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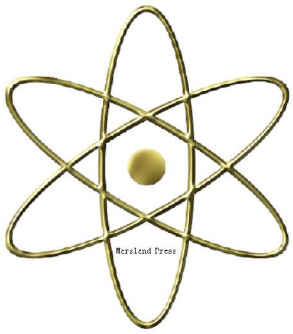

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The following manuscripts are presented as online first for peer-review, starting from February 1, 2011.
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	lagoon, between March and August, 2008 at five study sites. A total of 663 specimens of hermit crab in gastropod shells were collected. Shell occupation of *C. africanus* was limited to gastropod shells belonging to *Pachymelania* and *Tympanotonus* spp. The gastropod shell most inhabited was *Pachymelania* spp, which accounted for 60.96% of inhabited shells, while about 39% of the shell inhabited belonged to *Tympanotonus* spp. Shell preferences were characterized by shell length, weight and aperture width. Positive and statistically significant correlations were obtained between morphometric characteristics of *C. africanus* and those of the shells inhabited, suggesting that fitness of shell to crab dimension constitutes mainly the determinant for *C. africanus* shell utilization. Spatiotemporal variations in the type of shell occupied were not significant in this study. Analysis of the abundance of *C. africanus* in the study area indicates that, a relatively higher abundance of *C. africanus* was observed in site 3, due probably to the favourable environmental conditions provided by large percentage of sand fractions in the sediment. From the data recorded in this study, it may be concluded that shell selection by hermit crabs involves individual preferences related to the shell features that best provide protection, survival and opportunity for the enhancement of behavioural attributes that are necessary for the maximization of bio-ecological relationships. [Aderonke Lawal-Are, Roland Efe Uwadiae and Olayemi Ruth Owolabi. Shell selection of the hermit crab *Clibanarius africanus* (Aurivillus, 1898) (Decapoda: Diogenidae) in the Lagos lagoon: Aspects of behavioural and bio- ecology of benthos. Nature and Science 2011;9(3):7-15]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: Bioecological relationships, shell selection, hermit crabs, *Clibanarius africanus*		
3	**Meristic, Morphometric Characteristics of Frill-fin goby** (*Bathygobius soporator valenciennes, 1837)* **from Lagos Lagoon, Nigeria.** Adeboyejo, O. A. Department of Fisheries, Faculty of Science, Lagos State University, Lagos-Nigeria. adeboyejoakintade@yahoo.co.uk **Abstract:** During the period of March 2010 to August 2010, 500 specimens of *Bathygobius soporator* were collected from Makoko-Iwaya area of Lagos lagoon in mainland area of Lagos state, Nigeria. The number of females were 204 while males numbered 296 giving a sex ratio of 1.45 in favour of males. The size range for males was between 6.0cm and 34.10cm total length (mean = 14.28 ± 4.21 TL). However, the body weight measurement for the males ranged from 8.00 – 135.00g. The total length measurement for female was between 6.2cm and 32.5cm (mean = 15.61 ± 4.38) and from 8 - 135g body weight (mean = 39.97 ± 24.7). A positive correlation existed between length and weight for males (r = 0.67) and females (0.47). The b values were 4.58 and 3.99 for males and females respectively. The condition factor (K) were (1.15 – 1.20) and (2.00 – 2.30) for male and female respectively. The gonad weight ranged between (0.10 – 4.20) and the GSI ranged between 0.21 – 26.58 (mean = 3.77 ± 2.49). The food items of the species in the lagoon were Fish-fry, Worms, Shrimps, Crabs and Bulinous species. **[**Adeboyejo, O. A. **Meristic, Morphometric Characteristics of Frill-fin goby** (*Bathygobius soporator valenciennes, 1837)* **from Lagos Lagoon, Nigeria.** Nature and Science 2011;9(3):16-23]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words:** fish biology, meristic, morphometric, Lagos lagoon, Gonadosomatic index, and goby	Full Text	3
4	Identification and detection of a hepatitis C virus antigen in sera of patients with hepatocellular carcinoma El-Shahat A. Toson[1,*], Tamer E. Mosa [2] and Mohamed Maher [1] 1. Mansoura University, Chemistry Department (New Damietta), Egypt. 2. National Research Center, Cairo, Egypt eatoson@yahoo.com **Abstract**: Hepatocellular carcinoma (HCC) is a major cause of cancer death worldwide, accounting for over half a million deaths per year. Several lines of evidence indicate a strong causal association between hepatitis C virus (HCV) and HCC. The aim of the present study was to identify, purify and	Full Text	4

	partially characterized one of HCV antigens in sera of HCC patients. Also, the possibility of HCV infection play a role in the development of HCC will be tested. Therefore, serum samples of 75 HCC patients and of 25 healthy individuals as a negative control were included in this study. HCV antigen was identified in these samples using western blotting and quantified using enzyme linked immunosorbent assay (ELISA). Western blot analysis showed a single immunoreactive band in sera of HCC patients infected with HCV at 27-kDa. In addition, the 27-kDa purified immunoreactive bands were eluted, mixed and characterized using various physicochemical treatments. Briefly, after such treatements the antigen was found to have protein nature. Moreover, ELISA technique was used to quantify the 27-kDa antigen. The cutoff level of ELISA above or below which the tested sera were considered positive or negative was calculated and was found to be 150 ng/L. Based on such cutoff value a total of 61 out of 75 serum samples of HCC patients were positive for HCV antigen using ELISA. However, zero out of 25 serum samples of healthy individuals were positive for HCV antigen. In addition, ELISA showed sensitivity 81% and specificity 100%. The antigen detection method showed positive predictive value 100% and negative predictive value 64%. [El-Shahat A. Toson, Tamer E. Mosa and Mohamed Maher. Identification and detection of a hepatitis C virus antigen in sera of patients with hepatocellular carcinoma. Nature and Science 2011;9(3):24-30]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: HCV; Hepatocellular carcinoma ; ELIS		
5	**Overview on Hepatitis B virus** Engy Yousry Elsayed Ashor Department of Internal Medicine, Ain Shams University, Cairo, Egypt **Abstract:** Hepatitis B is an infectious illness caused by hepatitis B virus (HBV). The disease has caused epidemics in parts of Asia and Africa, and it is endemic in China. About a third of the world's population, more than 2 billion people have been infected with thehepatitis B virus. This includes 350 million chronic carriers of the virus. This paper offers an overview of hepatitis B virus. **[**Engy Yousry Elsayed Ashor. **Overview on Hepatitis B virus.** Nature and Science 2011;9(3):31-36]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Hepatitis B; infectious illness; epidemics; virus	Full Text	5
6	**Extraction Of Molybdenum (Vi) With 4-Adipoyl And 4-Sebacoyl Derivatives Of Bis (1-Phenyl-3-Methylpyrazolone-5) In Acid Media** Kalagbor, A. Ihesinachi [1]*, Uzoukwu, B. Augustus[2] and Chukwu, U. John[2] 1. Department of Science Laboratory Technology, Rivers State Polytechnic, Bori, P.M.B. 20, Bori, Rivers State, Nigeria. ksinachi@yahoo.com 2. Department of Pure and Industrial Chemistry, University of Port Harcourt, P.M.B 5323, Choba,Port Harcourt, Rivers State, Nigeria uzoukwupob331@yahoo.co.uk, lydiuche@yahoo.com **Abstract:** Liquid – liquid extraction of Molybdenum (VI) ions from various aqueous medium have been carried out using chloroform solution of 4-adipoylbis (1-phenyl-3-methylpyrazolone-5), H_2Adp and 4-sebacoylbis (1-phenyl-3-methylpyrazolone-5), H_2SP in acid media (HCl, H_2SO_4 and HNO_3) in the presence and absence of butanol as a synergist. The degree of extraction of Mo (VI) using H_2Adp was found to be in the range of 82 – 95% for HCl concentrations of 10^{-3}M to 10^{-1}M and 90 – 97% for HNO_3 (10^{-3}M to 10^{-1}M) while H_2SO_4 concentrations gave 70% extraction. On the other hand, the degree of extraction of Mo(VI) using H_2SP was comparatively lower in all acid media. Under all acid conditions studied, H_2Adp was found to be a better extractant for Mo(VI) than H_2SP, while optimal extraction was better in HCl followed by HNO_3 and least in H_2SO_4 concentrations. However, introduction of butanol into the organic phase resulted in enhanced extraction of Mo(VI) to above 98%	Full Text	6

	using both ligands in all three acid media for both H_2Adp and H_2SP. Statistical treatment using slope analysis show that the extracted specie is $M_OO_2(SP)_{(o)}$ and $M_OO_2(Adp)_{(o)}$. [Kalagbor, A. Ihesinachi , Uzoukwu, B. Augustus and Chukwu, U. John. Extraction Of Molybdenum (Vi) With 4-Adipoyl And 4-Sebacoyl Derivatives Of Bis (1-Phenyl-3-Methylpyrazolone-5) In Acid Media. Nature and Science 2011;9(3):37-]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Liquid – liquid extraction, chloroform, Molybdenum (VI), acid media		
7	**Comparative Response of Different Varieties of Maize (*Zea mays* L) to NPK 15:15:15 Compound Fertilizer and Poultry Droppings Applications** Okaka Victor, Alleh Eric, Ogedegbe Felix, Ayodele Emmanuel Department of Crop Science, Ambrose Alli University, Ekpoma, Nigeria E-mail: vbokaka@yahoo.com **ABSTRACT:** Two similar field experiments were conducted during the early cropping seasons of 2007 and 2008 in the Teaching and Research Farm of Ambrose Alli University, Ekpoma, to evaluate DMSR and TZSR improved varieties as well as a popular local maize for agronomic performance and yield responses to NPK 15:15:15 compound fertilizer and poultry droppings. The three varieties were fertilized with 100,200 and 300kg/ha NPK as well as 6t/ha poultry droppings and a control in a 3 × 5 factorial arrangement fitted into a randomized complete block design replicated three times. The three varieties differed significantly ($P<0.05$) in most of the vegetative parameters monitored including plant height, number of leaves and leaf area, in the order of the local > TZSR >DMSR. The positive and significant ($P<0.05$) response of the vegetative and yield parameters to fertilizer application mostly to 300kg/ha NPK, then poultry droppings and 200kg/ha NPK in that descending order, was independent of variety. The DMSR, the TZSR and the local variety produced tassels and silks in 63 and 73 days, in 66 and76 days, and in 72 and 83 days respectively. The three varieties had similar shelling percentage values (68.5-69.9%). The improved varieties: DMSR and TZSR varieties did not differ significantly, out yielded the local by 18.9% and 10.8% respectively and were found to be adapted to this ecological zone. The DMSR was highest yielding with a 100grain weight of 32.0grams and total grain yield of 4.4t/ha. **[**Okaka Victor, Alleh Eric, Ogedegbe Felix, Ayodele Emmanuel. **Comparative Response of Different Varieties of Maize (*Zea mays* L) to NPK 15:15:15 Compound Fertilizer and Poultry Droppings Applications.** Nature and Science 2011;9(3):43-48]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words**: improved varieties; popular local; NPK; Poultry droppings; adapted; ecological zone	Full Text	7
8	**Phytosanitary Protection in Horticultural Seed Production: A Bridge to National Seed Demand** Ihejirika, Gabriel Onyenegecha, Ibeawuchi, Izuchukwu Innocent, Obiefuna, Julius Chiedozie and Ofor, Marian Onomerhievurhoyen Department of Crop Science and Technology, Federal University of Technology, Owerri, P.M.B. 1526 Owerri, Imo State, Nigeria, ihegab@yahoo.com **Abstract:** The problem of quantifying seed losses and their effect on agricultural production and food availability is of primary importance to meet national seed demand. Through that, rational control measures can be developed and applied and resources can be better allocated as well. Seeds being living things, respire by absorbing oxygen and giving off carbon dioxide and water vapour, producing heat at the same time and these phenomena play a major role in its preservation as if not properly taken care off, could cause the seeds to stick together, coagulate as a mass, creating blockage in the store. Seed deterioration is due to a number of interrelated factors like physical e.g. temperature, humidity, water; Biological like microflora (mould, bacteria , fungi, yeast etc) or arthropods (insect, mites); Vertebrates (rodents, birds) or technical (conditions, methods, duration of storage) as well as state of seeds (broken, impurities, residues etc). Infestation in the field, during transportation, storage premises, sacks and containers as well as putting contaminated seeds in store and unhygienic store are inimical to phytosanitary protection and cause losses in seed viability. A reduction in these losses would lead to production of high quality and quantitatively valued seeds to meet national seed derived.	Full Text	8

	[Ihejirika, Gabriel Onyenegecha, Ibeawuchi, Izuchukwu Innocent, Obiefuna, Julius Chiedozie and Ofor, Marian Onomerhievurhoyen. **Phytosanitary Protection in Horticultural Seed Production: A Bridge to National Seed Demand.** Nature and Science 2011;9(3):49-52]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** Phytosanitary, protection, horticultural, seed, national demand		
9	**On the Chromosomes of two Cyprinid Fishes of the Subfamily Schizothoracinae from Kashmir.** [1]Farooq Ahmad Ganai, [2]Abdul Rahman Yousuf, [3]Narinder Kumar Tripathi, [4]Ummer Rashid Zargar. 1. Research Scholar, Limnology and Fisheries Laboratory, Centre of Research for Development, University of Kashmir, Hazratbal Srinagar-190 006 (India). email: farooqmd84@gmail.com 2. Dean Academic Affairs, Dean Biological Sciences, University of Kashmir, Hazratbal Srinagar-190 006 (India). email: aryousuf1951@gmail.com. 3. Dean Students Welfare, University of Jammu. email: narindertripathi@gmail.com 4. Research scholar, Centre of Research for Development. email: uzssummer2@gmail.com. Corresponding author: Farooq Ahmad Ganai. email: farooqmd84@gmail.com **Abstract:** Karyotypic study of two *Schizothorax* species viz. *Schizothorax plagiostomus* and *Schizothorax curvifrons* belonging to family Cyprinidae, from River Jhelum Kashmir, was carried out. Conventional KCl-acetomethanol air-drying protocol was followed for the chromosomal preparation. The diploid chromosome number in *S.plagiostomus* was 96 with a chromosomal formula of 24m+18Sm+54t and fundamental number (NF) =138. Diploid chromosome number in *S.curvifrons* was 94 with Karyotypic formula 26m+20Sm+20St=28t and fundamental arm number (NF) =140. The evolutionary significance of polyploidy and the role of chromosomal rearrangements was discussed. Both these fishes are new to cytological literature. [Farooq Ahmad Ganai, Abdul Rahman Yousuf, Narinder Kumar Tripathi, Ummer Rashid Zargar. **On the Chromosomes of two Cyprinid Fishes of the Subfamily Schizothoracinae from Kashmir.** Nature and Science 2011;9(3):53-61]. (ISSN: 1545-0740). http://www.sciencepub.net. Key words: *Schizothorax plagiostomus*, *Schizothorax curvifrons*, River Jhelum, Karyotype	Full Text	9
10	**Biotechnological potential of bacterial flora from Cheend juice: Alcoholic beverage from Bastar, India** Shukla P*, Vishwakarma, P and Gawri S G.D. Rungta College of Science and Technology, Bhilai 490024, Chhattisgarh, India prashant19782000@gmail.com **Abstract:** Cheend is an alcoholic beverage of tribal people of Bastar region of Chhattisgarh State in India. It is extracted from *Phoenix dactylefera*. From a sample of Cheend juice seven different bacteria were isolated which were of four genera: four of Bacillus, one each of Paenibacillus, Micrococcus and Streptococcus. All the seven bacteria produced ethanol, PHA and EPS in varying capacity. [Shukla P, Vishwakarma, P and Gawri S. Biotechnological potential of bacterial flora from Cheend juice: Alcoholic beverage from Bastar, India. Nature and Science 2011;9(3):62-66]. (ISSN: 1545-0740). http://www.sciencepub.net. Key words: Bacteria; ethanol; PHA; EPS; Cheend	Full Text	10
11	**Serum Trace Element Levels In Sickle Cell Disease Patients In An Urban City In Nigeria**	Full Text	11

