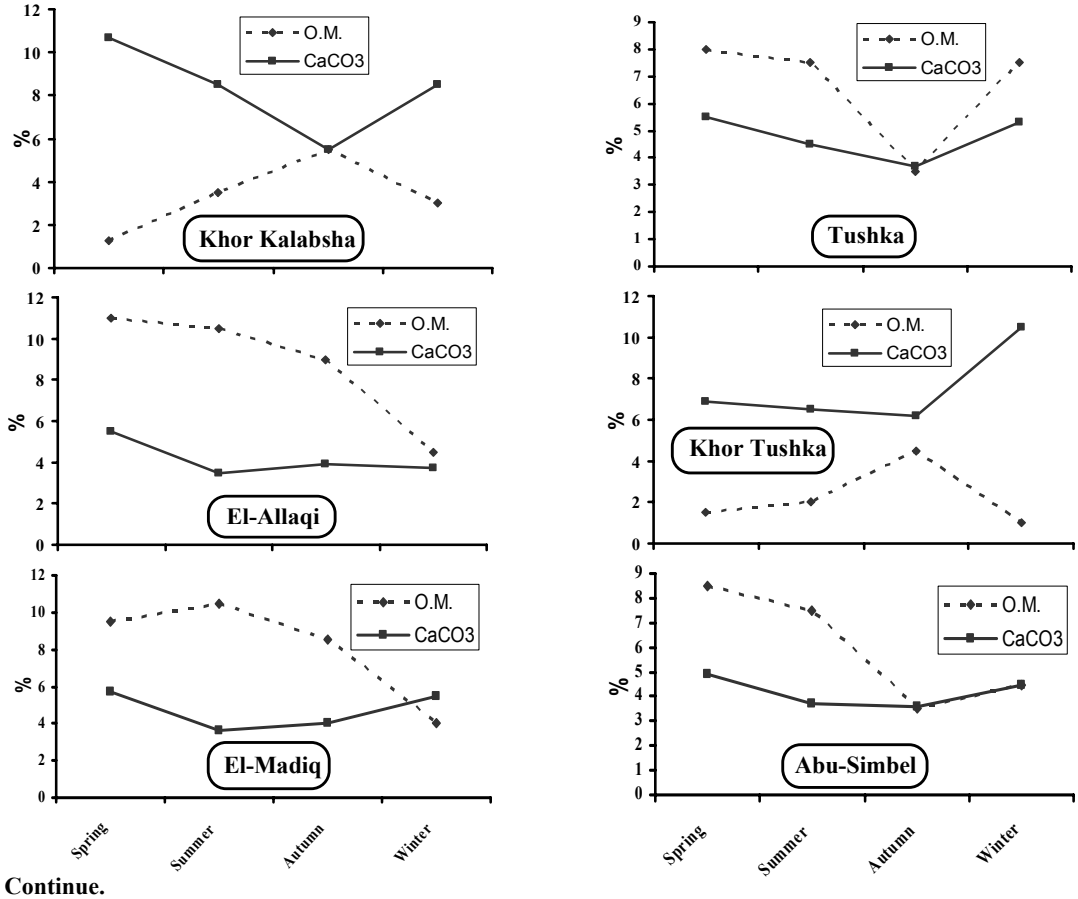


Fig. (3): Continue.

c- Calcium carbonate

The calcium carbonate content in the bottom sediments of Lake Nasser is represented in Fig. (3). In the main channel, $CaCO_3$ content was found to range from 4.9 – 7.3%, 3.5 – 7.0%, 3.6 – 6.5% and 3.7 – 9.0% during spring, summer, autumn and winter, respectively. These values increased in the khors to reach a range from 6.9 – 11.5%, 6.5 – 10.5%, 5.5 – 9.5% and 8.5 – 12.0% during the four above mentioned seasons. In the present study, $CaCO_3$ sediments in Lake Nasser was found to be not related to pH values variation in the water (Table 1 & Fig. 3). This indicates that the $CaCO_3$ abundance is not controlled by chemical precipitation. Consequently it is reliable to say that the $CaCO_3$ content is related to the encrusting organisms i.e. of biogenic origin. El-Dardir (1984) concluded that $CaCO_3$ concentration in the bottom sediments can indirectly help and participate in prognosing the primary productivity in Aswan High Dam Reservoir.

3.2. Bottom Fauna

3.2.1. Community composition, distribution and seasonal variations:

The macrobenthic fauna at the offshore zones of Lake Nasser embrases 10 species belonging to: oligochaetes (3 species), chironomid larvae (4 species) and molluscs (3 species). Oligochaetes were the most dominant group of benthic fauna (Fig. 4), constituting 83.9% (2017 org./m^2) and 91.7% (10.0 g.f.w./m^2) of their total numbers and biomass, followed by chironomid larvae (9.6% with 230 larvae/m^2 & 5.5% with 0.6 g.f.w./m^2) and Mollusca (6.5% with 157 org./m^2 & 2.8% with 0.3 g.f.w./m^2). The predominance of oligochaetes in the lake is possibly due to their ability to adapt to various habitats and to their tolerance to low oxygen content or anoxic conditions. The highest densities of benthic biota in the offshore zones were recorded in the main channel, particularly at Korosko and Tushka (4387 & 3978 org./m^2, respectively), accompanied by clay & silt grains (40.5 – 54.5%) and (37.0 – 46.0%), respectively (Table 2) and subsequently high content of organic matter (6.0 – 10.0%) (Fig. 3).

Contrary to that, they reached the lowest densities in the khors (634 – 1807 org./m^2) where the type of sediments were mainly sand (49.5 – 56.5%) with low organic matter (2.25 – 3.3%). The total biomass was nearly proportion to the numerical density (2.6 – 20.7 g.f.w./m2). The annual average density and biomass of bottom fauna for the whole offshore zones of the lake amounted 2404 org./m^2 and 10.9 g.f.w./m^2.

A marked difference in the bottom fauna stock was noticed during the four seasons (Fig. 4). They were more abundant in the main channel during spring with peaks at Kalabsha (11051 org./m^2 & 48.6 g.f.w/m^2) and at Korosko (10965 org./m^2 & 43.2 g.f.w./m^2) while the khors sustained the lowest densities (559 – 1462 org./m^2 and 1.3 – 2.5 g.f.w./m^2). On the other hand, the opposite occurred in summer during thermal stratification except at Tushka (6278 org./m^2 & 18.9 g.f.w./m^2) followed by another small ones in the khors (1204 – 2795 org./m^2 and 5.0 – 12.5 g.f.w./m^2). The community decreased more at most stations during autumn (86 – 2838 org./m^2 & 0.3 – 5.6 g.f.w./m^2) and winter (516 – 4171 org./m^2 & 0.7 – 25.0 g.f.w./m^2) with the continuity of the oxygen depleted layer and the falling of water temperature (Table 1), respectively. The abundance of bottom fauna in the main channel during spring and/or in the khors in summer was correlated with the amount of organic matter (8.0 – 12.5% & 4.5 – 5.5%, respectively) (Fig. 3).

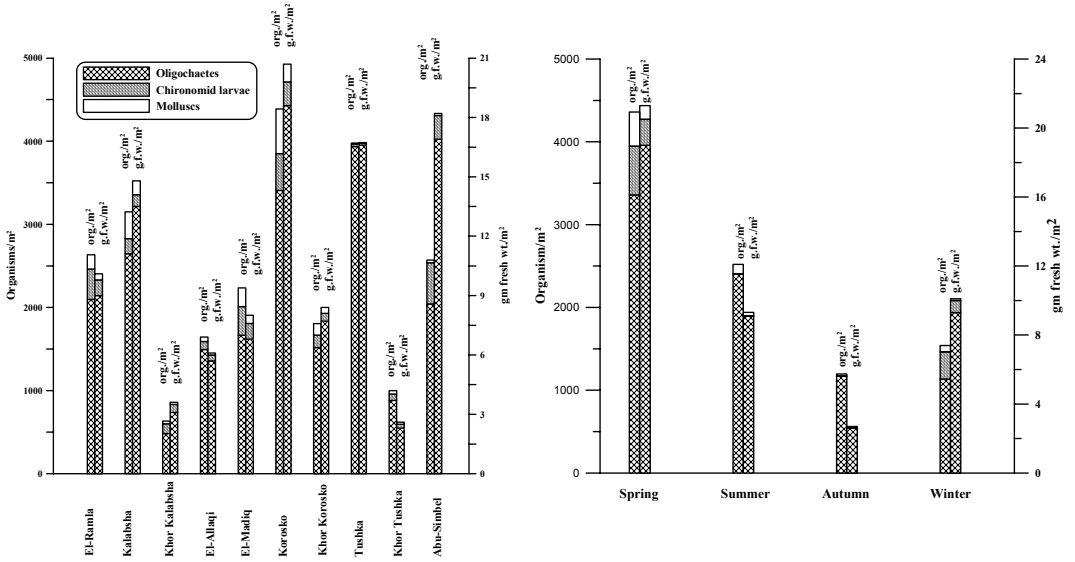


Fig. (4): Distribution and seasonal variations of bottom fauna (avg. org./m^2 & g.f.w./m^2) in Lake Nasser during the study.