	*[1]Idonije B.O, [2]Iribhogbe O.I, [3]Okogun G.R.A [1]Department of Chemical Pathology, [2]Department of Pharmacology and Therapeutics and [3]Department of Medical Microbiology, College of Medicine, Ambrose Alli University Ekpoma. *oignis@yahoo.com ABSTRACT: Assessment of serum trace element levels was carried out in a total of eighty (80) subjects comprising of forty (40) sickle cell disease patients attending the sickle cell centre, Benin City and forty (40) age and sex matched apparently healthy control subjects. Blood samples collected from participants were analyzed for trace elements using atomic absorption spectrophotometer. The mean serum level of magnesium, zinc, manganese, copper, selenium and chromium in sickle cell disease patients were 11.03±1.77mg/L, 120.85±10.29µg/dL, 68.30±3.63µg/dL, 68.54±10.49µg/L, 60.98±7.29µg/L and 62.90±5.97µg/L respectively. Serum magnesium, zinc and selenium levels were significantly lower ($p<0.05$) while serum manganese levels were significantly higher ($p<0.05$) in sickle cell disease patients when compared with apparently healthy control. Serum trace metal levels was not age or sex dependent, as similar pattern of serum trace metals was observed in both male and female sickle cell disease patients. Conclusively, assessment of trace element levels is vital in the management of sickle cell disease. Supplementation with deficient trace elements may reduce the severity of symptoms and complications associated with sickle cell disease, thereby improving the chances of survival in sickle cell disease. **[**Idonije B.O, Iribhogbe O.I, Okogun G.R.A. **Serum Trace Element Levels In Sickle Cell Disease Patients In An Urban City In Nigeria.** Nature and Science 2011;9(3):67-71]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key Words:** Sickle cell disease, Serum Trace Metals, Oxidative Stress.		
12	**Rice Husk Extract is Potentially Effective as a Phytopesticide against Root-/Soil-borne Fungal Pathogens of Cowpea** A.S. Killani[1, 2], R.C. Abaidoo [1*] and A.K. Akintokun [2] 1.International Institute of Tropical Agriculture PMB 5320, Ibadan,Oyo State, Nigeria 2. Department of Microbiology, College of Natural Sciences, University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria E mail: killani405@yahoo.com, skillani@cgiar.org; rabaidoo@cgiar.org ron_akintokun@yahoo.com **Abstact:** Phytopesticide produced from rice husk extract (RHE) was evaluated, in the laboratory and in the glasshouse as a potential biocontrol agent for controlling root- and soil-borne fungal pathogens isolated from field-grown cowpea in the northern Guinea savanna of Nigeria. The pathogenicity test was carried out in the glasshouse on the fungal species isolated from infected plants in cowpea field trials conducted in 2006 and 2007 cropping seasons. Five root- and soil-borne fungal pathogens: *Fusarium verticilloides, F.equiseti, F.solani, F. oxysporum* and *Rhizoctonia solani*, were the major highly virulent fungal pathogens which caused severe problems including damping off, root rot, reduction in nodulation, vascular wilt/discoloration, chlorosis, necrotic lesions, leaf blight, complete defoliation, seedling mortality, and death in cowpea. Plants from the glasshouse experiments (on the microbial antagonism study) were examined for disease incidence and severity symptoms. *In–vitro* and *in–vivo* studies revealed that RHE significantly ($P<0.05$) inhibited all the five fungal pathogens at 1.5% concentration. However, at 1% concentration of RHE did not inhibit mycelia radial growths of *F. verticilloides, F. equiseti* and *F. oxysporum* after 7 days incubation *in-vitro*. The RHE was phytotoxic on cowpea seedlings at 2% concentration. The RHE can thus be regarded as a potential bioprotectant as an alternative to chemical pesticides which are known to be environmentally unsafe for the management of common root- and soil–borne fungal pathogens of cowpea. **[**A.S. Killani, R.C. Abaidoo and A.K. Akintokun. **Rice Husk Extract is Potentially Effective as a Phytopesticide against Root-/Soil-borne Fungal Pathogens of Cowpea.** Nature and Science 2011;9(3):72-79]. (ISSN: 1545-0740). http://www.sciencepub.net.	Full Text	12

	Keywords: Phytopesticides; pathogenicity; bioprotectant; antagonism; fungal pathogens		
13	**Spatial distribution and habitat preferences of selected large mammalian species in the Nech Sar National Park (NSNP), Ethiopia** Aramde Fetene[1], Girma Mengesha[2] and Tsegaye Bekele[3] [1]Debre Markos University, Department of Natural Resources Management, P.O Box 269 email: aramdefetene@yahoo.com [2]Wondo Genet College of Forestry & Natural resource, Department of wildlife & Ecotourism Management, P.O.Box 128, Shshemene, Ethiopia [3]Associate Professor, Hawasa University, Planning and Programming Office, P.O. Box 05, Hawassa, Ethiopia, e-mail:bekele57@yahoo.com (corresponding author) **ABSTRACT.** A study on spatial distribution and habitat preferences of five large mammal species was conducted in the Nech Sar National Park (NSNP) for one year from January 2007-January 2008. The spatial distribution and habitat preference information is useful to propose appropriate patrol strategy for the management and conservation of the species with regard to attracting tourist and management of the park. The objective of this study was to determine the spatial distribution and habitat preference of five large mammals (Defassa waterbuck, Swayne's Hartebeest, Greater Kudu, Lesser Kudu and Black and White Colobus). For the purpose of this study, the park was divided in to three management zones and nine patrolling teams composed of six individuals were involved in the data collection. Each individual was assigned to a certain management zone to monitor the status and distribution of large mammals and the impact of human activities on the Park on daily basis. The patrolling team was equipped with Garmin Etrix Venture GPS receiver and Communication Radio and point sampling technique was used to collect the necessary information. The data was summarized and all spatial data were recorded and analyzed using GIS Software (DNRgarmin and ArcGIS9.1). DNRgarmin was used to transfer data from GPS receiver to computer. ArcGIS9.1 was used to analyze the spatial distribution of the wild animals, habitat association and human activities. Comparison of the mean on the observation of different wild animals in the NSNP was carried out using SPSS17. The results of the study showed that there were a total of 3340 observations of the five large mammals on 29013 km track movements in the NSNP. Observation in this sense does not mean the number of individuals, but the frequency of wild animals seen during the inspection. In this regard, Greater Kudu has shown a significant wider distribution in the three zones of NSNP ($P>0.05$), with high ecological amplitude and high tolerance range to different habitat factors. The other four large mammals were concentrated in a particular association of different habitats. Herds of Swayne's Hartebeest were restricted only to the Nech Sar Plain, Lesser Kudu, to west of the plain, on the mountain near to the hot spring, Defassa waterbuck on a hill of wooded grasslands near to Kulfo river and, the Black and white Colobus in the riverine forests of Kulfo and Sermele river valleys. Large numbers of peoples were observed in the Arba Minch forest and Lake Chamo collecting fuel wood and harvesting fish, respectively. The results of the study are important tools for the park managers, researchers and tourists, since it revealed clear species spatial distributions and habitat preferences. **[**Aramde Fetene, Girma Mengesha and Tsegaye Bekele. **Spatial distribution and habitat preferences of selected large mammalian species in the Nech Sar National Park (NSNP), Ethiopia.** Nature and Science 2011;9(3):80-90]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words:** habitat preferences, large mammals, monitoring, Nech Sar National Park, spatial distribution	Full Text	13
14	**Acute Toxicity Of Nile Tilapia (*Oreochromis niloticus*) Juveniles Exposed To Aqueous And Ethanolic Extracts Of *Ipomoea aquatica* Leaf** *****Simeon O. Ayoola[1], Kuton M.P[1], Idowu A.A[2] and Adelekun, A.B[1] [1]Department of Marine Sciences, University of Lagos, Akoka, Yaba, Lagos State, Nigeria [2]Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta, Ogun State. *****Email:soaayoola@yahoo.com, sayoola@unilag.edu.ng, Tel: +234(80)34650102	Full Text	14

	ABSTRACT: The differential acute toxicity of aqueous and ethanolic extracts of *Ipomoea aquatica* leaf on Nile Tilapia, *Oreochromis niloticus* were carried out under laboratory conditions. The LC_{50} after 96hr of exposure for aqueous and ethanolic extracts of *Ipomoea aquatica* were 2.659g/L and 0.196g/L respectively. These values showed that ethanolic extract of *Ipomoea aquatica* was more toxic than its aqueous extract. Signs of agitated behaviours, respiratory distress and abnormal nervous behaviors including eventual deaths were observed in exposed fish. Control fish neither died nor exhibited any unusual behaviour. The randomized analysis of variance (ANOVA) showed that there were significant differences ($P<0.05$) in the quantal response (mortality) of *O. niloticus* to aqueous and ethanolic extracts of *I. aquatica* at 24hrs, 48hrs, 72hrs and 96hrs of exposure period. It was investigated that leaf of *Ipomoea aquatica* has piscicidal property and can be put into use in the control and management of fish ponds to eradicate predators by farmers. **[**Simeon O. Ayoola, Kuton M.P, Idowu A.A and Adelekun, A.B. **Acute Toxicity Of Nile Tilapia (*Oreochromis niloticus*) Juveniles Exposed To Aqueous And Ethanolic Extracts Of *Ipomoea aquatica* Leaf.** Nature and Science 2011;9(3):91-99]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords**: Acute toxicity, *Ipomoea aquatica, Oreochromis niloticus*		
15	**Effect of Benzyladenine Foliar Sprays on Offsets Production and Root Growth of *Aloe Barbadensis* Miller.** Saeid hazrati[1], zeinalabedin Tahmasebi Sarvestani*[1], arman beyraghdar[2], faraz mojab[3] and Seyyed jaber hosseini[1] Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran[1] Department of Horticulture, Faculty of agriculture, Tarbiat Modares University, Tehran, Iran[2] Department of Pharmacognosy, Shaheed Beheshti University, Tehran, Iran [3] saeid.hazrati@yahoo.com; tahmaseb@modares.ac.ir **Abstract:** Aloe vera (*Aloe barbadensis*) is one of the most important medicinal plants and used world wide in drug and cosmetic industry. In order to determine the effect of different BA levels on offset production and root growth of Aloe vera, an experiment was conducted; the experimental design was RCBD with four replications placed in greenhouse condition. Treatments were included four different BA levels (0, 500, 1000, 1500 $mg.L^{-1}$). At the end of growth period, some characteristics such as offset number, offset leaf number, offset height; number of flowering stems, root length, root volume and root fresh and dry weight were measured. Resulted showed that Increasing hormone concentration cause increase offset number and decreased the root growth, so that the highest offset number was at 1500 $mg.L^{-1}$ which was 95.36% more than our control treatment. Thus BA spraying can be used as an appropriate way to increase offset production in Aloe vera, which has low offset production rate. [Saeid hazrati, zeinalabedin Tahmasebi Sarvestani, arman beyraghdar, faraz mojab and Seyyed jaber hosseini. Effect Of Benzyladenine Foliar Sprays On Offsets Production and Root Growth Of *Aloe Barbadensis* Miller. Nature and Science 2011;9(3):100-104]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:** *Aloe barbadensis* Miller, Benzyl Adenine, Offset, Root	Full Text	15
16	**Antimicrobial proteins and oil seeds from pumpkin (*Cucurbita moschata*).** A. B. Abd EI-Aziz and H.H. Abd EI-Kalek. Microbiology Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. abdelazizamany@gmail.com **Abstract:** The nutritive value and biological activity of pumpkin (*Cucurbita moschata*) seeds cultivated in Egypt were evaluated. Chemical analysis of fiber, protein, ash, carbohydrates, and fatty acids present	Full Text	16

	in the non irradiated and irradiated seeds was conducted. The results show that the values for the indices are within recommended levels for edible oils. Seeds were found to be rich in oil (44.45±2.83 %). The oil contains an appreciable amount of unsaturated fatty acids (71.10±4.32 %) and found to be a rich source of linoleic acid (52.64±0.90 %).Gamma irradiation of pumpkin increased significantly ($P<0.05$) the yield of free fatty acid, acid value and peroxide value of extracts. Results showed decreases in the iodine value after irradiation at doses up to 10kGy. The antimicrobial effect of irradiated and unirradiated pumpkin oil seeds was studied. Gamma radiation up to 10kGys don't affect on the antimicrobial activity of pumpkin oil. Three different proteins were extracted from the pumpkin rinds, seeds, and pulp. All the extracted proteins were screened for their antimicrobial activity against the tested microbial isolates. The total protein and antimicrobial effect of all extractions were decreased at gamma irradiation doses used. **[**A. B. Abd EI-Aziz and H.H. Abd EI-Kalek. **Antimicrobial proteins and oil seeds from pumpkin (*Cucurbita moschata)*.** Nature and Science 2011;9(3):105-119]. (ISSN: 1545-0740). http://www.sciencepub.net. **Key words:** pumpkin seed, Pumpkin seed oil, Oil Quality, fatty acid, Antimicrobial, Antibacterial protein		

Uncertainty determination of correlated color temperature for high intensity discharge lamps

A.B. El-Bialy[1], M.M. El-Ganainy[2] and E.M. El-Moghazy[3]

[1]University College for Woman for Art, science and education. Cairo , Egypt
[2] National Institute for Standards (NIS), Giza, code 11211, Egypt
[3] NIS and Ph.D. student in University College of Woman, Giza, code 11211, Egypt.
Email: emoghazy@yahoo.com Web site: www.nis.sci.eg

Abstract: Color temperature is a description of the color of light sources. The chromaticity coordinates of the light source lying on the Planckian locus which is called (Commission Internationale de l'Eclariage, referred to as CIE) CIE diagram and the source has color temperature (in Kelvin) equal to the blackbody temperature of the Planckian radiator. For light sources that don't have chromaticity coordinates that fall exactly on the Planckian locus but lie near it. In this case the chromaticity coordinates of such sources can be representing by correlated color temperature (CCT). Uncertainty of Correlated Color Temperature (CCT) or **(T_{cp})** for high intensity discharge lamps (HID) is derived from (u, v) color coordinates. The method of the International organization for standardization (ISO) Guide is applied by Gardner to drive analytical expression for uncertainty in **u** and **v** chromaticity coordinates and an uncertainty in CCT for few Kelvins can be achieved. The color temperature standard achieved with the uncertainty of NIS. (11.48 % for mercury lamp, 3.44 % for sodium lamp and 6.4 % for metal halide lamp).