3.2.2. Oligochaetae

As shown in Fig. 4, oligochaetes were most abundant in the main channel (1494 – 3935 org./m^2 & 5.7 – 18.6 g.f.w./m^2) than in the khors (484 – 1516 org./m^2 & 2.3 – 5.7 g.f.w./m^2). They were represented by 3 species, namely; *Limnodrilus udekemianus* Claparede, *L. hoffmeisteri* Claparede and *Branchiura sowerbyi* Beddard. Peaks of oligochaetes abundance were mainly recorded during spring (Fig. 4) at most main channel stations, particularly at Kalabsha (9761 org./m^2 & 47.0 g.f.w./m^2) and Korosko (7353 org./m^2 & 35.4 g.f.w./m^2) and/or in summer mainly at Tushka (6278 org./m^2 & 18.9 g.f.w./m^2). Such peaks produced large numbers of *Limnodrilus udekemianus* (Fig. 5) which contributed 72.3 & 86.2% (avg. 1738 org./m^2) of the total bottom fauna and oligochaetes, respectively. *Limnodrilus hoffmeisteri* contributed 6.2% of the oligochaetes (125 org./m^2). Its major occurance was recorded between El-Allaqi and Korosko (range 161–333 org./m^2) (Fig. 5) with a peak during spring (Fig. 5), particularly at the two above mentioned stations (1161 & 903 org./m^2, respectively). *Branchiura sowebyi* contributed 7.6% of the oligochaetes (154 org./m^2) where its major distribution was recorded at comparable densities at El_Ramla, Kalabsha and Abu-Simbel (398, 484 & 441 org./m^2, respectively) (Fig. 5) which also harboured the highest numbers during winter and spring being 817 and 1505 and 1376 org./m^2, respectively (Fig. 5).

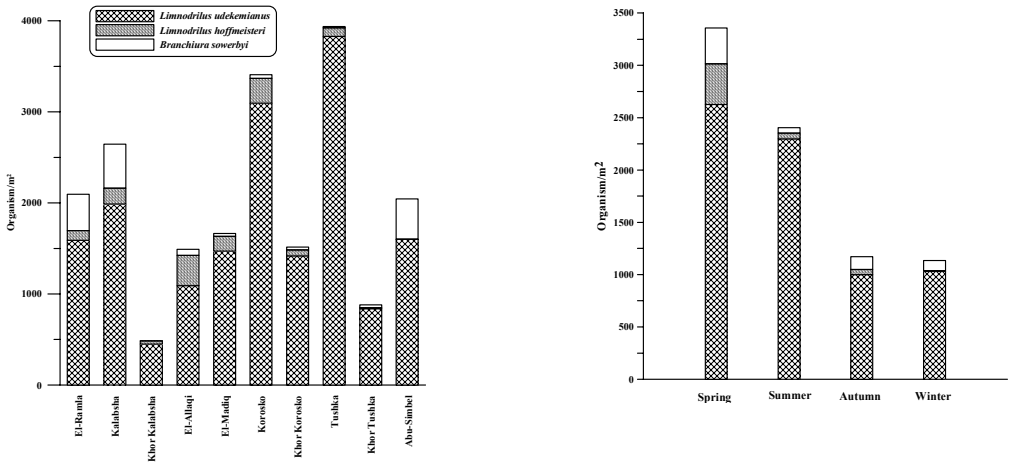


Fig. (5): Distribution and seasonal variations of Oligachaetae (avg. org./m^2) in Lake Nasser during the study.

3.2.3. Chironomid larvae

Chironomid larvae reached their highest density at comparable values at Abu-Simbel and Korosko (495 & 441 larvae/m^2, respectively & 1.2 g.f.w./m^2 for each) (Fig. 4) whereas, the low densities were detected between Khor Kalabsha and El-Allaqi and at Tushka and its khor (32 – 118 larvae/m^2 and 0.1 – 0.4 g.f.w./m^2). Larvae of Chironomidae were represented by *Procladius* sp., *Microtiendipes* sp., *Cryptochironomus* sp. and *Clinotanpus* sp. They were confined to spring (Fig. 4) with peaks at Abu-Simbel (1892 larvae/m^2 & 4.6 g.f.w./m^2) and at Korosko (1462 larvae/m^2 & 3.5 g.f.w./m^2) and during winter, particularly at El-Ramla (1462 larvae/m^2 & 3.2 g.f.w./m^2), mainly consisting of *Procladius* sp. (Fig. 6) which contributed 8.3 & 89.9% (avg. 199 larvae/m^2) of the bottom fauna yield and the chironomid larvae, respectively. *Microtiendipes* sp. was only encountered during spring at El-Allaqi, Korosko and its khor and Abu-Simbel (avg. 90 larvae/m^2) and at Kalabsha in summer (43 larvae/m^2). *Clinotanpus* sp. and *Cryptochironomus* sp. were recorded in few numbers during spring at Khor Kalabsha and El-Allaqi (172 & 86 larvae/m^2, respectively). Pupae probably of *Procladius* sp. were scarcely recorded during spring at Kalabsha (43 pupae/m^2).

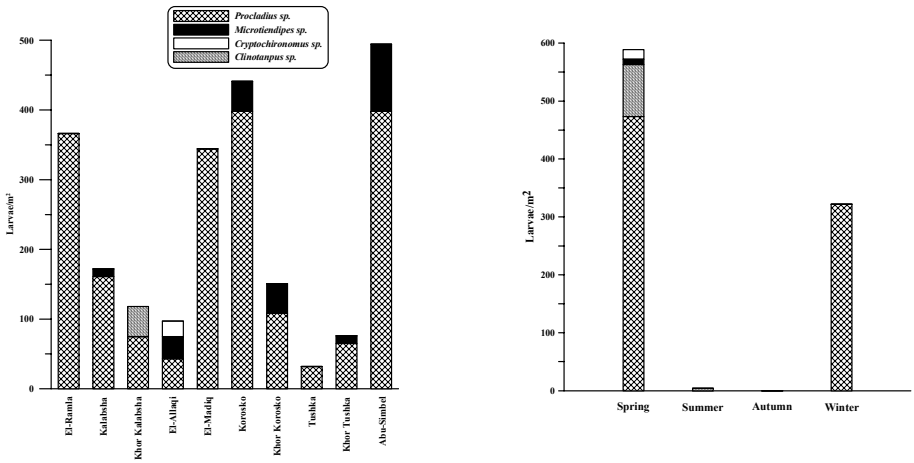


Fig. (6): Distribution and seasonal variations of the Larvae of Chironomidae (avg. larvae/m^2) in Lake Nasser during the study.

3.2.4. Mollusca

Mollusca attained its maximum abundance at Korosko (538 org. & 0.9 g.f.w.) followed by Kalabsha (322 org. & 0.7 g.f.w./m^2) (Fig. 4). The lowest record was at the south sector (range 11 – 43 org./m^2 & 0.03 – 0.1 g.f.w./m^2). Mollusc were represented by a single

gastropod *Valvata nilotica* Jickeli and two bivalvia *Pisidium pirothi* Jackeli and *Corbicula fluminalis* Müller. They were common during spring (Fig. 4), being more abundant at the two above mentioned stations (2150 org./m^2 with 4.3 g.f.w./m^2 and 1290 org./m^2 with 2.6 g.f.w./m^2, respectively), associated with an increase of phytoplankton standing crop, increasing amount of calcium carbonate (range 4.9 – 11.5%) (Fig. 3) when the water column was well saturated with dissolved oxygen (Table 1). They sharply declined during summer and autumn with free oxygen hypolimnion and in winter with the falling water temperature. *Valvata nilotica* (Fig. 7)was the major species of Mollusca, contributing 6.4 & 98.7% (avg. 155 org./m^2/year) of total the bottom fauna and mollusc, respectively. Few individuals of *Pisidium pirothi* and *Corbicula fluminalis* were recorded during spring and winter at Kalabsha and Abu-Simbel being 43 org./m^2 for each & 0.1 & 0.2 g.f.w. /m^2, respectively.

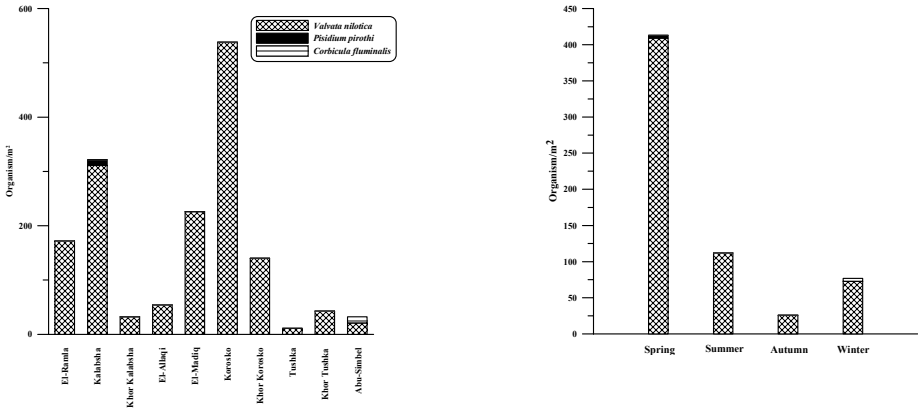


Fig. (7): Distribution and seasonal variations of Mollusca (avg. org./m^2 & g.f.w./m^2) in Lake Nasser during the study.

4. Discussion

In a complex natural environment, such as Lake Nasser where several factors operate simultaneously, it is not easy to generalize and designate certain factor as being more important than the other. The biological processes taking place at the lake bottom being the end result of the interactions of organisms present with the surrounding environment (Bishai et al., 2000). The benthic invertebrates in Lake Nasser exhibited marked variations from one station to another and this is attributed to the variations in the prevailing physico-chemical conditions as well as to their biological productivity. The substrate status of the bottom sediments are also of great importance.