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Key words: lamp, correlated color temperature, Uncertainty and luminous flux.

1. Introduction:

Color temperature is a characteristic of visible light that has important applications in photometry science (calibration and lighting), photography, videography, publishing, manufacturing, and other fields. The color temperature of a light source is the temperature of an ideal black-body radiator that radiates light of the same chromaticity as that light source. The temperature is usually stated in Kelvin (K). It is directly related to Planck's law and Wien's displacement law. The CIE color coordinates are derived by weighting the spectral power distribution (obtained by using a spectroradiometer). the chromaticity coordinates are usually given by normalized coordinates x and y. The (x, y) coordinates are called the chromaticity coordinates. (1) The CCT of a light source, also expressed in Kelvins, is defined as the temperature of the blackbody source that is closest to the chromaticity of the source in this case the CIE 1960 (Uniform Color Space) UCS (*u*, *v*) system is used .(2)

A "modified uniform chromaticity scale diagram" suggested, based on certain simplifying geometrical considerations where (u, v) chromaticity coordinates was used instead of (x,y) . This (u, v) chromaticity space became the CIE 1960 color space, which is still used to calculate the CCT. (3). Higher color temperatures (5,000 K or more) are cool (blueish white) colors, and lower color temperatures (3,000 K or lower) warm (yellowish white through red) colors. For incandescent lamp is called color temperature but for flourescent and high intensity discharge lamps is called Correlated color temperature. (4)

In physics and color science, the Planckian locus is the path or locus that the color of an incandescent black body would take in a particular chromaticity space as the blackbody temperature changes. It goes from deep red at low temperatures through orange, yellowish white, white, and finally bluish white at very high temperatures. (5)

In this work we have to calculate the uncertainty in u, v and CCT for one high pressure mercury lamp has symbol W1, one high pressure sodium lamp has the symbol W2, and one metal halide lamp has the symbol W3.

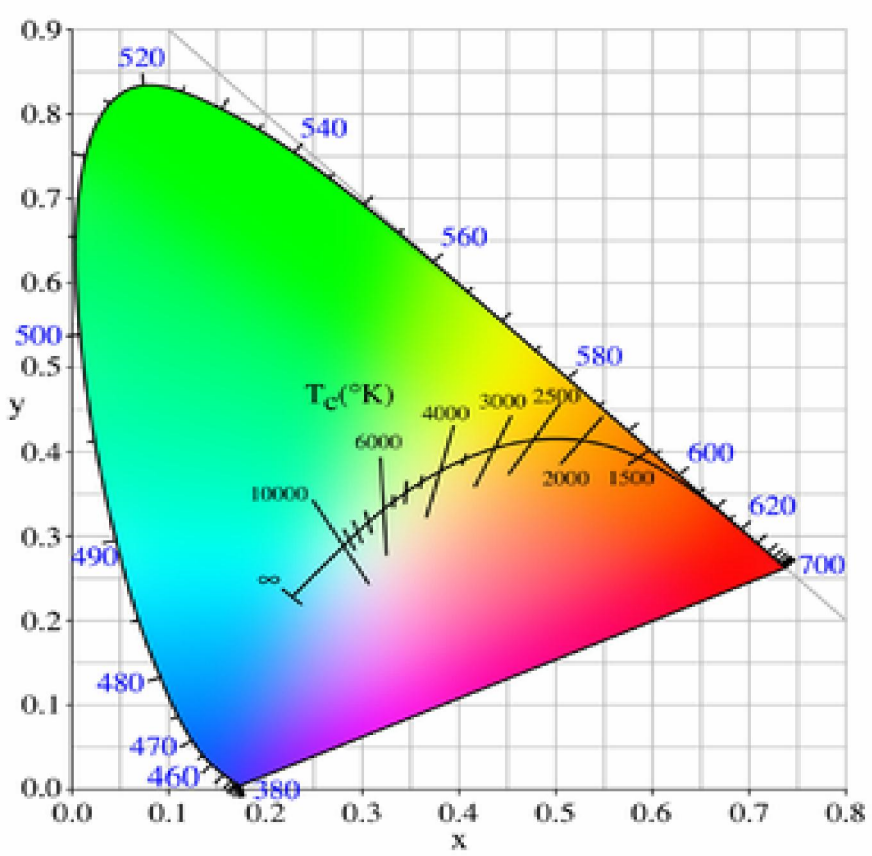

Figure 1.**The CIE 1931 x,y chromaticity space, also showing the chromaticities of black-body light sources of various temperatures (Planckian locus), and lines of constant correlated color temperature.**

2. The experiment technique

The measurements of CCT and u, v were done by HR 2000 spectroradimeter.

The spectroradiometer system is made up of several elements:

- input optics, (a source or sources, with power supplies and electrical measuring equipment).
- Polychromator (monochromator)/array detector,
- Data acquisition system (electronics for measuring detector output quantity combined with a data processing system).

The spectroradimeter ocean optics HR 2000

Irradiance uncertainty: 4.7%

In the present work we choose the one lamps of high pressure mercury 125 Watts,
one lamp of high pressure sodium 150 watts and one lamp of metal halide 150 watts
Such lamps have CCT from warm (≈ 2200 K) to cool light (≈ 6500 K). In the spectroradimeter measurements irradiance is total radiant flux incident on an element of surface divided by the surface area of elements in W/m^2. (7)
Before any work the lamps should be seasoned until the photometric and electric characteristics remain constant. In the present work the HID lamps must be seasoned for ***100*** operating hours and should be cycled ***11*** hours on and one hour off. The metal halide and high pressure sodium lamps should be stored in the same position as seasoned. (8)

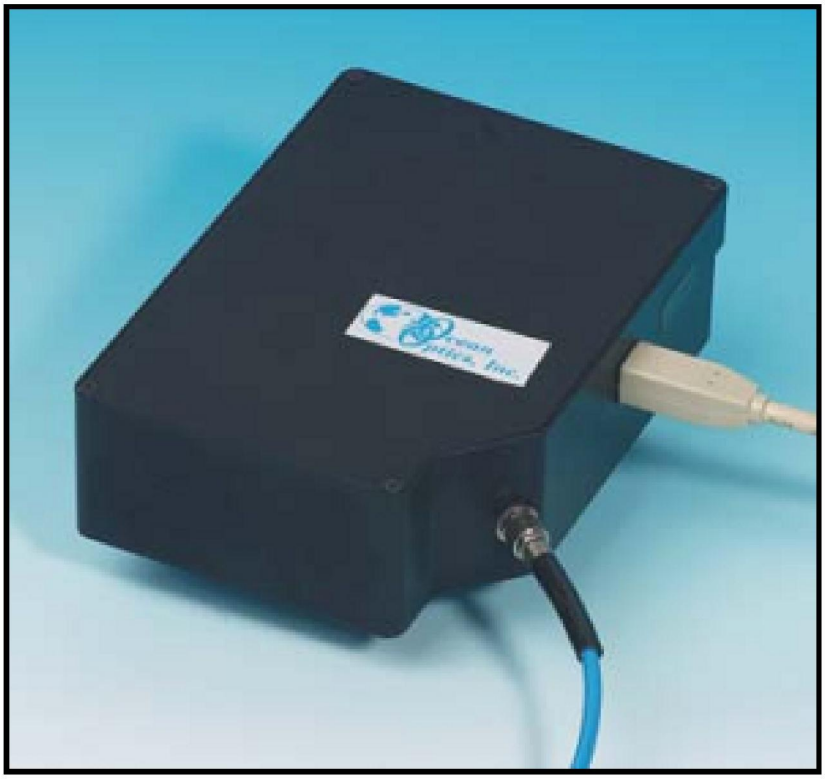

Figure 2. Spectroradiometer measurements where illuminant A is used to take as reference spectrum borrowed from manual (6) .

3.Theoretical background

3.1 The uncertainty of (u, v)

The uncertainty in u is

$$u_c(u) = \{ (u-4)^2 \Sigma u_c^2(E_i) x_i^2 + u^2 [225 \Sigma u_c^2(E_i) y_i^2 + 9 \Sigma u_c^2(E_i) z_i^2] + 30u (u-4) \Sigma u_c^2(E_i) x_i y_i + 6u (u-4) \Sigma u_c^2(E_i) x_i z_i + 90 u^2 \Sigma u_c^2(E_i) y_i z_i \}^{1/2} / (\Sigma E_i x_i + 15 \Sigma E_i y_i + 3 \Sigma E_i z_i).$$

And similarly

$$u_c(v) = \{9(5v-2)^2 \Sigma u_c^2(E_i) y_i^2 + v^2 [\Sigma u_c^2(E_i) x_i^2 + 9 \Sigma u_c^2(E_i) z_i^2] + 6v (5v-2) \Sigma u_c^2(E_i) x_i y_i + 6 v^2 \Sigma u_c^2(E_i) x_i z_i + 18v (5v-2) \Sigma u_c^2(E_i) y_i z_i \}^{1/2} / (\Sigma E_i x_i + 15 \Sigma E_i y_i + 3 \Sigma E_i z_i) \quad (9)$$

Correlated color temperature CCT:

The CCT of a general source is defined the temperature of the nearest point on the Black-body locus. The standard uncertainty u_c**(T)** in CCT is given by

$$u_c(T) = (\partial T/\partial u)^2 u_c^2(u) + (\partial T/\partial v)^2 u_c^2(v) + 2r_{uv} (\partial T/\partial u) (\partial T/\partial v) u_c(u) u_c(v). \quad (1)$$

Where r_{uv} is the correlation coefficient between u and v and

$$\partial T/\partial u = - 5918.47 + 9.69941 T - 0.00958899 T^2 + 1.88114x10^{-6} T^3 - 1.67343x10^{-10} T^4 + 5.42081x10^{-15}.$$

$$\partial T/\partial v = - 385.70 + 8.40689 T - 0.00362952 T^2 + 3.71034 x10^{-8} T^3.$$

The correlation coefficient between u and v is given by (1) is

$$r_{uv} = \Sigma (\partial u/\partial E_i) (\partial v/\partial E_i) u_c^2(E_i) / [\sqrt{\Sigma} (\partial u/\partial E_i)^2 u_c^2(E_i) \sqrt{\Sigma} (\partial v/\partial E_i)^2 u_c^2(E_i)] \quad (9)$$

x_i, y_i and z_i are color matching functions (description of a color by the spectral concentration of a radiometric quantity such as radiance or radiant power as a function of wavelength) from 360 nm to 770 nm and obtain from standard table . Radiant power is total emitted by a light source per unit time. (7)

Gardner obtains the uncertainty in CCT derived directly from systematic and random components of the spectral irradiadiance values. (10)

4. Results and discussions:

By setting the lamps at their nominal voltage at distance one meter from input fiber (optics). The u,v and CCT data of each lamp obtained from the computerized spectroradimeter, tabulated in tables (1-4). In table 1 the values of u,v and CCT for each lamp. In table 2 the values of uncertainty of u,v and their squares. In table 3 we obtained the uncertainty of CCT . Finally in table 4 the values of operating voltage, current and watt for each lamp. We found that photometrically and electrically the W1 is the high uncertainty in CCT for high pressure mercury lamp, the high pressure sodium lamps has lower uncertainty and W3 for metal halide lamps is intermediate . Gardner (9) uses this method for calculating CCT for a high pressure sodium lamp reaching uncertainty of CCT for this lamp as 3.1 K assuming an uncertainty of spectral irradiance $u_c(E_i)$ is 0.01 but we measure the uncertainty of spectral irradiance $u_c(E_i) = 4.7\%$.

Table 1. The values of CCT and u and v were obtained by using the spectroradimeter.

Lamps	**CCT**	**u**	**v**
W1	**6036**	**0.236**	**0.327**
W2	**2200**	**0.32**	**0.361**
W3	**6306**	**0.235**	**0.333**

Table 2. The values of uncertainty of u and v and their square

Lamps	$\partial T/\partial u$	$\partial T/\partial v$	$(\partial T/\partial u)^2$	$(\partial T/\partial v)^2$	Uncertainty of CCT (Kelvin) %
W1	-29488.2	-17849.6	8.7E+08	3.2E+08	11.48
W2	-8476.06	-376.64	71843513	141858	3.44
W3	-31058.5	-19833	9.65E+08	3.9E+08	6.4

Table 3. The values of uncertainty of CCT for lamps

Lamps	$u_c(u)$	$u_c(v)$	$u_c^2(u)$	$u_c^2(v)$
W1	0.03	0.018	0.000888	0.000324
W2	0.046	0.018	0.002116	0.00032761
W3	0.015	0.009	0.000228	8.14506E-05

Table 4. The values of Current, volt and power of the lamps

Lamps	Volt (V)	Current (A)	Power (W)
W1	119	1.18	125
W2	91	1.71	133
W3	97	1.87	149

5. Conclusion:

- For the first time in Egypt experimentally determination of the uncertainty of CCT for high intensity discharge lamps.
- The lamps under investigation may use as standard lamps for correlated color temperature
- By using the uncertainty for CCT we can obtain the uncertainty for mismatch factor, which is very important for calculation of luminous flux uncertainty.

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Evaluation of pattern of shell utilization of the hermit crab *Clibanarius africanus* (Aurivillus, 1898) (Decapoda: Diogenidae) in the Lagos lagoon: an example of bioecological relationship in benthos.