The decrease in water's transparency in Lake Nasser is mainly caused by two factors: allochtonic inorganic silt and mud of riverine origin and autochtonic suspended organic matter (plankton & detritus). The northern and middle parts of the lake with their adjacent khors were showed the highest transparency (Fig. 2), particularly during autumn and winter (maximum Secchi depth 450 cm) and lower values during spring and summer (minimum Secchi depth 150 cm) due to phytoplankton blooming. On the other hand, the southern end of the lake exhibited the lowest transparency, thus coinciding with the introduction of flood turbid water in late summer and autumn.

A direct relation appeared to exist between the density of the molluscs and the rate of silting. Thus, the higher density of molluscs at the north (avg. 145 org./m^2 with 0.3 gm/m^2) and middle stations (avg. 301 org./m^2 with 0.5 gm/m^2) (Fig. 4) is attributed to the negligible amount of the silt that may reach these areas whereas the poorest values were observed at the southern ones (avg. 29 org./m^2 with 0.1 gm/m^2) in relation to the instability of the substrate, resulting from deposition of silt load carried by the flood. Present work results are in good accordance with those given by El-Dardir (1984) who mentioned that the maximum sedimentation in Lake Nasser takes place at the south sector. Brown & Lemma (1970) and Kloose & Lemma (1974) concluded that the influx of inorganic material in Awash River (Ethiopia) is inhospital for most molluscs because of their heavy silt load.

The grain size analysis (Table 2) in Lake Nasser revealed that the studied sediments in the main channel are mainly composed of sandy silty clay (60%) and sandy clayey silt (10%) whereas khors samples are clayey silty sand (30%). Consequently, the values of the total organic content (Fig. 3) in the sediments of the lake are higher in the main channel stations (4.0 –

12.5%) than those of the khors (1.0 – 5.5%). This may be attributed to the main channel sediments fine texture which can sustain high content of those elements. According to Tjoe-Awie (1975), the fine detrital fraction is usually richer in organic material than the coars ones which acts as a dilutant. Therefore, the total organic content of the lake sediments is well correlated with the silt and clay fractions size (r = 0.360 & 0.811, respectively), contrary to sand (r = -0.786)(Table 3).

The nature of the bottom sediments has a selective influence on quality and quantity of benthos and it is considered the most significant factor determining their distribution (Welch, 1952 and Brinkhurst & Jamieson, 1971). A direct relation was recorded between the magnitude of the standing stock of benthos and the type of sediments (Table 2 & Fig. 4). Thus, a positive correlation was found between most bottom fauna species abundance and the silt and clay fractions (r = 0.112 – 0.355 & 0.167 – 0.275, respectively) (Table 3). Our results were emphasized by Wirth & Stone (1968) and Brinkhurst & Jamieson (1971) who stated that the Tubificidae and Tanypodinae are more often found in mud or soft black mud of rivers and lakes.

Most of the benthic fauna probably derive the bulk of their nutritional requirements from micro-organisms, ingested along with allochthonous and autochthonous organic matter in sediments (Payne, 1986). The increased of total organic content in the sediments during spring (avg. 7.3%) and summer (avg. 7.5%) was generally in concomitance with a parallel density of bottom fauna (Fig. 3 & 4). Thus a positive correlation stands for the abundance of the bottom fauna and the total organic content (r = 0.208 – 0.457) (Table 3). This indicates that the organic content may be an important relative to the population growth of the bottom fauna in Lake Nasser. Brinkhurst & Jamieson (1971) and Brown (1980) findings confirm present work results as they stated that the growth and reproduction of the oligochaetes and molluscs are apparently related to available food supply. Della Croce (1955) and Brinkhurst (1965 & 1967) concluded that there exist few clear correlations between the variations in particle size or total organic matter present and the distribution and abundance of bottom fauna, particularly concerning oligochaetes which have been demonstrated.

The present work results indicate that temperature, electrical conductivity, dissolved oxygen and pH proved to be of variable effect in controlling the flourishing and existence of different species in Lake Nasser. However, Brinkhurst & Jamieson (1971) agree in demonstrating an overall lack of correlation between the data obtained from chemical analysis of the overlying water and the sort and proportion of the species encountered. This may be attributed to other environmental conditions prevailing there and not prevailing in Lake Nasser.

Table (3): Correlation coefficient between the ecological parameters and the main bottom fauna species in Lake Nasser during the study.

	T	E.C.	D.O.	pH	O.M.	Sand	Silt	Clay	Limn. ud.	Limn. ho.	Bran. so.	Proc.
EC	0.530											
DO	0.162	-0.161										
pH	0.647	0.030	0.428									
O.M	-0.287	0.273	-0.312	-0.175								
Sand	0.536	-0.098	0.145	0.161	-0.786							
Silt	-0.204	-0.352	-0.123	0.071	0.360	-0.660						
Clay	-0.526	0.200	-0.137	-0.199	0.811	-0.982	0.508					
Limn. ud.	0.122	0.065	-0.186	0.112	0.395	-0.289	0.355	0.243				
Limn. ho.	-0.047	-0.037	-0.100	0.052	0.457	-0.247	0.035	0.275	0.420			
Bran. so.	-0.167	0.071	0.060	0.041	0.387	-0.269	0.126	0.277	0.337	0.193		
Proc.	-0.245	-0.225	0.347	0.136	0.208	-0.176	0.112	0.174	0.122	0.230	0.410	
Valv. ni.	0.019	0.001	-0.040	0.091	0.291	-0.149	0.012	0.167	0.592	0.651	0.243	0.339

T.: Temperature; E.C.: Electrical conductivity
D.O.: Dissolved oxygen; O.M.: Organic matter
Limn. ud.: *Limnodrilus udekemianus*
Limn. ho.: *Limnodrilus hoffmeisteri*
Bran. so.: *Branchiura sowerbyi*
Proc.: *Procladius* sp.
Valv. ni.: *Valvata nilotica*

The bottom fauna in the deep areas of Lake Nasser was poor in number of species, being mainly oligochaetes (3 species), chironomid larvae (4 species) which are the only temporary residents of the lake bed

together with the gastropods (1 species) and the bivalves (2 species). This lack of species diversity is found to be due to the unstable and deoxygenated nature of the mud. Similar status have been encountered in Lake George (Uganda) (Burgiess et al., 1973) and in central areas of the Varzea Lakes on the Amazon (Reiss, 1977). However, in the littoral areas af Lake Nasser and among the marginal vegetations, the diversity increases as a wide variety of insects appear, particularly the larvae of Chironomidae (17 species), Pupae of Chironomidae, Nymph of Odonata (8 species), Nymph of Ephmeroptera (1 species), larvae and adult of Coleoptera (1 species for each), adult Hemiptera and Larvae of Trichoptera together with Oligochaetae (4 species), Hirudinea (1 species), Crustacea (3 species), Mollusca (10 species), Hydrozoa (1 species) and Phylactolamata (1 species) as shown in Table (4).

Table (4): Cheklist of benthic inverlebrates recorded in Lake Nasser by different authors. (+ = present, - = not recorded).

Taxa & species	Iskaros (1988 & 1993)	Fishar (1995 & 2000)	The present work (2008)
Phylum: Cnidaria			
Class: Hydrozoa			
Hydra vulgaris Pallas	-	+	-
Phylum: Bryozoa			
Class: Phylactolaemata			
Fredericulla sultana Blumenbach	-	+	-
Phylum: Arthropoda			
Class: Insecta			
Procladius sp.	+	+	+
Clinotanpus sp.	+	-	+
Coelotanpus sp.	+	+	-
Pelopia sp.	+	-	-
Conchapelopia sp.	+	-	-
Tanpus sp.	+	-	-
Ablabesmyia sp.	+	+	-
Einfeldia sp.	+	-	-
Nilodorum sp.	+	+	-
Tanytarsus sp.	+	+	-
Dicrotiendipes modestus	+	-	-
Polypedilum sp.	+	+	-
Cryptochironomus sp.	+	+	+
Microtiendipes sp.	+	+	+
Microchironomus sp.	+	-	-
Chironomus sp.	+	+	-
Circotopus sp.	+	+	-
Pupae of Chironomidae	+	+	+
Ischnura sp.	+	+	-
Pseudagrion niloticum Dumont	+	-	-
Perithemis sp.	+	+	-
Libellula sp.	+	-	-
Neurocordulia sp.	+	+	-
Gomphus sp.	+	-	-
Enallagma sp.	-	+	-
Plathemis sp.	-	+	-
Caenis sp.	-	+	-
Dytiscus sp.	-	+	-
Hydrovatus sp.	-	+	-
Larvae of Tricoptera	+	+	-
Adult Hemiptera	+	-	-
Class: Crustacea			
Cardinea nilotica Roux	-	+	-
Chlamydotheca unispinosa Baird	-	+	-
Stenocypris malcolmosoni Braird	-	+	-

Table (4): Continue

Taxa & species	Iskaros (1988 & 1993)	Fishar (1995 & 2000)	The present work (2008)
Phylum: Annelida			
Class: Oligochaetae			
Branchiura sowerbyi Beddard	+	+	+
Limnodrilus udekemianus Claparede	+	+	+
Limnodrilus hoffmeisteri Claparede	+	+	+
Pristina sp.	-	+	-
Class: Hirudinea			
Helobdella conifera Moore	+	+	-
Phylum: Mollusca			
Class: Gastropoda			
Bulinus truncates Audouim	+	+	-
Physa acuta Darparnaud	+	+	-
Melanoides tuberculata Müller	+	+	-
Valvata nilotica Jickeli	+	+	+
Cleopatra bulimoides Olivier	-	+	-
Gyraulus ehrenbergi Beck	-	+	-
Class: Bivalvia			
Corbicula consobrina Cailliaud	-	+	-
Corbicula fluminalis Muller	+	-	+
Pisidum pirothi Jickeli	+	+	+
Eupera ferruginea Krauss	-	+	-
Total 48	33	38	10

Worthmentioning that Lake Nasser is considered among the highly eutrophic lake and its productivity ranged from 4.32 – 128.15 mg c/m3/h during 1990 (Abdel-Monem, 1995). The community of planktonic algae during the period 1981 – 1993 was fairly diversified, belonging to 5 classes: Chlorophyceae (54 species), Cyanophceae (34 species), Bacillariophyceae (33 species), Dinophyceae (13 species) and Euglenophyceae (1 species) (Bishai, et al., 2000). The standing crop of the phytoplankton in the upper water layer tended to increase southwards from 3.405 x 10^6 algal units/l at El-Ramla to 15.272 x 10^6 algal units/l at Adindan. Also, zooplankton was rich and diversified (Ali et al., 2007 & Iskaros et al., 2008) where its standing crop in the upper water layer amounts to 73521 org./m^3 with 57 species belonging to Copepoda (4 species), Rotifera (39 species), Cladocera (12 species) and Protozoa (2 species) of which the former group constitutes 62.2% of the zooplankton populations. The benthic fauna represents the third component among the food chain in the fresh water habitats. It converts sediments detritus, micropes and other small preys onto their body's flesh that is available to capture. Iskaros (1988) pointed out that the average annual number and biomass of the bottom fauna recorded for Lake Nasser as a whole during 1986 – 1987 amounted to 2659 org./m^2 with 13.1 gm/m^2 at the littoral stations and only 288 org./m^2 with 1.9 gm/m^2 at the offshore ones. In the present study, however, the standing crop of bottom fauna at the offshore stations increased much to 2404 org./m^2 with 10.9 gm/m^2. This may be attributed to the gradual accumulation of organic matter during the last 24 years where the organic matter fluctuated between 1.96 and 3.39% in the period 1981 – 1983 (Latif, et al., 1989). Because in Lake Nasser, there is a prominent phytoplankton population and slow circulation, there can be a continual rain of plankton on to the lake bed providing organically rich sediments, like the same found by Payne (1986) when he investigated some tropical lakes. Latif et al., 1979 and Iskaros (1988 & 1993) found that in Lake Nasser the different groups of benthos serve as an important food for various fish species, therefore it participate in enriching an economic fish yield. Members of the Tanypodinae are regarded as eutrophic species (Rosenberg et al., 1984) while Brinkhurst (1964) postulated to the possibility of using the natural tubificid population in lakes to classify them in regards to their annual fish yield.

The fish crop in any water mass represents the final link culminating the food cycle. More than 55 fish species belonging to 15 families were recorded from Lake Nasser (Latif 1974 & 1977). The annual yield fluctuated between 30000 ton in 1983 and 17563 ton in 2008. *Tillapia* spp. (*Sarotherodon galilaeus* and *Oreochromis niloticus*) formed 90% of the total production. These species mainly caught from the coastal areas and khors of the lake. The offshore area

(about 80% of the total lake area) is not well utilized, except for a minor catch of *Hydrocynus* spp., *Alestes* spp. and others. El-Shabrawy & Dumont (2003) suggested the possibility of introducing a pelagic freshwater fish species (the Tanganika sardine, *Limnothressia miodon*) in to Lake Nasser – to consume the large quantities of plankton in the open water area. This species has been introduced to and successfully exploited in Lake Kariba (25000 ton/annum) and spread down the middle Zambezi to Lake Cahora Bassa (Skelton, 1993). Also, it was introduced to Lake Kivu, but it has decimated the zooplankton (Dumont, 1986). A phase of careful testing should therefore precede its possible introduction to Lake Nasser.

5. Conclusions

Three main benthic fauna groups were identified in the offshore area of Lake Nasser (Oligochaetae, Larvae of Chironomidae and Mollusca) with 10 identified species of which the former group contributed 83.9 & 91.7% of their number and biomass, respectively. The highest standing crop of benthos was recognized during spring while it decreased in its density in summer during thermal stratification and more in autumn and winter with the continous of oxygen depleted layer and the failing of water temperature, respectively. Spatially, the highest standing crop of benthos appeared at the main channel stations accompanied by the silt and clay fractions and subsequently high content of organic matter. Contrary to that, it reached the lowest density in the khors where the type of sediments were mainly sand with low organic matter. Also, transparency, temperature, dissolved oxygen, calcium carbonate and hydrogen ion concentrations proved to be of variable effect in controlling the assemblage of bottom fauna species. The physico-chemical features as well as the characteristics of the bottom sediments in Lake Nasser are in favour for producing high standing crop of benthos which in turn, provide the main food items for the various fish inhabitants of the lake.

6. References

Abdel-Monem, A.M.A. (1995): Spatial distribution of phytoplankton and primary productivity in Lake Nasser. Ph.D. Thesis, Univ. College for Girls, Ain Shams Univ. 161 pp.

Ali, M.M., Mageed, A.A. & Heikel, M.T. (2007): Importance of aquatic macrophyte for invertebrate diversity in large subtropical reservoir. Limnologica 37, 155 – 169.

APHA, AWWA & WEF. (1995): Standard methods for the examination of water and wastewater (18 th ed). Washington, DC: APHA, AWWA, WEF., 1042pp.

Awadallah, R.M. & Moalla, S.M.N. (1996): Seasonal variation of High Dam Lake Water. J. Env. Sci. H., A31, 4, 731 – 746.

Bishai, H.M., Abdel_Malek, S.A. & Khalil, M.T. (2000): Lake Nasser (567 pp.). Publication of National Biodiversity Unit – No. 11 – 2000. Nat. Cons. Sec., Egypt.

Brinkhurst, R.O. & Jamieson, B.G.M. (1971): Aquatic Oligochaetae of the world. Uni. Toronto press, Ont; 860 pp.

Brinkhurst, R.O. (1964): Observations on the biology of lake dwelling Tubificidae. Arc. Hydr., 60: 385 – 418.

Brinkhurst, R.O. (1965): The biology of the Tubificidae in relation to pollution. Proc. 3rd seminar on Water Quality Criteria Cincinnati, 1962, 57.

Brinkhurst, R.O. (1967): The distribution of aquatic Oligochaetae in Saginaw Bay, Lake Huron. Limnol. Oceanogr., 12, 137.

Brown, D.S. & Lemma, A. (1970): The molluscan fauna of the Awash River, Ethiopia, in relation to the transmission of schistosomiasis. Ann. trop. Med. parasit.64: 533 – 538.

Brown, D.S. (1980): Fresh water snails of Africa and their medical importance. Lond., Taylor & Francis Ltd, 478 pp.

Brown, D.S., Fison,T., Southgate, V.R.& Wright, C.A. (1984): Aquatic snails of the Jonglei region, Southern Sudan, and transmission of trematode parassites. Hydrobiol., 110: 247-271.

Burgis, M.J., Darlington, J.P.Ec., Dunn, I.G., Ganf, G.G., Gwahaba, J.J. & Mc Gowan, L.M. (1973): The biomass and distribution of organisms in Lake George, Uganda. Proc. Roy. Soc. Lond., 184: 271 – 298.

Della Croce, N. (1955): The conditions of sedimentation and their relationships with Oligochaetae populations of Lake Maggiore. Memorie Ist. ital. Idrobiol. Suppl., 8, 39.

Dumont, H.J. (1986): The Lake Tanganika sardine in Lake Kivu: another ecodisaster for Africa? Envir. Conserv. 13: 143 – 148.

El Dardir, M. (1994): Sedimentation in Nile High Dam Reservoir, 1987 – 1992, and sedimentary futurologic aspects. Sedimentology of Egypt, 2, 23 – 39.

El-Dardir, M. (1984): Geochemical and sed. studies on the sediments of Aswan High Dam Reservoir. Ph.D. Thesis. Fac. Sci., Al Azhar Univ., Cairo, Egypt, 238pp.

El-Shabrawy, G.M. & Dumont, H.J. (2003): Spatial and seasonal variation of the zooplankton in the coastal zone and main khors of Lake Nasser (Egypt). Hydrobiologia 491:119-132.

Entz, B.A.G. (1978): Sedimentation processes in the Reservoir Lake Nasser-Nubia during 1965 – 1974 and future aspects. Symposium of Ecological studies on the River Nile. Egyptian Society of Environment, Cairo.

Fishar, M.R.A. (1995): Studies on bottom fauna in Lake Nasser. Ph.D. Thesis, Fac. Sci., Suez Canal Univ. Egypt, 267 pp.

Fishar, M.R.A. (2000): Macrobenthic fauna, p. 75 – 79. In J.F. Craig (ed.) Sustainable fish production in Lake Nasser: Ecological Basis and Management Policy. ICLARM Conf. Proc. 61, 184 pp.

Gindy, N.N. and El Dardir, M. (2008): Sedimentological and mineralogical studies and organic carbon of Lake Nasser sediments, Egypt. J. of Aquat. Res. 34(2): 179 – 205

Hanna, A. (1965): Organic matter in soil: Chemistry of soil. F.E. Bear (Ed.), Amer. Chem. SOC. Monog. Ser.: 515 pp.