Aderonke Lawal-Are, Roland Efe Uwadiae* and Olayemi Ruth Owolabi

Department of Marine sciences, University of Lagos, Akoka , Yaba, Lagos, Nigeria
*Corresponding author: eferoland@yahoo.com. Tel: +2348063145723

Abstract. Evaluation of pattern of shell utilization by *Clibanarius africanus* was investigated in the intertidal area of the Lagos lagoon, between March and August, 2008 at five study sites. A total of 663 specimens of hermit crab in gastropod shells were collected. Shell occupation of *C. africanus* was limited to gastropod shells belonging to *Pachymelania* and *Tympanotonus* spp. The gastropod shell most inhabited was those of *Pachymelania* spp, which accounted for 60.96% of inhabited shells, while about 39% of the shell inhabited belonged to *Tympanotonus* spp. Shell preferences were characterized by shell length, weight and aperture width. Positive and statistically significant correlations were obtained between morphometric characteristics of *C. africanus* and those of the shells inhabited, suggesting that fitness of shell to crab dimension constitutes mainly the determinant for *C. africanus* shell utilization. Spatiotemporal variations in the type of shell occupied were not significant in this study. Analysis of the abundance of *C. africanus* in the study area indicates that, a relatively higher abundance of *C. africanus* was observed in site 3, due probably to the favourable environmental conditions provided by large percentage of sand fractions in the sediment. From the data recorded in this study, it may be concluded that shell utilization by hermit crabs involves individual preferences related to the shell features that best provide protection, survival and opportunity for the enhancement of behavioural attributes that are necessary for the maximization of bio-ecological relationships.
[Aderonke Lawal-Are, Roland Efe Uwadiae and Olayemi Ruth Owolabi. Evaluation of pattern of shell utilization in the hermit crab *Clibanarius africanus* (Aurivillus, 1898) (Decapoda: Diogenidae) in the Lagos lagoon: aspects of behavioural and bio- ecology of benthos. Nature and Science 2011;9(3):7-15]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: Bioecological relationships, shell utilization, hermit crabs, *Clibanarius africanus*

1. Introduction

Bioecological relationship is an important phenomenon in benthic assemblages and therefore plays significant roles in the pattern of structural parameters of benthic communities such as density, biomass, richness, species diversity and spatiotemporal distribution (Tait and Dipper, 1998). Benthic taxocoenosis of the Lagos lagoon system is controlled by the populations of two gastropod mollusc taxa; *Tympanotonus* and *Pachymelania* (Oyenekan, 1975; Brown, 2000; Uwadiae, 2009), which provide important biotope for encrusting benthic species and cover for hermit crabs.

Hermit crabs (Anomura: Diogenidae) are crustaceans adapted to use empty gastropod shells as shelter, preventing mechanical damage to their abdomen (Barnes, 2003), and as protection against predation (Leonard *et al.*, 2001). They need increasingly larger shells during their life cycle, a fact that keeps them in constant activity searching for suitable shells (Bertness, 1981b). The life cycle of hermit crabs, therefore depends mostly on the availability of suitable gastropod shells (Hazlett, 1981). Shell utilization appears to be based on a complex and interactive factors, including shell weight, architecture, volume, height, width, colour and aperture size (Garcia and Mantelatto,2000, 2001).

Often, availability of empty gastropod shells is a limiting factor to populations of many species of hermit crabs (Scully, 1979) and the sizes of shells occupied by hermit crabs are usually well correlated with crab size owing to mechanisms such as mutual gain shell exchange (Hazlett, 1981, 1989). Hermit crabs seem to utilize among the available empty gastropod shells the most suitable one for their size and shape (Koutsoubas *et al.*, 1993). Shell selection and utilization are not by chance, but based on adequacy and availability of resources (Reese, 1969, Conover, 1978), and is affected by both shell size and species (Conover, 1978; Lively, 1988; Ohmori *et al.*, 1995; Rodrigues *et al.* 2000; Mantelatto *et al*., 2007). Moreover, the fitness of a particular shell may differ between hermit crab species, reflecting several selection pressures associated with different habitats which act in different ways on each crab species (Bertness, 1981a) in different areas.

As hermit crabs grow they require larger shells. Since suitable intact gastropod shells are sometimes a limited resource, there is often vigorous competition

among hermit crabs for shells. The availability of empty shells at any given place depends on the relative abundance of gastropods and hermit crabs, matched for size. An equally important issue is the population of organisms that prey upon gastropods and leave the shells intact (Tricarico and Gherardi, 2006).

A hermit crab with a shell that is too small cannot grow as fast as those with well-fitted shells, and is more likely to be eaten if it cannot retract completely into the shell (Angel, 2000). Most species of hermit crabs have long, spirally curved abdomens, which are soft, unlike the hard, calcified abdomens seen in related crustaceans. The vulnerable abdomen is protected from predators by shell carried by the hermit crab, into which its whole body can retract (Ingle, 1997). The tip of the hermit crab's abdomen is adapted to clasp strongly onto the columella of the gastropod shell (Chapple, 2002).

This habit of living in a second hand shell gives rise to the popular name "hermit crab", by analogy to a hermit who lives alone. Several hermit crab species use “vacancy chains” to find new shells: when a new, bigger shell becomes available, hermit crabs gather around it and form a kind of queue from largest to smallest. When the largest crab moves into the new shell, the second biggest crab moves into the newly vacated shell, thereby making its previous shell available to the third crab, and so on (Randi *et al.*, 2010).

Although the utilization and selection process of gastropod shells by hermit crabs have been investigated by many authors (Bertness, 1980, 1981a, b, 1982; Blackstone, 1985, 1989; Gherardi and Vannini, 1989; Hazlett, 1989, 1990, 1992; Gherardi, 1991; Ohmori *et al.*1995; Garcia and Mantelatto, 2001; Meireles and Mantelatto, 2005) around the world, there is scarcity of information on shell utilization in hermit crabs in Nigerian aquatic environment. *Clibanarius africanus* (Aurivillus, 1898) is a species of hermit crab in the family Diogenidae common on the shores of the Lagos lagoon. Despite its abundance, wide distribution and easy accessibility to its habitat, ecological information on the organism is still scarce. In this study, shell utilization by *C. africanus* in a stretch of intertidal area in the western part of the Lagos lagoon is evaluated.

2. Description of study area

Lagos lagoon is located in the West African Coast of Nigeria. It lies between longitude 3° 54" and 4° 13"E and latitude 6° 25" and 6° 35" N. The Lagoon is more than 50 km long and between 3 to 13 m in width, it is separated from the Atlantic Ocean by a long sand spit of 2 to 5 km wide. The lagoon is characterised by fresh and brackish water conditions occasioned by the heavy input of rainfall run-offs and river discharges during the rainy season, and the influences of tidal incursion and increased surface water evaporation during the dry seasons. The Lagoon is fed by a number of rivers including Ogun and Majidun rivers and Agboyi creek. These water bodies altogether have a drainage area of 103, 637 km^2 (Oyewo, 1998).

The study sites (Table 1; Figure 1) used for this study were selected along the intertidal area of the Lagos lagoon close to the University of Lagos. This part of the lagoon has been under the stress of human activities because of its accessibility to man. Major stressors include garbage overload arising from indiscriminate dumping of litter during major events such as picnics and students’ activities around the lagoon. Modification of the shoreline arising from the construction of brick wall to serve as shoreline protection is evident in the study area. Another prominent feature of the study area is the presence of burrows of crabs scattered all over the shoreline which are most visible at low tides. This area was chosen for this study because of the high biological activities that can be observed in this area. Table 1 presents some physical characteristics of the study sites.

Table 1: Some physical characteristics of the study sites.

Station	Longitude	Latitude	Water colour	Sediment colour
1	003º24´01E	06º31´42"	Brown	Brown
2	003º24´ 02E	06º31´13"	Brown	Brown
3	003º24´06E	06º31´07"	Brown	Brown
4	003º24´08E	06º31´04"	Brown	Brown
5	003º24´ 10E	06º31´00"	Gray	Black

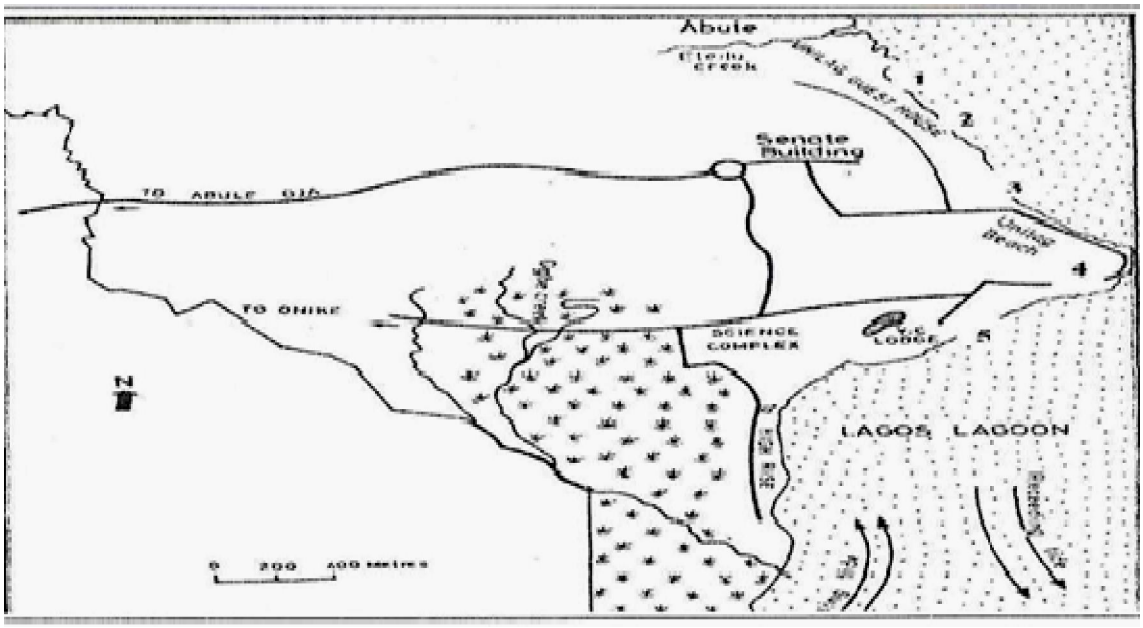


Figure 1: Map showing sampling sites (1-5)

3. Materials and methods

3.1 Collection and analysis of samples

Specimens of *C. africanus* in gastropod shells were collected using a van Veen grab at five locations along the lagoon. Three grab hauls were taken from each site and the collected materials washed through a 0.5mm mesh sieve. The residue in the sieve for each station was preserved in 10% formalin solution and kept in labelled plastic containers for further laboratory analysis. Preserved benthic samples were washed with tap water to remove the preservative and any remaining sediment. Specimens of *C. africanus* were sorted out, counted and recorded for all the sampling months and study sites, gastropod species were identified based on the works of Olaniyan (1975), Edmund (1978) and Yoloye (1994).

Specimens of *C. africanus* in gastropod shell and after removal from the shell were weighed with an electronic scale of 0.001g sensitivity. Prior to weighing, the animals were drained on a fine sieve, air dried for 5 minutes on absorbent paper and exposed to air until liquid is no longer visible. The lengths of gastropod shell, width of aperture and length of *C. africanus* were measured in centimeter using a graduated meter rule. Collection of sediment samples and grain size analysis followed the methods described in Holme and Mclntyre (1970).

3.2 Statistical analysis

To determine morphometric relationships and correlations between characteristics of hermit crabs and their preferred shells, regression analyses were computed. All statistical analyses were performed using SPSS 10 and Microsoft Excel 2003 for Windows.

4. Results

4.1 Shell type preference of *C. africanus*

The spatial and temporal variations in the frequency of shell type used by *C. africanus* are presented in figure 2. A total of 663 specimens of hermit crab in gastropod shells were collected. Shell occupation of *C. africanus* was limited to gastropod shells belonging to *Pachymelania* and *Tympanotonus* spp. The gastropod shell mostly inhabited were those of *Pachymelania* spp, which accounted for 60.96% of inhabited shells, while about 39% of the shell inhabited belonged to *Tympanotonus* spp. Occurrence of *C. africanus* in the shells of *Pachymelania* spp shell was highest (43) in the month of March while its lowest occurrence (30) in the same shell type was observed in the months of August and April. The number of shells of *Tympanotonus* spp inhabited by *C. africanus* was highest (34) in the month of April and least number (9) of shells occupied in June.

There were slight variations (41- 64) in the total monthly number of individuals of *C. africanus* recorded during the sampling months. The month of April recorded the highest number (64) of *C. africanus* in monthly samples, while the least number of individuals of *C. africanus* was observed in June. Sixty - one and forty - eight individuals were recorded in the months of July and August respectively, while 59 specimens of *C. africanus* were observed for the months of March and May respectively.

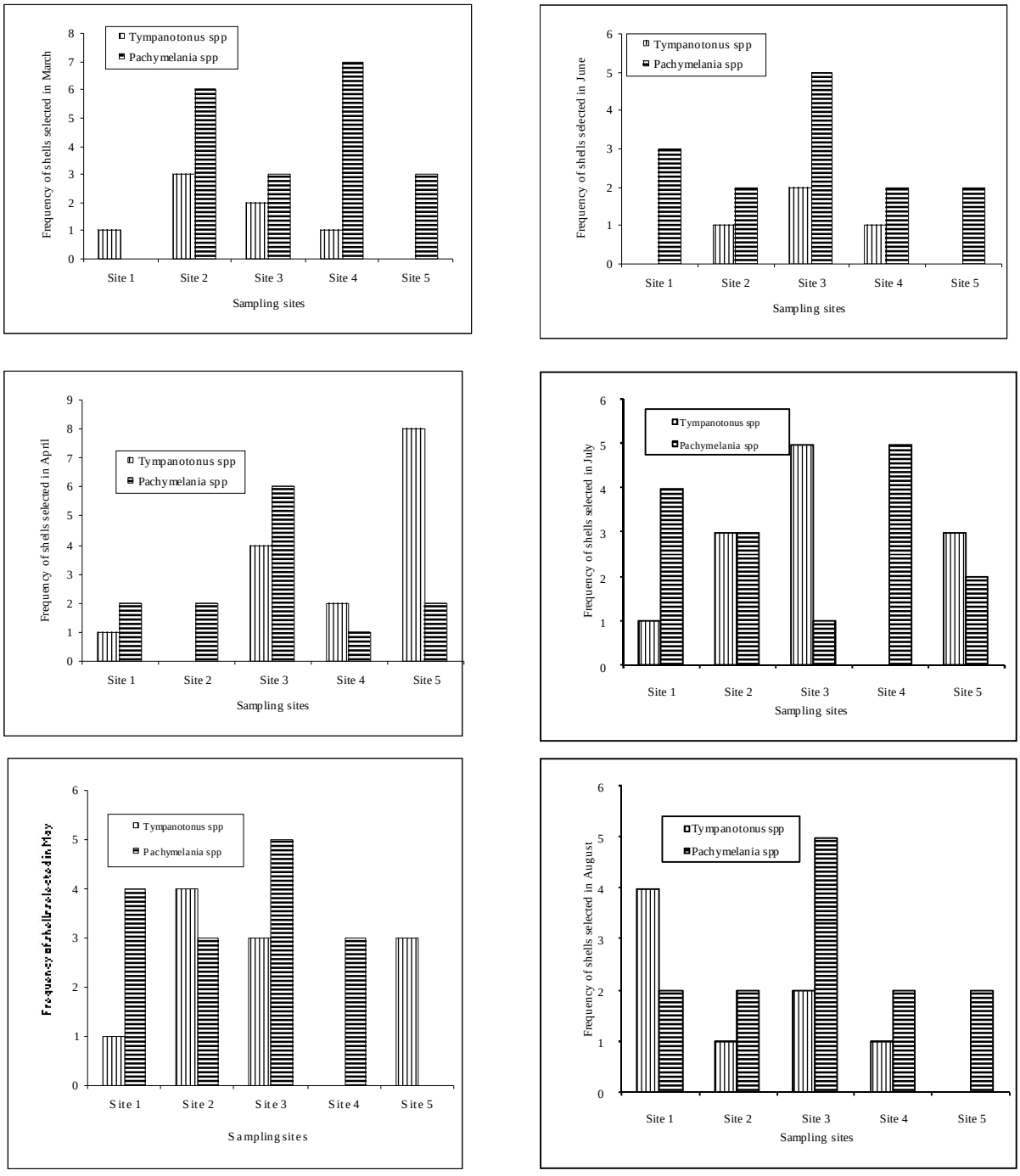


Fig. 2. Spatial and temporal variations in the frequency of shell type selected by *C. africanus.*

4.2 Shell size preference

Specimens of *C. africanus* collected during this study varied between 0.5 and 4.3 cm in length while the length of shell occupied ranged from 1.8 to 5.5 cm. These figures show an overlap indicating that the lengths of *C. africanus* observed are closely related to the length of gastropod shell inhabited. This relationship is clearly depicted in figure 3a where a positive correlation between lengths of shell and that of *C. africanus* is shown in a regression model. Statistically, the relationship between the length of *C. africanus* and the length of shell it occupied was found to be significant (F= 145.58, df =1, P <0.001). The length of *C. africanus* was also found to be related to the width of the aperture of the mollusc shell it occupied. Regression analysis indicates that length of *C. africanus* correlated positively (Figure 3b) with width of shell aperture, and this relationship was observed to be statistically significant (F = 38.58, df = 1, P<0.05).