Hilsenhoff, W.L. (1975): Aquatic Insecta of Wisconsin. With generic keys and notes on biology, ecology and distribution. Tech. Bull. no. 89. Dept. Nat. Res., Mdison Wisconsin, 55pp.

Iskaros, I.A. (1988): Biological studies on bottom fauna of Lake Nasser and adjacent waters. M.Sc. Thesis, Fac. Sci. Alex. Univ. 184 pp.

Iskaros, I.A. (1993): Ecological studies on the distribution of zooplankton and bottom fauna in Khor Kalabsha in lake Nasser. Ph.D. Thesis, Fac. Sci, Mansoura Uni., 304 pp.

Iskaros, I.A., Bishai, R.M.& Mokhtar, F.M.(2008): Seasonal variations of zooplankton and physico-chemical factors in Lake Nasser. Egypt. J. Aquat. Res. 34 (2): 232-259.

Jackson, M.L. (1958): Soil chemical analysis. Constable, London.

Kloos, H. & Lemma, A. (1974): Bilharziasis in the Awash valley. 2. Molluscan fauna in irrigation farms and agricultural development. Ethiopian Med. J.12: 157 – 173.

Latif, A.F.A. (1974): Fisheries of Lake Nasser. Aswan Reg. Plan., Lake Nasser Develop. Cent., Aswan, 235 pp.

Latif, A.F.A. (1977): Report on surveys to Lake Nasser. Lake Nasser and the River Nile Project. Acad. Sci. Res. Technol., Cairo, 286 pp.

Latif, A.F.A., El-Etreby, S.G. & Iskaros, I.A. (1979): Bottom fauna of the High Dam Reservoir. Report, River Nile and Lake Nasser Project. Acad. Sci., Technol., Cairo.

Latif, A.F.A., Elewa, A.A., Hassan, M.M. & El-Dardir, M. (1989): Relation between sediment calcium carbonate, organic matter and productivity in Aswan High Dam Reservoir. Bull. Nat. Ins. Oceanogr. & Fish. A.R.E., 15 (2): 191 – 199.

Mageed, A.A. and Heikal, M.T.: Factors affecting seasonal patterns in epilimnion zooplankton community in one of the largest man-made lakes in Africa (Lake Nasser, Egypt). Limnologica, 36, 91 – 97.

Payne, A.I. (1986): The ecology of tropical lakes and rivers. John Wiley & Sons. Chichester, New York, Tornoto, Brisbane and Singapore, 300pp.

Pennak, R.W. (1978): Fresh water invertebrates of the United States. 2nd edition. John Willey & Sons. New York, 803 pp.

Reiss, F. (1977): Qualitative and quantitative investigations on the macrobenthic fauna of central Amazon lakes. I. Lago Tupe, a blackwater lake on the lower Rio Negro. Amazoniana, 6: 203 – 235.

Rosenberg, D.M., Bilyi, B. & Wiens, A.P. (1984): Chironomidae (Diptera) emerging from the littoral zone of reservoirs, with special reference to Southern Indian Lake, Manitoba, Can. J. Fish. Aquat. Sci., 41: 638 – 647.

SECSF (1996): Studies of the environmental characteristics, sediments and fisheries of Lake Nasser. 1St Annual Report, National Institute of Oceanography and Fisheries to General Authority for Development of High Dam Lake, Aswan, 165 pp. (In Arabic).

Skelton, P. (1993): A complete guide to the freshwater fishes of Southern Africa. Southern Book Publisher, (Pty) Ltd.

Tjoe-Awie, E.C. (1975): A reconnaissance survey of the Amazon mouth region. Netherlands, Institute of Sea Research Publication Report 19, 51pp.

Welch, P.S. (1952): Limnology. 2nd edition. New York, Toronto, London, Mc. Grew, Hill Book Company, Inc., 538pp.

Wirth, W.W. & Stone, A. (1968): Aquatic Diptera, P. 372 – 482. In: R.L. Usinger (Ed.), Aquatic Insecta of California. Press Berkelley & Angeles; 155 – 169.

Floristic Composition and Biological Spectrum of Vegetation in Alpine Meadows of Kedarnath: Garhwal Himalaya

Kusum Pharswan *, J. P. Mehta and Subodh
Department of Botany, Post Box-22, H. N. B. Garhwal University, Srinagar (Garhwal), 246174, India
*kkusumpharswan@gmail.com, Research fellow

Abstract: The present paper gives an account of flora of Kedarnath which have very harsh climatic conditions. 80 species, belonging 36 families were estimated from the study area. Notes on phenological pattern, life form (biological spectrum), plant type and uses have also been studied. Asteraceae was the dominant family (11.25%) recorded under present investigation. The genus and species are arranged alphabetically with in a family with correct nomenclature. The major class of life form was found to be Chamaephytic. Besides Chamaephytes (36.25%), the other life forms enumerated were Therophytes (28.75%), Cryptophytes (18.75%), Hemicryptophytes (11.25%) and Phenerophytes (5%). Most of the plant species had flowering and fruiting in rainy season, followed by summer season and very few species in winter season. [Nature and Science 2010;8(7):109-115]. (ISSN: 1545-0740).

Key words: Kedarnath, life forms, biological spectrum, floristic list, altitude.

Introduction:

Himalaya is a mega diversity centre of world. It supports about 18,440 species of plants, of which 25.3% are endemic to Himalaya (Singh and Hajra, 1997, Samant *et.al.*, 1998). In which most of the plant species are used as medicine and food. High mountain ecosystem are comparatively thrilling and sensitive at least at the upper elevation levels, and are determined by abiotic climate related ecological factors (Gaur *et.al.*,2005). Species richness increases remarkably partially due to the invasion of plant species from alpine belt (Gottfried, 1998). The well known cause for declining plant species diversity are habitat loss, narrow distribution range, low population size, fragmentation degradation of population and genetic variation (Allen and Allen, 1990; Weekly and Rau, 2001; Vergar *et.al.*,2003; Kala, 2000,2005a). To save this precious natural wealth, protected areas have been established and within the Indian Himalaya there are many protected areas that contain rich medicinal plant diversity (Kala, 2005a). KWLS (Kedanath Wild Life Sanctuary) in Uttarakhand state of India is one of the protected area which have a lot of traditional knowledge of medicinal plants and a very high diversity.The flora and its ecological characters such as life forms were studied in this paper. Life form etc. indicates climate and human disturbance of a particular area (Cain & Castro, 1959). Very little work is available on this aspect.

The climatic conditions of alpine zones of India include dense frost, fog, heavy hailstorms, extremely low temperature, high intensity of light and high wind velocity and lower oxygen and carbon dioxide concentration. There is sharp fluctuation regarding these weather conditions, even in the same day. Monthly max and minimum temperature ranges between 24-14 c and 7.5-3 c respectively (Maikhuri *et.al.*, 1998). Comparatively very high rainfall was observed in this area. Average annual rainfall (1475mm) occurs over a short period of two months (July-Aug), featuring a strong monsoonic influence.

The diverse topographic features of the Himalayas sustain an enormous perennial reservoir of vegetation resources (Gaur *et al.*, 1995).The high altitude of this region have unique vegetation due to their diverse geo-morphology which provides different microhabitats for specific plant growth. The alpine vegetation of this part has many characteristic features in connection with the separation zone from timber line, seasonal succession and distributional pattern. On the basis of distribution the alpine plants represent distinct habitats. They are found on exposed dry rocks crevices, ravines and on much fertile loamy soils constituting the alpine meadows (Semwal & Gaur, 1981).

The well known species of this alpine meadow are *Hypericum hookerianum, Thalictrum alpinum, Angeleca glauca, Primula denticulate, Gentiana* spp., *Caltha palustris, Gagea lutea, Anemone*

obtusiloba and species of *Potentilla, Polygonum, Delphinium* and *Taraxacum officinale* are observed.

Material and Methods:

Study area:

KWLS in Uttarakhand that are situated in central Himalayan region, covers an area of 975 km², which lies between 29 26' and 31 38'N latitude and 77 49' and 80 6' E, longitude, at an elevation of 1160m to 7068 m.asl in district Rudraprayag (Figure-1). The elevation of study area between 2500m to 4000 m.asl. The slope of the study area is lies between 30-60 c and towards the South-East aspect .

The region is rich in bioresources and fascinating folk culture as well as diverse flora and fauna due to its distinct meteorological, geographical, geological and ecological patterns (Gairola and Biswas, 2008).The alpine habitat usually starts at timberline or the tree line i.e. 3500 m.asl and are characterized by the complete absence of tree. The soil of the Kedarnath valley is dark brown to brown at surface and brown to yellowish brown in the sub soil and endodynomorphic (Singh and Singh, 1992).

For the estimation of vegetation structure and composition random sampling was done taking 0.5 m² quadrates. The size of the sampling plot was arrived at by the method given by Misra (1968). After counting the number of individual species, they were clipped at the ground level and identified. Classification was done as per the Raunkiaer (1934) and species belonging to respective life-form were arranged.