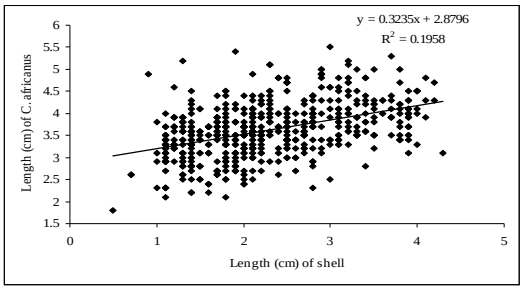
This study also revealed that another factor of importance in the utilization of shell by *C. africanus* is the weight of the gastropod shell. The study observed a positive correlation (Figure 3c) between the weight of *C. africanus* and the weight of gastropod shell selected. This relationship was also found to be of statistical significance (F = 27.540, df = 1, P<0.005).

4.3 Sediment characteristics and abundance of *C. africanus.*

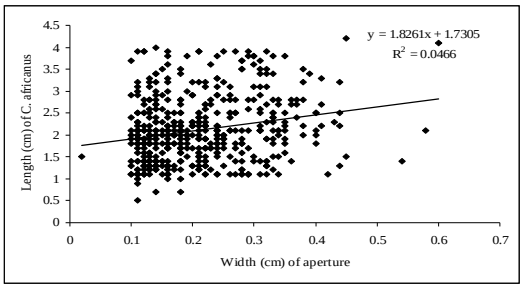
In the study area, it was observed that sand and mud intermixed in varying proportions (Figure 4). The percentage of sand ranged from 45.7 to 99.4% while mud varied between 0.5 and 63.9%. In site 1, sand ranged between 45.7 and 87.4%, and mud between 5.7 and 63.9%. Lowest values observed for sand in sites 2 to 5 were 58.8%, 66.2%, 63.7% and 65.2% respectively, while those of mud for the same stations were 4.3%, 0.5%, 3%, and 1.5% respectively.

Variation in the percentage of sand fractions in the sediment of the study area was a major physical parameter that affected the abundance of *C. africanus* recorded in the study sites. Figure 5 depicts a positive correlation between abundance of the animal and percentage sand in sediment. The overall results indicate that, although there were higher percentage sand fractions than mud in sediment of all the study sites, values for site 3 were relatively higher. Site 3 also recorded the highest number of individuals of *C. africanus*. The lowest mud content was recorded in site 1

3a



3b



3c

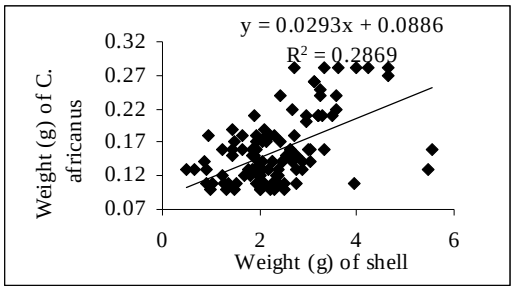


Figure 3a-c. Relationships between morphometric characteristic of *C. africanus* and those of the shells inhabited.

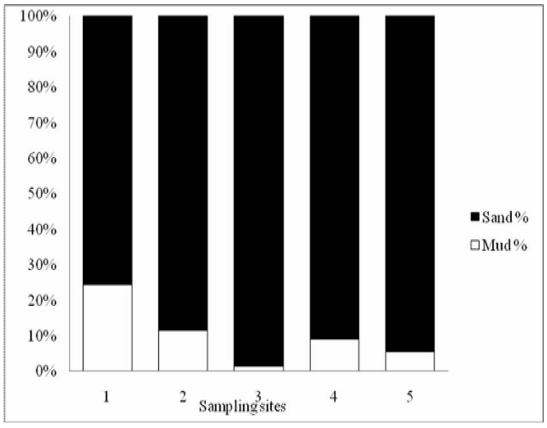


Figure 4 . Grain size composition of the study sites.

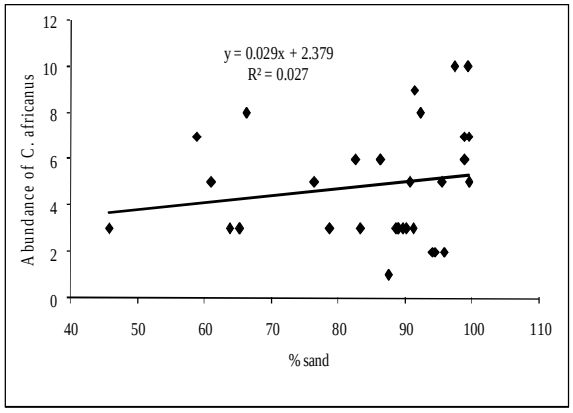


Figure 5. Relationship between percentage of sand in sediment and abundance of *C. africanus*

5. Discussion

The most striking feature of the result obtained in this study is the positive and significant relationships among morphometric qualities of *C. africanus* and that of the gastropod shell inhabited. This is a confirmation of the views of many Crustacean biologists that shell utilization in hermit crabs is based on the dimension of the crab (Hazlett, 1981, 1989, Koutsoubas *et al*., 1993, Ates *et al.*, 2007, Mantelatto *et al*., 2007, Nakin *et al*., 2007).

Several factors have been adduced and hypotheses put forward to explain the discrepancy in gastropod shell occupancy in hermit crabs (Bertness, 1980; 1981a, b, 1982): 1) Gastropod life cycle; availability of different shell types (species) in nature is determined by the relative abundance of different live gastropods and their mortality rates (Meireles *et al*., 2003), 2) Environmental conditions; differences in abiotic characteristics of the area in terms of water dynamics (wave activity, intensity of currents, food supply) are determinant of installation of some invertebrate species (Fransozo and Mantelatto, 1998), 3) Predation pressure; several combined actions from natural and artificial predators can act in different ways to reduce the diversity of gastropod shells in the region.

This study reveals that shell dimension constitutes mainly the determinant for *C. africanus* shell utilization. In hermit crabs a well-fitted shell is essential for maintaining low evaporation rates and carrying ample water (Angel, 2000). An appropriately sized shell in good condition allows for effective movement and provides competitive edge than ill – fitted shells. Hermit crabs with broken, ill-fitted shells are restricted to the coast and appear to be in relatively poor conditions (Koutsoubas *et al.*, 1993; Angel, 2000; Ates *et al.*, 2007).

Bertness (1980), reported that hermit crabs do not necessarily live in shells they prefer, the availability of different shell types and contact with competitors for empty shells influences shell occupation. This study revealed that shells of *Tympanotonus* spp provided a low specimen adequacy to *C. africanus*, this may be connected with the relatively higher abundance and availbilty of *Pachymelania* spp in the study area (Oyenekan, 1975, 1979, 1988; Brown, 2000; Uwadiae, 2009), which provided larger number of shells for *C. africanus*. Although, Yoshino *et al.* (1999), posited that there is a trade-off between shell size and species preference and that less preferred shell species are actively chosen when the more preferred shell species the crabs encounter frequently in the field are of a less suitable size, in this study, considering the great availability (in size and number) of shells of *Pachymelania* spp in the study area , we may infer that the assertion proposed by Yoshino *et al.* (1999) may not be applicable because availability of shell was considered as the overriding factor determining shell usage in this particular study. Shell morphology and morphometric qualities of *Pachymelania* and *Tympanotonus* spp would not have been significant in preferential utilization of shell by *C. africanus* since the two taxa bear similar qualities. Previous studies including Lively (1988) found that shells generally used by hermit crabs were found in the same frequency as the gastropod fauna, revealing a close relationship between shell use and availability of the resources.

The availability of shells in the environment plays a fundamental role in the population dynamics and distributional pattern of hermit crabs (Meireles *et al.* 2003). Although we did not evaluate the shell

availability to the hermit crab community in this present study, the availability of empty shells at any given place depends on the relative abundance of gastropods and hermit crabs matched for size. An equally important issue is the population of organisms that prey upon gastropods and leave the shells intact (Tricarico and Gherardi, 2006). The families Potamiididae and Melaniidae are major components of the benthic communities in the lagoons, estuaries and mangrove swamps in West Africa (Buchanan, 1954). The two families are euryhaline and adapts perfectly to freshwater and brackish water conditions, hence, their survival and continuous presence all year round, providing shells for *C. africanus*. The sedimentary characteristics of the study area have also enhanced the continued presence of these gastropods. Uwadiae *et al* (2009) reported that *P. aurita* preffered sedimentary conditions similar to those observed in this study

Most shells collected have their columela and callosity modified, this agrees with the report of Wolcott (1988), which observed that, shells are modified by hermit crab use, the new shells in some cases are small to accommodate big crabs, so relatively bigger crabs may inhabit shell whose internal volume have been modified by the occupation of another hermit crab. A similar observation has been made by Kinosita and Okajima (1968) on shells of *Nerita striata* occupied by *Coenobita rugosus* from Japan.

Differences in gastropod shells utilization can occur as a function of the area of occurrence of the hermit crabs (Garcia and Mantelatto, 2000). *Clibanarius africanus* was found in places with small percentage of mud, and high concentrations of sand. The nature of the substratum influences the frequency and ability of the hermit crabs in burying themselves, in a way, that they rarely choose another substrate that is not sand (Oyenekan and Adediran, 1987). This fact corroborates our observations in the present study, the animal was registered principally in locations with the highest percentage of sand.

Besides the interactions with the abiotic factors, the animals share the environment with other organisms from the benthic community. The individuals of a community are in various ways interdependent, and some organisms thrive only in the presence of particular associated fauna. These inter-relations can interfere in the population and distribution of the organism (Pardo *et al*., 2007). The populations of *C. africanus* like other benthic organisms are threatened by the intensive unregulated human activities in the Lagos lagoon. This suggests that the data gathered may be reflecting both responses to biotic and abiotic environmental features. Further studies are encouraged to analyse the consistency of the patterns depicted here and explore the causal mechanisms.

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Meristic, Morphometric Characteristics of Frill-fin goby (*Bathygobius soporator valenciennes, 1837)* from Lagos Lagoon, Nigeria.

Adeboyejo, O. A.

Department of Fisheries, Faculty of Science, Lagos State University, Lagos-Nigeria.
adeboyejoakintade@yahoo.co.uk

Abstract: During the period of March 2010 to August 2010, 500 specimens of *Bathygobius soporator* were collected from Makoko-Iwaya area of Lagos lagoon in mainland area of Lagos state, Nigeria. The number of females were 204 while males numbered 296 giving a sex ratio of 1.45 in favour of males. The size range for males was between 6.0cm and 34.10cm total length (mean = 14.28 ± 4.21 TL). However, the body weight measurement for the males ranged from 8.00 – 135.00g. The total length measurement for female was between 6.2cm and 32.5cm (mean = 15.61 ± 4.38) and from 8 - 135g body weight (mean = 39.97 ± 24.7). A positive correlation existed between length and weight for males (r = 0.67) and females (0.47). The b values were 4.58 and 3.99 for males and females respectively. The condition factor (K) were (1.15 – 1.20) and (2.00 – 2.30) for male and female respectively. The gonad weight ranged between (0.10 – 4.20) and the GSI ranged between 0.21 – 26.58 (mean = 3.77 ± 2.49). The food items of the species in the lagoon were Fish-fry, Worms, Shrimps, Crabs and Bulinous species.

[Adeboyejo, O. A. **Meristic, Morphometric Characteristics of Frill-fin goby** (*Bathygobius soporator valenciennes, 1837)* **from Lagos Lagoon, Nigeria.** Nature and Science 2011;9(3):16-23]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: fish biology, meristic, morphometric, Lagos lagoon, Gonadosomatic index, and goby.

1. Introduction

Bathygobius soporator is a bony fish that belong to the family Gobiidae. Members of this family are *Signogobius ocellarus* (Twinspot Goby), *Pleurosicya mossambica* (Toothy goby), *Istigobius rigilius* (orange-spotted goby), *Gnatholepsis thomponi* (Gold-spot goby) etc. The family are mostly small sizes, in which the two pelvic fins are united to form a cup-like sucking disc, which they use to hold on to rocks (Nelson, 1994). A few gobies occur in deep water and a few are found at the surface, but the vast majority live in rock-pools between tidal-marks where their modified pelvic fin is most useful. Many of the species enter estuaries and a number are permanently resident in freshwater. They are usually carnivorous e.g. crustaceans, small fishes, worms etc. Very few of them are used as food because of their size. Many genera and species are known to endemic tropical and subtropical regions (Hoese, 1998). *Bathygobius soporator* as a species of the Gobiidae family has two separate dorsal fins; the body is not very elongated. The teeth in the lower jaw are arranged in more than one row; the eyes are not prominent, or erectile; the pectoral fins are normal. The snout is rounded, the scales are of moderate size, and there being less than 50 in a row from the gill-opening to the base of the caudal fin. The body is feebly compressed; the caudal fin is shorter than the head; the upper rays of each pectoral fin are free and silk-like (figure 1). They are either bottom-dwellers or hovers in the water column, a short distance above the bottom. It is associated with a variety of substrata. The depth range includes tide-pools, or shallow waters next to shore and offshore areas down to at least 50m.

Varying sizes were obtained during a period of six months. Even though the specie is poorly represented in the open market, the area of capture has indicated their area of abundance. The scarcity may be due to the high patronage enjoyed by the fish, but it's usually exhausted before reaching the market. The sexually immature specimens occurred in similar area to where the juveniles were found i.e. shallow water close to the shore but were also found (matured adult) in the slightly deeper waters of the lagoons many of the mature specimens were caught in the lower end of the set net, indicating that they occurred near the bottom. Behaviour typically involves intermittent swimming, with short darting when disturbed.

B. soporator is one of the most common surface inshore fishes in West Africa and deserve necessary attentions by researchers. Work on the Age and Growth of the species is however scanty; Diaz *et al.* (2000) studied the food and feeding habits, Miller (1986) reported that the specie dwells on or near the bottom in epibenthic ecotypes and Thresher (1984) studied the reproduction in reef fishes. This study is aimed at providing information on the age and growth, food and feeding habits and the reproductive biology on the following areas: To deter mine the growth pattern of the fish, to present the characterization [Meristic and morphometric], the condition factor (K), and determine the sex ratio and gonadosomatic index of the fish.

2. Study Area

Lagos is a coastal state situated between ($6^0$22" - $6^0$42"N, $2^0$42" - $4^0$20"E). Lagos state covers an area of about 3,577km^2 thus occupying about 0.4% of the total land area of Nigeria (Ajao, 1990). Lagos lagoon as shown in figure 1, is an expanse of shallow water which in most areas is between 0.5 – 2.0m with a maximum of 5m depth in the main body and 18 – 25m in some dredge portions of the Lagos harbour. Lagos lagoon is moderately large water body that stretches from Lagos harbor in the south, Ikorodu in North, Epe in the East and University of Lagos in West (figure 1a and b). Irvine (1931) has shown that the specie is widely distributed throughout the Gold Coast (Ghana) inshore and offshore, and also penetrating the inland waters with openings into the sea.

3. Materials and Methods

3.1 Sampling: Specimens of *Bathygobius soporator* were obtained from well monitored fishermen at Iwaya-makoko area of Lagos lagoon, about 2km from University of Lagos campus, Lagos-Nigeria. Total length was taken from the tip of the snout to the termination of the caudal fin. Head length was taken from the tip of the snout to the posterior end of the operculum and the eye diameter was measured across the eye socket. Body depth was taken to be the distance from the ventral region and the base of the dorsal fin. Sexes of the fishes were also determined.

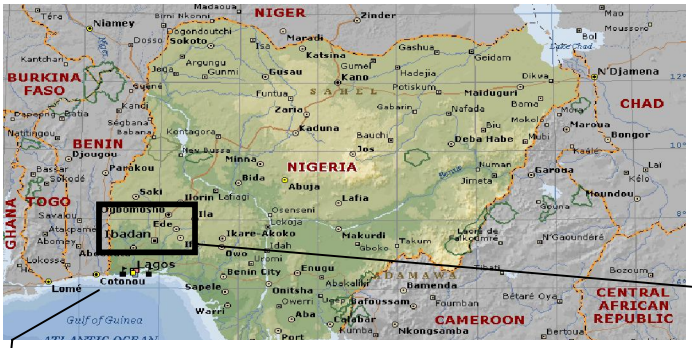

Figure 1a: Map of Nigeria Inset Lagos Lagoon,

Figure 1b: Map of Lagos Lagoon,

Figure 2a

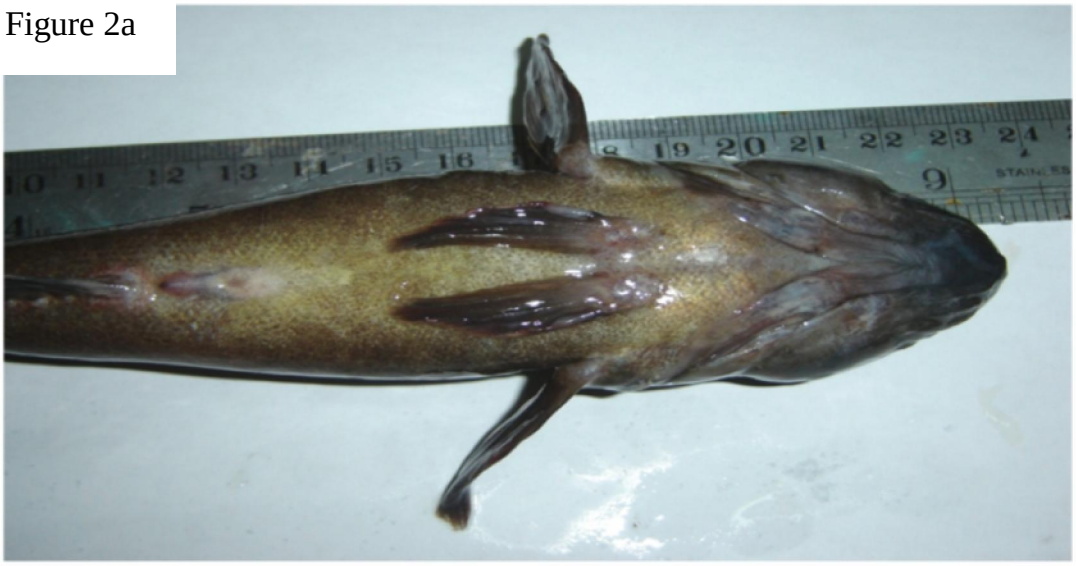

Figure 2b

Figure 2a and b: Ventral and Dorsal view of *Bathygobius soporator* from Lagos lagoon, Nigeria.

3.2 Age and growth: A total of 500 specimens of *B. soporator* were caught from the Lagos lagoon, with a set of barrier traps between March 2010 and August 2010. They were preserved in 10% formalin for laboratory analysis. Data on the sex, total length and body weight measurements of the specimens were recorded in the laboratory. The growth studies were based on the analysis of length frequency data of Petersen method and that of von Bertalanffy (Bagenal, 1968; Pauly, 1979; 1980).

The von Bertalanffy growth formula was expressed as:

$$L_t = L\infty \{1 - e^{k(t-t_0)}\}$$

Where : L_t = *length-at-age t*

$L\infty$ = *length the fish would reach, if they were to grow to a very old age.*

t_o = *the age the fish would have had at length zero, if they have always grown according to the equation.*

K = *growth coefficient.*

3.3 Lengths-Weight Relationships: The length weight relationship also known as growth index has been widely used in fish biology with several purposes like estimating the mean weight of fish, based on known (Beyer, 1987). It is also used in the conversion of length equation in weight, morphometric; inter specific and intra population comparison to assess the index of well being of fish population (Bolger and Conolly, 1989). The length-weight relationships were obtained and the linear regression analysis was determined.

The intercept (a) was expressed as:
$$a = \{\sum Y/n - (b.\ \sum X/n)\}.$$

The slope (b) as:
$$b = \frac{\sum XY - (\sum X)(\sum Y)/n}{\sum X^2 - (\sum X)^2/n}$$

And the correlation coefficient (r) as:
$$r = \frac{[\sum XY - (\sum X)(\sum Y)/n]^2}{[\sum X^2 - (\sum X)^2/n]\ [\sum Y^2 - (\sum Y)^2/n]}$$

Where: X = lengths of fish (mm), Y = weight of fish (g), n = number of specimen

The length-weight relationships for males and female specimens were obtained and a scattered diagram was drawn to determine the statistical relationships. The relationships were expressed as:

$$W = a + b.L$$

The same data was converted to Logarithms and a straight line graph was drawn and the relative slope (b) was obtained from the relationship.

$$\mathbf{Log_{10}\ W = a + b\ log_{10} L}$$

Condition Factor (k): Fulton's condition factor (k) obtained for both sexes was expressed as:

$$K = 100W/L^3$$

Where W = Weight of the fish (g), L = Length of the fish)

The condition factor (k) was used to compare the condition, "well-being" of both sexes.

4. RESULTS

4.1 Length - weight relationship: Results obtained for Length – weight relationships in the linear regression analysis of *Bathygobius soporator* are shown in the relationship for both sexes below in table 1.

Table 1: Length - weight relationship of *Bathygobius soporator*

Sex	Normal data	Logarithm transformation
Combined sex	W = -28.36 + 4.41L (n=500; r=15.18)	W = -0.93 + 2.09L (1.17)
Male	W = -31.60 + 4.58L (14.90)	W = -0.66 + 1.85L (1.16)
Female	W = -22.67 + 3.99L (15.61)	W = -0.73 + 1.91L (1.18)

The value of b (slope) was 4.41 for combined sex which indicates that the fish becomes heavier for its length as it increases in size (figure 1). The correlation coefficient 'r' obtained was 0.66 and 'a' (intercept) obtained from equation (2) was -28.36. Positive value obtained for the intercept (r) shows that there is an increase in the body weight with increasing lengths. While the slope (b) computed from the logarithm transformation, though indicated allometrism but shows the fact that the specie is feebly depressed and mostly of small sizes.

4.2 Length - Frequency Analysis: The length – frequency based on the specimens of *Bathygobius soporator* obtained from Lagos lagoon is shown in the figure 3. (*Where* n=500): It is clearly show that the highest percentage frequency of occurrence of 36% comes from the length class (11–17) cm which indicate that these length class is predominant in catch during the period of study.

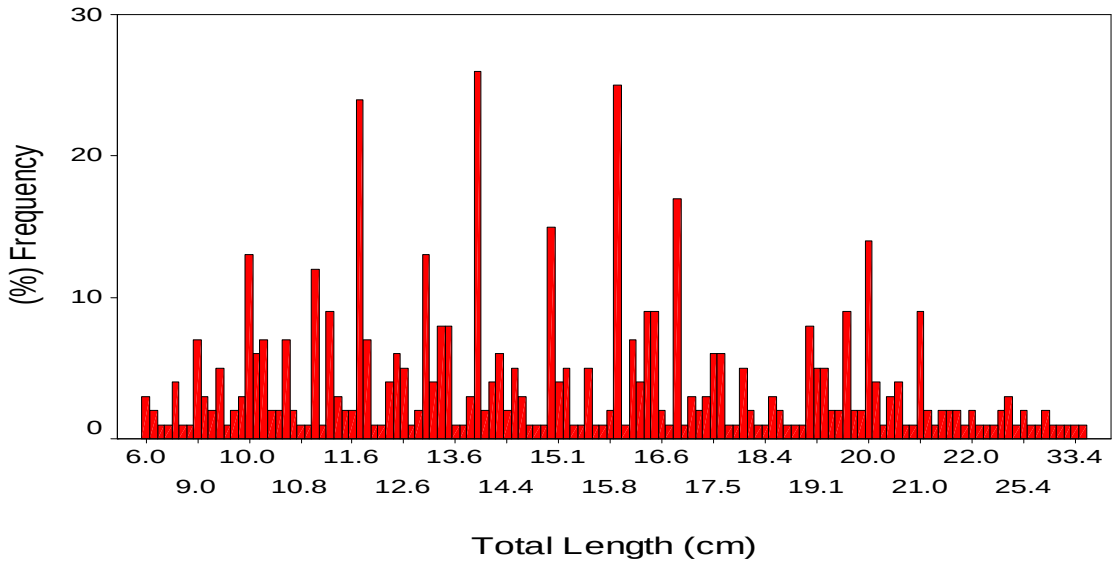

Figure 3: Length-Frequency Distribution of B. soporator in Lagos lagoon.

4.3 Meristic and morphometric: All the specimens meristic and morphometric characters were summarized in table 2. Values of correlation coefficient were very low indicating low meristic features. All the fins of *B. soporator* were not supported with rays. The relationship studied for the meristic characters and body length (SL), showed a positive relationship, however rather low in all the features. Morphometric characters are presented in table 2. The relationship analyzed are the morphometric character and standard length (SL), the head length (HL) as well as the mean values. The most stable morphometric were the Anterior Dorsal Fin and the Pelvic Fin to the body shape. However the morphometric relations showed a tendency toward allometrism. Characterization of growth of body parts using the calculated slope (b) value from the regression equation indicated that only the head length had slope (b) value 16.28 (table 3) and 4.65 for body weight (table 3) indicating a positive allometric growth with the standard length, while other parts had (b) value of less than 3 indicating a negative allometric growth.

5. DISCUSSION

As derived from the linear regression analysis, the slope (b) indicated that the body of *Bathygobius soporator* is feebly compressed (b= 4.41). The correlation coefficient (r =14.90) shows that there is an increase in body weights with lengths; as indicated in the positive value of (r). The significant difference recorded between L_∞ and L_{max} are most probably related to the fact that several relatively large specimen were included in the samples analyzed, these fact however coincide with the result reported for *Pseudotolithus elongates* by Haimonvia *et al. (2000).* A scrutiny of the evaluated length-frequency data and length-weight relationship reveals that the growth of the fish specie is allometric. Specimen of *B. soporator* shows variation in sizes, from 6.0cm and 34. 1cm (mean = 14.28 $\pm$ 4.21 TL) and 8.00 – 135g (BW) for males, while females were between 6.2cm and 32.5cm (mean = 15.61 $\pm$ 4.38TL) and 8.00 - 135g (BW). Males were of bigger sizes than females, attaining early growth and maturity than the females. The growth of males (b = 4.58) shows positive allometrism indicating that the rate of increase in body weight is relatively higher than increase in body length, while b=3.98 for females indicates that fish becomes heavier for its length as it increases in size. Positive correlation of r= 0.90 for male and 0.97 for females indicate a strong relationship between the total length and body weight for both male and female in Lagos lagoon. The fish of length class (14 – 17cm) occurred most in May and June within study period, when they are fully matured and are ready to spawn. The length of the body parts increased with the standard length and body weight of fish. The mean head length was larger in males than females but the difference observed in both sexes could not be used as distinguishing character because the difference was not significant; statistically. Similar observation has been made in *Sarotherodon galilaeus* by Nzeh, (1994). The non-significant differences in the head length of the two sexes thus suggest that the populations are homogenous.

Table 2: Summary of Meristic and Morphometric features of *Bathygobius soporator Lagos* lagoon [n=500].

Parameters	**Mean**	**Range**	**Variance**	**Standard deviation.**	**Standard Error**
Total length (cm)	15.21	34.10 – 6.00	18.32	4.28	0.19
Standard length (cm)	12.61	31.00 – 3.00	16.71	4.09	0.18
Body weight (g)	38.61	135.00 – 6.47	570.45	23.88	1.06
Head length (cm)	2.87	5.50 – 1.00	1.00	1.00	0.04
Eye diameter (cm)	0.23	0.60 - 0.10	0.01	1.00	0.004
Body depth (cm)	2.46	4.50 – 1.00	0.47	0.68	0.03
Anterior dorsal fins	VI	VI	0	0	0.012
Posterior dorsal fins	IX	VIII - XI	0	0	0.035
Anal fins	X	VIII - XI	0	0	0.033
Caudal fins	XV	XIII - XXI	0	0	0.041
Pectoral fins	XV	XII - XVII	0	0	0.044
Pelvic fins	V	V	0	0	0.0
Body width (cm)	2.26	4.70 – 1.00	0.54	0.73	0.032
Stomach weight (g)	1.49	7.60 – 0.11	0.98	0.99	0.0

Table 3: Mean values of morphometric characters of *Bathygobius soporator* examined, expressed as proportion of body length (T.L.) in the length classes.