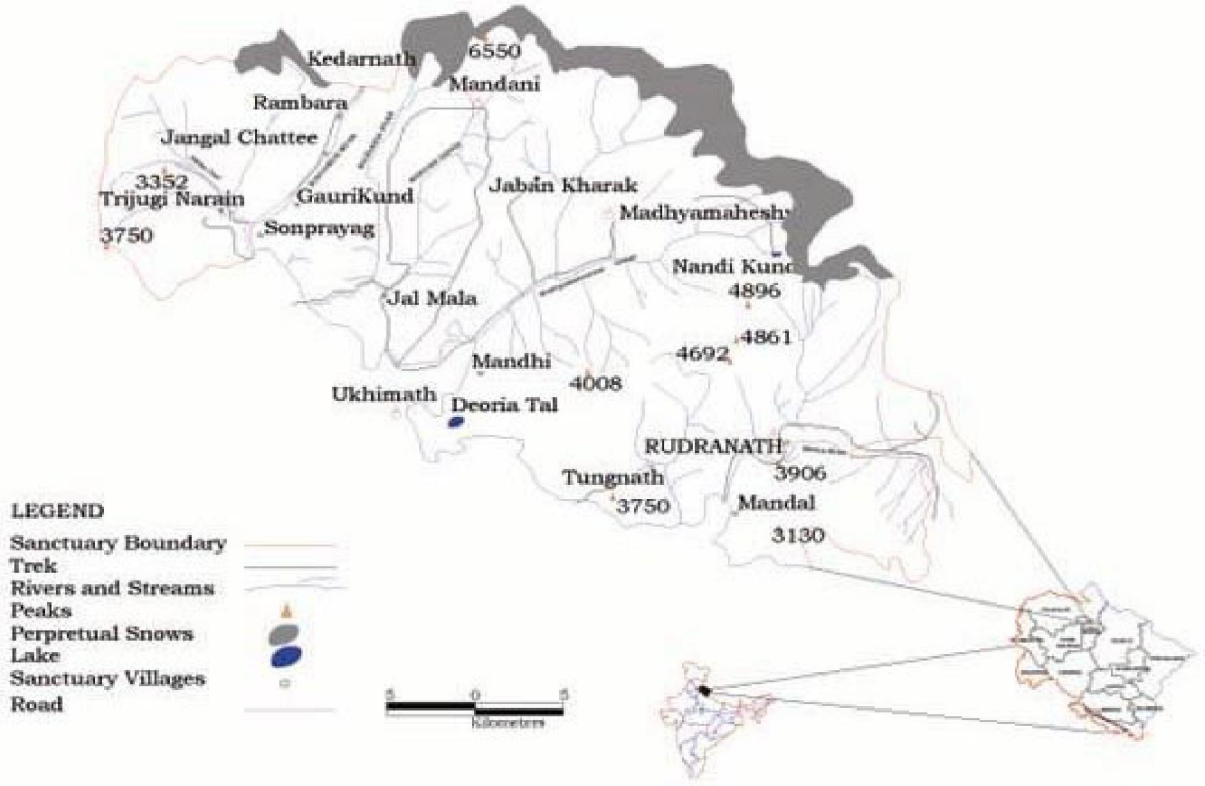


Figure-1. Complete map of KWLS by Semwal *et.al.*

Occurrence of plant species on the study site from May 2009 to October 2009 were recorded and assigned to various life-form classes following Raunkiaer (1934). The percentage life form was calculated as follows :

$$\% \text{ Life-form} = \frac{\text{Number of species in any life form}}{\text{Total number of species of all life forms}} \times 100$$

Result:

A complete list of the plant species encountered in the study area Kedarnath is given in Table-1. There are 80 plant species recorded which belong to 36 families. Monocots represented by six families (Liliaceae, Poaceae, Orchidaceae, Araceae, Amaryllidaceae and Dioscoreaceae) while the remaining thirty families represented the dicots. The dominated families were Asteraceae, 9 spp.(11.25%), Ranunculaceae, 8 spp.(10%), Apiaceae and Poaceae were represented by 5 spp.(6.25%) each, Lamiaceae and Polygonaceae had 4 spp. each. The majority of the species ,67.5% were recorded from 2500 to 4000m. altitude, 17.5% species from below to 2500m. and 15% of the total species have a broad range of occurrence, recorded from 1000 to 4000m. altitude.

The biological spectrum (Figure-2) showed that Chamaephytes, 29spp. (36.25%) and Therophytes, 23spp. (28.75%) were the dominant followed by Hemicryptophytes, 9spp. (11.25%), Cryptophytes, 15spp. (18.75%) and Phanerophytes, 4spp. (5%). The annuals, biennials, perennials and annual-perennials were 13.75%, 1.25%, 72.5% and 12.5% respectively, of the total plant species.There are three distinct climatic season of Kedarnath, rainy season, summer season and winter season. Majority of the plant species having flowering and fruiting in rainy season (50spp., 62.5%), summer season is represented by lesser no plants (18spp., 22.5%) than rainy season and the rest and very low plant species are represent to winter season (12 spp., 15%).

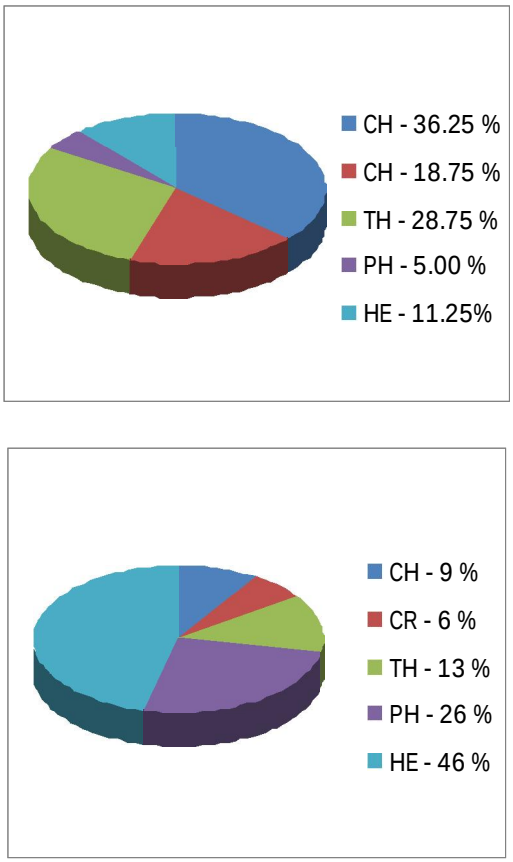


Figure-2. Biological Spectra of Kedarnath Raunkiaer's Normal Spectra

Table 1: List of plant species, life forms, plant type, phenolgy and medicinal uses encountered in the study area of Kedarnath

S.No	*Plants Name*	Vern. Name	Family	Altitude	Life-form	Plant Type	Fl-Fr	Part used	Uses
1	*Allium corolianum*	Faran	Amaryllidaceae	3000-4000	CR	Ann	Jun-Sept	BB, LF	Indigestion, joint pain
2	*Allium.humile*	Faran	-	3000-4000	CR	Pere	June-Aug	LF,BB	Asthma, Jaundice
3	*Angelica glauca*	Choru	Apiaceae	3000-4000	CH	Pere	Jul-Oct	RT, FR, ST	Flatulence, constipation, bronchitis, gastric, dyspepsia
4	*Bupleurum longicaule*		-	2800-4900	CH	Pere	July-Sept	RT	RAMP
5	*carum carvi*	Jangli dhaniya	-	2500-4500	CH	Pere	Jul-Sept	SD	Cold, fever, cough
6	*Foenculum vulgare*	Saunf	-	2500-3400	TH	Ann/Pere	Feb-Jun	WP	Vomiting
7	*Arisaema flavum*	Meen	Araceae	2400-3800	TH	Pere	May-Aug	BB	Skin diseases
8	*Arisaema jacquemontii*	Khaprya	-	2000-3000	CR	Pere	May-Aug	BB	Ringworm-killer, snakebite
9	*Anaphalis lineasis*		Asteraceae	3500	TH	Ann	May-Sept		
10	*Artimisia capillaries*	Marva	-	2400-5600	TH	Pere	Jul-Dec	WP	Hypertension, Typhoid, dyspepsia