Characters	Length Class (cm)							
	6.00-9.99	10.00-13.99	14.00-17.99	18.00-21.99	22.00-25.99	26.00-29.99	30.00-33.99	34.00-37.99
Total length (TL)	7.88	11.79	15.70	19.99	24.46	26.97	32.13	34.10
Standard length (SL)	5.94	9.63	12.87	16.83	21.24	24.00	29.23	31.00
Head length (HL)	1.69	2.27	42.74	3.51	3.72	4.33	4.50	3.50
Eye diameter (ED)	0.15	0.18	3.18	0.29	0.32	0.33	0.36	0.30
Body depth (BD)	1.50	2.11	0.24	3.03	3.06	3.33	3.00	4.00
Anterior dorsal fins (ADF)	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Posterior dorsal fins (PDF)	8.76	9.54	6.01	9.63	9.89	9.33	8.00	9.00
Anal fins (AF)	9.12	9.58	9.48	9.60	9.56	9.66	9.00	10.00
Caudal fins (CF)	14.88	14.95	9.56	15.00	14.89	15.33	15.33	16.00
Pectoral fins (PF)	14.35	14.70	15.12	15.05	14.44	15.00	14.66	15.00
Pelvic fins(PEF)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Body width (BW)	1.38	1.75	2.40	2.85	3.36	3.50	2.80	4.00
Frequency of occurrence	25	151	185	117	18	5	3	1
% Frequency of occurrence	5	30.2	36	23.4	3.6	1	0.6	0.2

Table 4: Characterization of growth of body parts in *B. Soporator* in relation to weight using Linear Regression quations.

Body parts	**a**	**b**	**r**	**Type of growth**
Standard length (SL)	y = 7.35	+ 0.14 x	0.79	Allometry
Head length (HL)	y = -9.75	+ 16.82 x	0.70	Allometry
Eye diameter (ED)	y = 0.15	+ 0.0018 x	0.54	Allometry
Body depth (BD)	y = 1.73	+ 0.019 x	0.66	Allometry
Anterior dorsal fins (ADF)	y = 6.00	+ 0.0006 x	0.05	Allometry
Posterior dorsal fins (PDF)	y = 9.43	+ 0.0016 x	0.049	Allometry
Anal fins (AF)	y = 9.48	+ 0.0018 x	0.059	Allometry
Caudal fins (CF)	y = 14.92	+ 0.0027 x	0.069	Allometry
Pectoral fins (PF)	y = 14.51	+ 0.0086 x	0.21	Allometry
Body width (BW)	y = 1.45	+ 0.021 x	0.68	Allometry

Table 5: Summary of Food items in the stomach of 500 specimens of *B. soporator* from Lagos lagoon.

Food items	**Specific name of items**	**Numerical method** Number	%	**Frequency Method** Number	%
Fish-fry	*Gopy spp.*	160	31.8	269	72.31
Worms	*Ascaris sp.*	148	29.4	380	102.15
Shrimps	*Penaeus notialis*	38	7.60	85	22.85
Crabs	*Calinectes sp.*	74	14.70	121	32.53
Bulinous	*Bulinous sp.*	83	16.50	138	37.10
Total		**503**	**100**	**344**	**266.93**

The fish exhibit positive allometric growth especially in the body weight as reflected by the b value (4.65) obtained in the regression of the standard length against length of external body parts (table 3 & 4). This suggests that the fish becomes heavier for its length as it increases in size. However, the allometric growth pattern ($b < 3$) observed in respect of the other parts such as eye diameter, pectoral fin, dorsal fin, caudal fin and anal fin, indicates that as the fish stagnate in length, those other parts increases. Although the growth pattern of the body in relation to standard length was allometric. The non-significant difference in the mean condition factor of male 0.28 – 3.80 (mean 1.46 $\pm$ 0.56) and female 0.27 – 4.28 (mean 1.44 $\pm$ 0.56) showed that both sexes were in good condition. The values are similar to results (2.28 and 3.27) obtained by Sadiku and Oladimeji (1991). However females are in better condition than males. This may be due to the energy expended on milt production by the males that may likely be higher than the one expended on the production of eggs in females. Fagade and Adebisi (1997) opined that the difference may be due to fatness and gonadal development which was attributed to females in *Chrysichthys nigrodotatus* having more fat accumulation than the male.

The foods found in the stomach of *B. soporator* were Fish-fry, Worms, Shrimps, Crabs (Bulinous *spp.*). Fish frys constituted the most important food item making up 31.8% followed by Worms (29.4%) using numerical percentage analysis. While study of frequency of occurrence shows that Worms (102.15%) are the dominant food item followed by Fish frys (35%). The result of food and feeding habit in table 5 shows that fish fry are most predominant and this agrees with the findings of Adebisi (1989). One hundred and twenty-eight (25.6%) had empty stomachs. High percentage of empty stomachs (11) obtained in *P. obscura* correlates with this work (Ogunlaru *et al.*1997) and this confirm that these species are predatory and their digestion rate is rapid.

Two hundred and five (205) sex data were collected for the specimen. The distribution of the sexes shows a significant difference between the number of males and females in all the age groups. The sex ratio shows that the males are fewer than the females which could be due to the migratory pattern of the fish and sexual differences. The calculated X^2 test on the sex ratio gave a value of 16.93 (at 5% significance level). This was higher than the tabulated value of 3.84 (5% significance level). This showed that the males were fewer than the females. This finding may indicate that Lagos lagoon is unfavorable to the fish and with the current prevailing environmental conditions in the region of the lagoon.

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Correspondence to:
Adeboyejo, O. Akintade

Department of Fisheries
Lagos State University, Lagos-Nigeria
PMB 11419, Ikeja, Lagos, Nigeria

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Identification and detection of a hepatitis C virus antigen in sera of patients with hepatocellular carcinoma

El-Shahat A. Toson[1,*], Tamer E. Mosa [2] and Mohamed Maher [1]

1. Mansoura University, Chemistry Department (New Damietta), Egypt.
2. National Research Center, Cairo, Egypt
eatoson@yahoo.com

Abstract: Hepatocellular carcinoma (HCC) is a major cause of cancer death worldwide, accounting for over half a million deaths per year. Several lines of evidence indicate a strong causal association between hepatitis C virus (HCV) and HCC. The aim of the present study was to identify, purify and partially characterized one of HCV antigens in sera of HCC patients. Also, the possibility of HCV infection play a role in the development of HCC will be tested. Therefore, serum samples of 75 HCC patients and of 25 healthy individuals as a negative control were included in this study. HCV antigen was identified in these samples using western blotting and quantified using enzyme linked immunosorbent assay (ELISA). Western blot analysis showed a single immunoreactive band in sera of HCC patients infected with HCV at 27-kDa. In addition, the 27-kDa purified immunoreactive bands were eluted, mixed and characterized using various physicochemical treatments. Briefly, after such treatements the antigen was found to have protein nature. Moreover, ELISA technique was used to quantify the 27-kDa antigen. The cutoff level of ELISA above or below which the tested sera were considered positive or negative was calculated and was found to be 150 ng/L. Based on such cutoff value a total of 61 out of 75 serum samples of HCC patients were positive for HCV antigen using ELISA. However, zero out of 25 serum samples of healthy individuals were positive for HCV antigen. In addition, ELISA showed sensitivity 81% and specificity 100%. The antigen detection method showed positive predictive value 100% and negative predictive value 64%.
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Key words: HCV; Hepatocellular carcinoma ; ELIS

1. Introduction

HCC is the third deadliest and the fifth most common cancer worldwide and its mortality is almost equal to its morbidity (Thorgeirsson *et al.*, 2002). Carcinogenesis of HCC is a multi-factor, multi-step and complex process, which is associated with a background of chronic and persistent infection of HCV and hepatitis B virus (HBV) (Yu *et al.*, 2003). Their infections along with alcohol and aflatoxin B1 intake are widely recognized as etiological agents in HCC (Tang, 2001). However, the underlying mechanisms that lead to malignant transformation of infected cells remain unclear. Therefore, early detection of cancer offers the best chance for cure; this is why regular screening for HCC is recommended (Thorgeirsson *et al.*, 2002). The global prevalence of HCV infection is approximately 3% (170 million people). Chronic HCV infection progresses at a variable rate to cirrhosis in 15 to 20% of patients, who then have a 1 to 4% annual risk of developing HCC (Lauer *et al.*, 2001). For these reasons the early diagnosis of HCV infection is crucial to prevent further transmission in high-risk groups and to allow for a rapid decision about its treatment (Gerlach *et al.*, 2003). Antibody tests are unable to identify subjects in the early stage of infection, during which specific antibodies have not yet been produced, but the virus is present in the plasma, sometimes in large quantities (van der Poel *et al.*, 1994). For HCV-RNA molecular tests, several generations of qualitative and/or quantitative HCV nucleic acid amplification technology (NAT) assays have been in use. All of such molecular tests are expensive. In addition, the identification and detection of native antigens may prove very useful tool in the diagnosis of the acute phase of infection by HCV as well as during re-infections and could pave the way for early treatment and consequently effective control of the disease (Muller-Breitkreutz *et al.*, 1999; Poljak *et al.*, 1997). Therefore, the aim of the present study was to identify, purify and characterize one of HCV antigens in sera of HCC patients to illustrate if HCV infection is associated with development of HCC or not.

Materials and Methods

Serum samples

Serum samples were obtained from 75 HCV-infected Egyptian patients (56 males and 19 females, aged 39 to 80 years, mean age 59.7 ± 9.5) at the Internal Medicine University Hospital, Mansoura University, Mansoura, Egypt. In addition, sera of 25 healthy volunteers (17 male and 8 female) were used as negative controls. All sera were stored at −20 ◦C until used. All patients were selected based on the pathological finding of thier previously taken liver biopsies which were positive for HCC. The HCV

infection was diagnosed based on biochemical, serologic and histologic criteria. Such criteria include negative tests for hepatitis B virus (HBV; HBsAg, HBeAg, anti-HBe, anti-HBc, and anti-HBs), a positive test for anti-HCV antibody (Ortho HCV EIA: Ortho Diagnostics, Raritan, USA), HCV serotype IV (MUREX HCV SEROTYPING 1–6 assay, Abbott Diagnostics), and HCV-RNA level greater than 2000 copies per milliliter on PCR analysis (Cobas Amplicor HCV Monitor [version 2.0], Roche Diagnostics, Branchburg, USA).

An informed consent was obtained from all patients participated in the present study and they were fully informed concerning the diagnostic procedures involved and the nature of the disease. The present study was approved by the ethical committee of the Internal Medicine University Hospital, Mansoura University, Mansoura, Egypt.

Polyacrylamide gel electrophoresis and gel electroelution

25µg/well of each serum sample were mixed (v/v) with sample buffer containing 0.125M tris base, 4% (w/v) sodium dodecyl sulphate (SDS), 20% (v/v) glycerol, 10% (v/v) mercaptoethanol and 0.1% (w/v) bromophenol blue. The mixture was loaded into 10% vertical slab SDS-PAGE according to the method of Laemmli (Laemmli, 1970). A mixture of reference proteins (BioRad Laboratories, CA) was run in parallel. Gels were then stained with Coomassie blue.

Western immunoblotting

The serum samples which were separated by SDS-PAGE were transferred from the polyacrylamide gel to nitrocellulose (NC) sheet according to the method of Towbin (Towbin *et al.*, 1979). The NC membrane was blocked using 5% (w/v) non-fat dry milk in 0.05 M Tris-buffered saline (TBS) containing 200 mM NaCl (pH 7.4). The NC paper was rinsed in TBS and incubated with anti-HCV IgG antibodies (ABC Diagnostics, New Damietta, Egypt) which raised against the purified HCV antigen with constant shaking (Attallah *et al.*, 2003b). The NC membrane was washed 3 times (30 min each) in TBS, followed by incubation for 2 h with goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma) diluted 1: 500 in TBS. After washing 3 more times with TBS (15 min each), the NC membrane was soaked in premixed NBT/BCIP substrate (ABC Diagnostics). The color was observed within 10 min, and the reaction was stopped by dipping the NC membrane in distilled water.

Purification and elution of HCV antigen

The target HCV antigen band (27-kDa) was cut and electroeluted from preparative polyacrylamide gels at 200 volts for 3 h in a dialysis bag according to Attallah et al. (Attallah *et al.*, 2003a). After dialysis, HCV antigen was concentrated using polyethylene glycol and 40% trichloroacetic acid, then centrifuged at 10,000 rpm for 15 min. The precipitate was washed twice using diethyl ether. The excess diethyl ether was removed by gentle drying and the pellet was reconstituted in phosphate buffered saline (PBS, pH 7.2). The protein content of the purified HCV antigen was determined according to Lowry et al. (Lowry *et al.*, 1951) and stored at −20 ◦C.

Detection of HCV antigen using ELISA:

After optimization of the reaction condition, polystyrene microtiter plate was coated with 50 µl/well of serum sample diluted 1:250 in coating buffer (pH 9.6) and was incubated overnight at 4 ºC. After washing, 50 µl/well of 1:50 diluted specific anti-HCV IgG antibody (ABC Diagnostics) in PBS-Tween 20 (PBS-T20) were added and incubated at 37 ºC for 2 h. After washing, 50 µl/well of anti-rabbit IgG alkaline phosphatase conjugat (Sigma) diluted 1:350 in 0.2% (w/v) BSA in PBS-T20, were added, and incubated for 1 h at 37 ºC. The amount of coupled conjugate was determined by incubation with p-nitrophenyl phosphate substrate (Sigma). After that the reaction was stopped by adding NaOH and the absorbance was read at 490 nm using ∑960 microplate autoreader (Metreiteck, Germany). Serial purified HCV antigen were used to establish a standard curve for quantitative determination of HCV antigen in serum. Cut-off level of ELISA concentration (150 ng/ml) above or below which the tested sample is considered positive or negative was calculated as the mean concentration of 16 serum samples from healthy volunteers ± 3 (S.D.)