11	*Artimisia maritima*	Pati	-	3000-4000	CH	Pere	Jun-Oct	LF,WP	Cuts, gastric, anthelmintic
12	*Artimisia nilagirica*	Kunjo	-	3000-3500	CH	Pere	Aug-Oct	LF,WP, AP	Ulcer, Wound, cut
13	*Doronicum falconeri*		-	3300-4800	TH	Pere	Aug-Sept		
14	*Echinops cornigerus*	Kandala	-	1000-2500	CH	Pere	Sept-Oct	RT	Urinary trouble, fever
15	*Inula cuspidata*	Jhuri	-	2000-3400	CH	Pere	Sept-Dec	RT	Dyspepsia, colic
16	*Jurinea dolomiacea*	Bis-kandara, guggul	-	3200	CH	Pere	Aug-Oct	RT	Colic, sores, antiseptic
17	*Taraxacum officinale*	Kanphuliya Karatu	-	1800-4200	TH	Pere	Feb-Oct	WP, LF,RT	Blisters, tonic, blood purifier, kidney disorder, migraines,
18	*Impatiens scarbida*	Ban-til, tillua	Balsaminaceae	2700-3000	TH	Ann/Pere	Jul-Oct	SD	Hair tonic, abortion
19	*Cynoglossum glochidiatum*	Kuri	Boraginaceae	1500-4000	TH	Pere	Jul-Nov	RT,LF	Wound, Ulcer
20	*Arabis amplexinule*	Ban-sarsaun	Brassicaceae	2500-3000	TH	Pere	Mar-May	LF	Burns, scratches
21	*Cyanthus lobatus*		Campanulaceae	3200-4200	CH	Pere	Jul-Oct	RT	Liniment in chronic rheumatism
22	*Arenaria orbiculata,*		Caryophylaceae	1500-3200	HE	Ann/Pere	Mar-Aug		
23`	*Cerastium cerastoides*	Pangein	-	2400-4700	TH	Pere	Apr-Sept	WP	Body ache, Headache, cough
24	*Chenopodium ambrasioides*	Kirmiri	Chenopodiaceae	2600-3200	TH	Ann/Pere	Aug-Oct		Hook-worm killer, piles
25	*Dioscorea deltoidea*	Gethi	Dioscoreaceae	Up to 3100	CR	Ann	Jul-Sept	RZ	Rheumatism, spermatorrhoea
26	*Goltheria tricophyla*	Goltheria (Bhuinla)	Ericaceae	2700-4600	TH	Ann	May-Jul	OL, LF, RT	Rheumatism, simulative, carminative, cough
27	*Euforbia stracheyi*	Dudhibish	Euforbiaceae	3500	TH	Ann	Jun-Jul	LT	RAMP
28	*Androchne cordifolia*	Bhatia	-	1000-2500	PH	Pere	Jan-Dec		
29	*Indigofera heterontha*	Sakina	Fabaceae	1500-3000	NP	Pere	May-Oct	LF,SD	Diarrhea, dysentery
30	*Trifolium repens*	Tipatiya	-	2300-3500	CR	Pere	Apr-Jul	LF	As astringent
31	*Corydalis govaniana*	Bhut-keshi	Fumariaceae	3000-4000	CH	Pere	Jul-Sept	RT	Diuretic, liver trouble
32	*C.cornuta*	Indra-jatta	-	2500-3700	CH	Pere	Jun-Oct	RT	Veterinary medicines
33	*Swertia ciliata*	Sfed Chirota	Gentianaceae	2000-4000	CH	Ann/Pere	Jul-Oct	WP, RT	Malaria, fever, vermifuge
34	*Gentiana argentea*		-	Up to 3300	CH	Pere	May-Sept	LF,FL	Sore throat
35	*Geranium wallichianum*	Ratanjot	Geraniaceae	1800-4200	CH	Pere	Jul-Oct	RT, FL	Dysentery, cough, eye trouble, toothache, opthalmia
36	*Geranium nepalense*	Lal jhari	-	1500-4000	HE	Pere	Aug-Nov	WP	Jaundice, ulcer, eczema
37	*Hypericum oblongifolium*	Peali	Hypericaceae	1500-2500	PH	Pere	Mar-Jul	FL	Wounds, boils, facilitate delivery
38	*Hypericum elodeoides*	Basanti	-	3200	PH	Pere	Aug-Oct	LF	Tertiary fever
39	*Ajuga bracteosa*	Neelkanth	Lamiaceae	1200-5100	HE	Pere	May-Aug	LF,RT	Jaundice, malaria, tonic
40	*Lucas lanata*	Bis-kapra	-	Up to 1800	CR	Pere	Jan-Dec	LF	Wound, check bleeding, heating
41	*Micromeria biflora*	Ban-ajwain	-	1000-4000	CH	Pere	Aug-Nov	WP, LF	Eczema, cold
42	*Stachys sericea*		-	2400-3000	CH	Pere	Sept-Nov		

43	*Lilium polyphyllum*	Kandmul	Liliaceae	1500-3400	CR	Ann	Jun-Jul	BB	Diuretic, antipyretic, tonic
44	*Epilobium lactum*		Onagraceae	2500-3800	TH	Pere	Jul-Oct	RT	Ringworm
45	*Habenaria intermadia*	Ridhi-virdhi	Orchidaceae	2800-3200	CR	Pere	Jul-Sept	WP	Tonic
46	*Dactylorhiza hatagirea*	Hattazari	-	2800-4000	CR	Pere	Jun-Oct	RT, TU	Diarrhea, bone fracture, wounds
47	*Oxalis corniculata*	Khatti-ghas	Oxalidaceae	Up to 2700	CR	Pere	Feb-Nov	LT,WP	Snakebite, jaundice, wart
48	*Meconopsis aculeate*	Kalihari	Papaveraceae	3200	CH	Pere	Jun-Sept	WP, RT, LF, FR	Renal pain, fever, colic, wound
49	*Plantago major*	Isabgol	Plantaginaceae	1200-3300	CR	Pere	Mar-Dec	SD, LF	Fever, Tonic, intestinal injury
50	*Cynodon dactylon*	Doob	Poaceae	1500-3000	HE	Pere	Jun-Dec	WP	Anti-Abortive, haemostatic
51	*Eulatia contorta*		-	1500-2600	HE	Ann	Sept-Oct		
52	*Festuca gigantean*	Tall broom	-	2000-3500	CR	Pere	Aug-Dec	LF	Fodder
53	*Heteropogon contortus*	Kumra	-	Up to 2600	HE	Ann/Pere	Aug-Dec	RT	Stimulant, diuretic
54	*Sporobulous diander*	Sitya	-	Up to 1500	CR	Pere	Mar-Sept	LF	Burns, pimples
55	*Podophyllum hexandrum*	Bankakri	Podophyllaceae	3200-4000	CR	Pere	Apr-Sept	RT, FR, SD, RH	Cancer, skin disease
56	*Polygala crotalariodes*		Polygalaceae	1800-3000	TH	Pere	Apr-Nov	WP,RT	Catarrhal affections, cough
57	*Polygonum amplexicaule*	Kutrya	Polygonaceae	2500-3000	CH	Ann/Pere	Jul-Sept	RT,LF	Stomach trouble, wound, cough.
58	*P. vaccinifolium*	Inuri	-	3000-3600	CH	Ann/Pere	Jul-Aug		
59	*P.macrophyllum*	Kukhri	-	3000-3400	CH	Ann	Jul-Oct	WP	RAMP
60	*Rheum emodii Wall.ex.*		-	3200	CH	Pere	Jun-Jul	RZ/RT	Asthma, abdominal pain, fever
61	*Androsace rotudifolia*		Primulaceae	2000-3000	TH	Pere	Jun-Sept	ST	Stomach pain
62	*Primula denticulata*	Jalkutra	-	3100	TH	Pere	Apr-Jul	LF, RT, FL	Urinary problems, cough, cold
63	*Aconitum heterophyllum*	Meeth Bish	Ranunculaceae	3350-4500	CH	Pere	Aug-Nov	RT, TU	Diarrhea, vomiting, digestive disorder, cough
64	*Aconitum balfourii*	Meeth bish	-	3300-4100	HE	Bi	Aug-Nov	TU	Neuralgia, paralysis, rheumatic fever
65	*Animone obtusiloba*	Kanch-phool	-	3400-4200	CH	Pere	May-Aug	RT, SD	Rheumatism, diarrhea
66	*Animone rivularis*	Mirchile Angeli	-	1600-4000	CH	Pere	Jun-Oct	LF, WP, RT	Ear pain, fever, bone fracture
67	*Caltha palustris*	Kushnya	-	2500-4200	TH	Pere	Jul-Sept	WP, FL	Warts, anemia, tincture, diuretic
68	*Delphinium denudatum*	Nirbishi	-	2000-3500	TH	Ann/Pere	Apr-Nov	RT	Ulcer, cold, cough
69	*Ranunculus hirtellus*	Simariya	-	2800-3600	CH	Ann/Pere	Jul-Sept	ST,LF	Anthelmintic, wounds
70	*Thalictrum alpinum*		-	3000-4000	CH	Pere	Jul-Aug	RT,LF	Fever
71	*Duchesnia indica*	Bhikafal	Rosaceae	Up to 2500	HE	Ann	Apr-Jun	LF,FL,RT	Leucoderma, diarrhea
72	*Potentilla fulgens*	Bajra-danti	-	1500-3000	TH	Pere	Jul-Nov	LF, RT	Toothache, urinary disorder, burns
73	*Potentilla nepalensis*		-	1800-3000	TH	Pere	Jul-Oct	RT	Burns
74	*Galium aparine*	Kuri	Rubiaceae	3000-4000	CH	Ann	Aug-Sept	WP,LF	Ant scorbutic, skin disease
75	*Bergenia ciliate*	Pasha nbhed	Saxifragaceae	2000-3600	TH	Pere	Jun-Sept	RZ	Febrifuge, digestive disorder

76	*Saxifraga brachipoda*		-	3000-4500	CH	Pere	Jul-Dec		
77	*Valeriana jatamansi*	Samewa	Valerianaceae	2000-3000	CR	Pere	Mar-Jun	RT,LF,FL	Epilepsy, hysteria, mental disorder
78	*Nardostachys grandiflora*	Jata-mansi	-	3400-5000	CH	Pere	Jun-Oct	RZ, WP	Hysteria, Epilepsy, Diuretic, blood purifier
79	*Viola biflora*	Bana-ksha	Violaceae	2300-3600	HE	Ann	Jun-Aug	RT, FL, WP, LF	Emetic, constipation
80	*Viola betonicifolia*	Banfasa	-	3000	HE	Ann	Aug-Sept	LF,WP	Blood disease, diaphoretic

CH-Chamaephytes, TH-Therophytes, CR-Cryptophytes, HE-Hemicryptophytes, PH-Phanerophytes, Ann-annual, Bi-biennial, Pere-perennial, WP-whole plant, LF-leaf, FL-flower, RZ-rhizome, RT-root, TU-tuber, SD-seed, FR-fruit, BB-bulb, OL-oil.

Discussion:

The analysis of the flora of the study area indicates that the meadows are affected by intense biotic interference. The number of Chamaephytes is maximum while that of Phanerophytes is minimum. According to the biological spectrum given by Raunkiaer (1934), the flora of the present meadows may be called as the Chamaephytic. The high percentage of Chamaephytes characterized the colder climate and high altitude (Braun-Blanquet, 1932). But Hagerup (1931) explained that higher percentage of Thamaephytes is an indication of semi-desert condition at altitude. Chamaephytes were followed by Therophytes which indicate that heavy biotic pressure due to grazing and men's interference.

Nearly thirty species from the Garhwal Himalaya have been listed in various categories under threat in the Indian Red Data Book (Nayar and Sastry, 1887-90), of which 24 species are from high altitude alpine regions. Recently, Rawat *et.al.* (2001) listed 45 more species (excluding Red Data Books) which need special attention for conservation and this list also contains as many as 30 species from high altitudes, for example *Aconitum heterophyllum, Angelica glauca, Arnebia benthamii, Dactylorhiza hatagirea, podophyllum hexandrum* and *picrorhiza kurrooa*, all possessing high medicinal damands and thus are over-exploited from the wild.

Acknowledgment:

Authors are grateful to Head of the Department, Department of Botany for providing laboratory facilities and encouragement.

Corresponding to:
Kusum Pharswan
Department of Botany, Post Box-22,
H. N. B. Garhwal University,
Srinagar (Garhwal), 246174, India
Phone No- 9410952673
Emails- kkusumpharswan@gmail.com

References:

Allen RP and Allen CP. How many plants feed world? Conservation Biology. 1990; **4**: 365-374.

Bhandari BS, Mehta JP and Tiwari SC. Floristic composition, biological spectra and diversity of burnt and unburnt submontane grazing lands of Garhwal Himalaya. J Indian bot Soc**.** 1999; **78**:107-110.

Braun-Blanquent J. Plant Sociology (Transl. by fuller,G.D.& Conard,H.S.). McGraw Hill New York. 1938.

Cain SA and GM de Oliveria. Castro Manual of Vegetation Analysis. Harper & Brothers, New York. 1959.

Gairola Y and Biswas S. Bioprospecting in Garhwal Himalayas, Uttarakhand. Current Science. 2008; **94(9):** 1139-1143**.**

Gaur UN, Raturi GP and Bhatt AB. Current vegetation pattern along Glacial Landscape in Central(Garhwal) Himalaya, India. Journal of Mountain Science. 2005; **2(3):** 255-264.

Gottfried M, Pauli H and Grabherr G. Prediction of Vegetation Patterns at the Limits of Plant Life: a new view of the alpine-Nival Ecotone. Arctic and Alpine Research.1998; **3**: 207-221.

Hagerup O. Etude des types biological de Raunkiaer dans la flora autour de Tombouctou. Daske Videnskah Selskab. Biol Medd. 1931; ix 4.

Kala CP. Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. Biological Conservation. 2000; **93**: 371-379.

Kala CP. Indigenous uses, population density and conservation of threatened medicinal plants in

protected areas of the Indian Himalayas. Conservation Biology. 2005a; **19(2)**: 368-378.

Maikhuri RK, Nautiyal S, Rao KS and Saxena KG. Medicinal plant cultivation and biosphere reserve management: A case study from the Nanda Devi Biosphere Reserve, Himalaya. Current Science. 1998; **74(2):** 157-163.

Naithani BD. The Flora of Chamoli, Vols. I & II BSI, Howrah. 1984-1985.

Ram J and Arya P. Plant forms and vegetation analysis of an alpine meadow of Central Himalaya, India. Proceeding of The Indian National Science Academy. 1991; **57**: 311-318.

Raunkiaer C. The life forms of plants and statistical plant geography. The clarendon press, Oxford. 1934; 632 pp.

Samant SS Dhar U and Palni LMS. Medicinal Plants of Indian Himalaya: Diversity, Distribution and Potential values, Gyanodaya Prakashan, Nainital. 1998.

Semwal DP, Saradhi P Pardha, Nautiyal BP and Bhatt AB. Current status,distribution and conservation of rare and endangered medicinal plants of Kedarnath Wildlife Sanctuary, Central Himalayas, India. Current Science. 2007; **92(12).**

Semwal JK and Gaur RD. Alpine Flora of Tungnath In Garhwal Himalaya. J. Bombay Nat. Hist. Soc. 1981; **78(3):** 498-512.

Semwal JK, Gaur RD and Purohit AN. Floristic pattern of Tungnath alpine zone in Garhwal Himalaya. Acto Botanica Indica. 1981; **9**:110-114.

Shah R. Nature's MEDICINAL PLANTS OF UTTARANCHAL (Herbs, Grasses & Ferns)Vol. II, B L Consul for Gyanodaya Prakashan, Mallital, Nainital. 2006.

Sher Zaman and Khan Zaheer-ud-Din. Floristic composition, life form and leaf spectra of the vegetation of Chagharzai valley, District Buner. Pak. J. Pl. Sci. 2007; **13(1):**57-66.

Sikarwar RLS. Life forms and biological spectrum of the flora of Morena District, Madhya Pradesh. J Indian bot Soc. 1996; **75**: 275-277.

Singh, D K and Hajra, P K (1997) Floristic diversity. In Gujral (Ed.). Biodiversity Status In the Himalaya. 23-38. British Council, New Delhi.

Singh JS and Singh SP. Forest of Himalaya: Structure, Functioning and Impact of Man, Gyanodaya Prakashan, Nainital. 1992.

Sundriyal RC, Joshi AP and Dhasmana R. Phenology of high altitude plants at Tungnath in the Garhwal Himalaya. Trop Ecol. 1987; **28:** 289-299.

Vergeer PR, Rengelink AC and Ouborg NJ. The interacting effects of genetic variation, habitat quality and population size on performance of Succisa pratensis. Journal of Ecology. 2003; **91**: 18-26.

Weekley CW and Race T. The breeding system of Ziziphus celata Judd and D.W.Hall (Rhamnaceae), a rare endemic plant of the Lake Wales Ridge, Florida, USA: Implication for recovery. Biological Conservation. 2001; **100(2):** 207-213.

19/3/2010

Nature and Science

ISSN 1545-0740

The ***Nature and Science*** is an international journal with a purpose to enhance our natural and scientific knowledge dissemination in the world under the free publication principle. Any valuable papers that describe natural phenomena and existence or any reports that convey scientific research and pursuit are welcome, including both natural and social sciences. Papers submitted could be reviews, objective descriptions, research reports, opinions/debates, news, letters, and other types of writings that are nature and science related.

1. General Information

(1) Goals: As an international journal published both in print and on internet, *Nature and Science* is dedicated to the dissemination of fundamental knowledge in all areas of nature and science. The main purpose of *Nature and Science* is to enhance our knowledge spreading in the world. It publishes full-length papers (original contributions), reviews, rapid communications, and any debates and opinions in all the fields of nature and science.

(2) What to Do: *Nature and Science* provides a place for discussion of scientific news, research, theory, philosophy, profession and technology - that will drive scientific progress. Research reports and regular manuscripts that contain new and significant information of general interest are welcome.

(3) Who: All people are welcome to submit manuscripts in any fields of nature and science.

(4) Distributions: Web version and the printed copy of the journal are opened to the world. For the subscription please contact with editor@sciencepub.net.

(5) Advertisements: The price will be calculated as US$400/page, i.e. US$200/half page, US$100/quarter page, etc. Any size of the advertisement is welcome.

2. Manuscripts Submission

(1) Submission Methods: Electronic submission through email is encouraged.

(2) Software: The Microsoft Word file will be preferred.

(3) Font: Normal, Times New Roman, 10 pt, single space.

(5) Manuscript: Don't use "Footnote" or "Header and Footer".

(6) Cover Page: Put detail information of authors and a short title in the cover page.

(7) Title: Use Title Case in the title and subtitles, e.g. "**Debt and Agency Costs".**

(8) Figures and Tables: Use full word of figure and table, e.g. **"Figure 1.** Annul Income of Different Groups**", Table 1. Annual Increase of Investment".**

(9) References: Cite references by "last name, year", e.g. "(Smith, 2003)". References should include all the authors' last names and initials, title, journal, year, volume, issue, and pages etc.

Reference Examples:

Journal Article: Hacker J, Hentschel U, Dobrindt U. Prokaryotic chromosomes and disease. Science 2003;301(34):790-3.

Book: Berkowitz BA, Katzung BG. Basic and clinical evaluation of new drugs. In: Katzung BG, ed. Basic and clinical pharmacology. Appleton & Lance Publisher. Norwalk, Connecticut, USA. 1995:60-9.

(10) Submission Address: editor@sciencepub.net, Marsland Company, P.O. Box 21126, Lansing, Michigan 48909, The United States.

(11) Reviewers: Authors are encouraged to suggest 2-8 competent reviewers with their name and email.

2. Manuscript Preparation

Each manuscript is suggested to include the following components but authors can do their own ways:

(1) Title page: including the complete article title; each author's full name; institution(s) with which each author is affiliated, with city, state/province, zip code, and country; and the name, complete mailing address, telephone number, facsimile number (if available), and e-mail address for all correspondence. **(2) Abstract**: including Background, Materials and Methods, Results, and Discussions. **(3) Keywords**. **(4) Introduction**. (**5) Materials and Methods**. (**6) Results**. (**7) Discussions**. **(8) Acknowledgments**. **(9) References**.

Journal Address:

Marsland Press
PO Box 180432, Richmond Hill, New York 11418, USA
Telephone: (347) 321-7172
E-mail: editor@sciencepub.net; sciencepub@gmail.com; naturesciencej@gmail.com
Websites: http://www.sciencepub.net

ISSN 1545-0740

9 771545 074344

Marsland Press