Biochemical characteristics HCV antigen

The purity of the HCV antigen from serum samples was assessed using SDS-PAGE techniques. To characterize the HCV antigen, the antigen was treated with protolytic enzymes or several other chemical reagents and then its reactivity was retested using ELISA. The periodate oxidation was carried out overnight with 20 mM sodium meta-periodate at RT and the reaction was then inhibited by adding an equal volume of 130 mM glycerol. The purified HCV antigen (at 50 µg/ml) was mixed with an equal volume of 20, 60, or 180 mM β-Mercaptoethanol. In the test with protolytic enzymes, the purified antigen (0.1 mg/ml) was incubated at 37 °C with α-chymotrypsin (0.1 mg/ml; Sigma) for 5, 10, 15, 30 or 45 min. Also, the serum samples from healthy individuals were tested in parallel as controls.

Statistical analysis

All statistical analyses were done by a statistical software package (SPSS 15.0 for Microsoft Windows, SPSS Inc.). Descriptive results were expressed as mean ± SD and range or number (percentage) of patients with

a condition. Differences in continuous variables were assessed using student *t-test* or ANOVA and X^2 test for categorical variables. The statistical significance (two-tailed) was assessed at the 0.05 level for the all tests. The diagnostic sensitivity, specificity, efficiency, and positive predictive values (PPV) and negative predictive (NPV) values were also calculated.

Results

Identification of a HCV antigen in HCC serum samples

***SDS-PAGE*:**

Figure 1 showed the Coomassie Brilliant blue stained SDS-PAGE of 3 serum samples of HCC patients infected with HCV (lanes 1-3), versus those of 3 healthy non-infected controls (lanes 4-6). The resolved bands were identified only in serum samples after staining the gel by Coomassie Brilliant Blue R-250 dye.

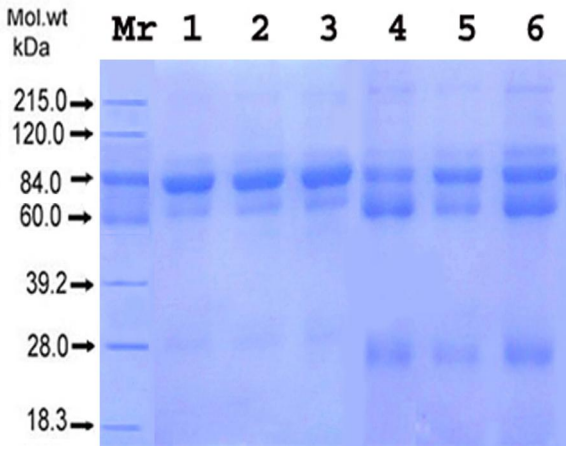

Fig. 1: Coomassie Brilliant blue stained SDS-PAGE showing the polypeptide pattern of serum samples of HCC patients. Lanes (1-3): 3 Serum samples of healthy individuals. Lanes (4-6): 3 HCC serum samples and molecular weight marker (Mr.) which includes: Myosin (215.0 kDa), phosphorylase B, (120.0 kDa), Bovine serum albumin (84.0 kDa), Ovalbumin (60.0 kDa), carbonic anhydrase (39.2 kDa), trypsin inhibitor (28.0 kDa), and lysozyme (18.3 kDa).

Isolation and purification of HCV antigen by SDS-PAGE:

After immunoblotting and electroelution of the target 27-kDa HCV antigen from sera of HCC patients, the antigen was precipitated by trichloroacetic acid (TCA) and both precipitate and supernatant were tested using 16% SDS-PAGE for the presence of the target antigen or not. Figure 3 showed that the 27-kDa was found only in the precipitate indicating that the antigen is a protein.

Detection of HCV in serum using ELISA technique

Quantitation of HCV antigen in unknown samples was performed using ELISA technique. As shown in table 1 a total of 61 out of 75 HCC serum samples (81 %) were positive for the target 27-kDa HCV antigen using ELISA. However, zero out of 25 serum samples (0 %) of the healthy individuals were positive for HCV antigen. Based on the previous data, the detection rate of the target HCV antigen is highly significantly ($p<0.0001$) differ than that of the healthy control group. Based on the data of table 1 the sensitivity, specificity, efficiency, positive predictive value and negative predictive value were calculated to be 81%, 100%, 86%, 100% and 64% respectively. These results indicated that the ELISA technique based on the detection of the target 27-kDa is sensitive and highly specific for detection of HCV infection.

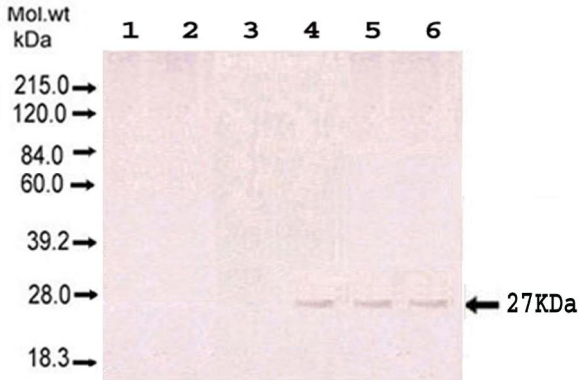

Fig. 2: Immunoblots of mono-specific antibody in serum samples of HCC patients. Lanes (1-3): 3 Serum samples of healthy individuals, Lanes (4-6): 3 HCC serum samples.

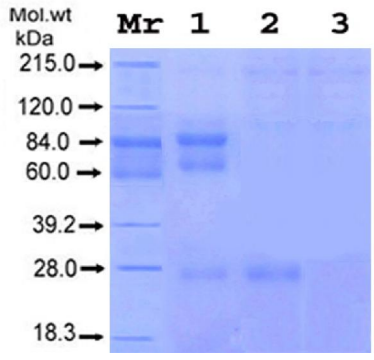

Fig. 3: Coomassie blue stain of 16% polyacrylemide gel under reducing condition in presence of SDS. Lane 1: Serum sample from HCC patient, lane 2: The trichloro acetic acid (TCA) precipitates of the purified fraction from serum samples of HCC patients. Lane 3: The TCA supernatant of the purified fraction from serum samples of HCC patients.

Table 1: Detection rate of HCV target antigen in sera of HCC patients and healthy individuals using ELISA technique.

Group	No	HCV antigen		% of positivity	*P* value	Sens.	Spec.	PPV	NPV	Effe.
		Positive	Negative							
Patients *(HCC/HCV)*	75	61	14	81	< 0.0001	81%	100%	100%	64%	86%
Control	25	0	25	0						

Sens: Sensitivity, Spec.: Specificity, PPV: Positive predictive value, NPV: Negative predictive value and Effe.: Efficiency.

Partial characterization of 27-kDa HCV antigen from HCC samples

Effect of heat:

Figure 4 A show the reactivity of the target HCV antigen using ELISA was lost starting from 56 ºC.

Effect of Periodate:

Figure 4 B illustrate the pattern of reactivity of the target antigen with its specific antibody after periodate treatement. The specific anti-HCV antibody still show high reactivity towards the target HCV serum antigen after treatement with periodate.

Effect of β Mercapto-Ethanol:

The reactivity of specific anti-HCV antibody towards mercaptoethanol (Zero, 20, 60 & 180 mM) (Figure 4 C). As shown from such figure the antigen reactivity was lost above 60 mM β-Mercaptoethanol treatment after 1hr. the purified HCV antigen from HCC serum samples after addition of different concentrations of β–

Effect of Proteolysis:

The specific anti-HCV antibody showed high reactivity at 15 and 30 minutes after antigen treatment with α-chymotrypsin, while it was lost after antigen treatment with α-chymotrypsin for 45 minutes (**Figure 4 D**).

Effect of 0.2 M HCl and 0.2 M NaOH :

The reactivity of the target HCV antigen was lost after acid or alkali treatment. i.e. the concentration value of HCV antigen was lower than that of the cut-off value which is 150 ng /L (Data not shown).

DISCUSSION

The identification and detection of native antigens may prove very useful in the diagnosis of acute phase of infection by HCV as well as during reinfections and could pave the way for the disease. Monoclonal antibodies (MAb) were used for the identification of antigenic determinants of native non structural protein (NS4) (Brody *et al.*, 1998). However, some MAb and polyclonal antibodies to recombinant proteins and synthetic HCV peptides do not react with native viral antigens (Masalova *et al.*, 2002). A highly specific antibody to recombinant HCV-NS4 protein was generated and the target antigen was detected in sera from patients with chronic HCV using a simple and rapid dot-enzyme immunoassay with high degrees of sensitivity and specificity (Attallah *et al.*, 2003b). In the present study, HCV antigen was identified in sera from HCC patients at 27-kDa molecular weight which contains several epitopes of both linear and conformation-dependent nature. The molecular weight (27-kDa) of the target HCV native antigen is similar to that of the HCV-NS4B protein (Konan *et al.*, 2003). Zheng (Zheng *et al.*, 2005) found that the expression profile of HeLa cells which are stably transfected by HCV non-structural protein 4B was identified at a molecular weight of 27-kDa by using an immunoblot analysis technique based on he use specific monoclonal antibody. I general, the NS viral sequences may represent valuable immunogens for the preparation of therapeutic or prophylactic vaccines (Leroux-Roels, 2005).

Tabll (Tabll *et al.*, 2008) established hybridoma cells secreting monoclonal antibodies against E1 synthetic peptide of HCV. BALB/c mice were immunized with HCV E1-synthetic peptide (GHRMAWDMM) and its pleenocytes were fused with the P3NS1 myeloma cell line. Two highly reactive and specific mAbs (10 C7 IgG2b mAb, and 10B2 IgG1 mAb) were generated. The target HCV E1 antigen was identified at approximately 38 kDa in serum of infected individuals. **El Awady** (El Awady *et al.*, 2006) used Western blot to demonstrate the presence of the core and E1 target antigen in serum samples. Western blot analysis based on monospecific antibodies against core and E1 recognized the 38-kDa and 88-kDa bands respectively in the sera of all infected patients. No specific reaction was observed with the sera from uninfected individuals.

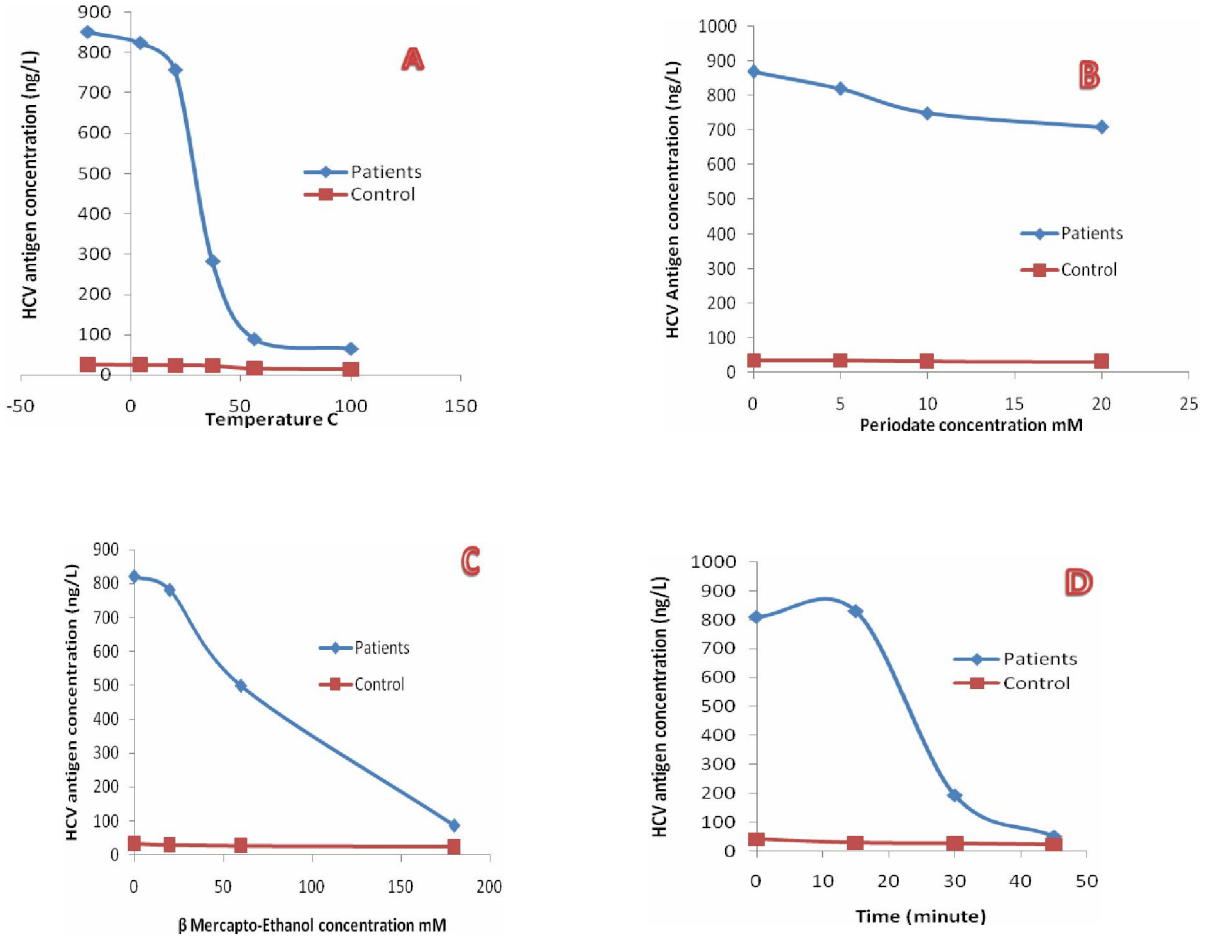

Fig. 4 Partial characteristics of HCV antigen. A: Effect of temperature, B: Effect of periodate concentration, C: Effect of β Mercapto-Ethanol and D: Effect of incubation time with α chemotrypsin.

In the present study, the purified HCV antigen from sera of HCC patients was analyzed by 16% SDS-PAGE and stained with Coomassie blue stain. The results showed that a polypeptide chain at 27-kDa was bound only in serum samples from HCC patients. In addition, enzyme linked immunosorbent assay (ELISA) format based on antisera to the purified 27-kDa HCV antigen was used for the detection of the native serum antigen in sera of Egyptian patients. Based on the a total of 61 out of 75 serum samples (81 %) of HCC patients were positive for the target HCV antigen.

However, zero out of 25 serum samples (0 %) of healthy individuals were positive for HCV antigen. These results indicate that HCV infection may be one of the major factors participating in HCC development (Anzola, 2004).

Antibody tests fail to identify HCV infected subjects before seroconversion or during the window period, when specific antibodies have not yet been produced or are in low titers. However, the virus continues to replicate and RNA can be detected in the plasma using polymerase chain reaction (PCR). In the present study, HCV antigen assay was used as a tool of HCV detection. This is due to its simplicity in use compared with PCR. Also, its high sensitivity and specificity can confirm its use.

A false negative HCV antigen result was obtained in only 14 HCC patients out of 75 patients in this study. This is may be due to the reduction of hepatocytes in the end stages of livers diseases (HCC or hepatic-cirrhosis) phenomena which is associated with lower HCV replication rates than the patients with less severe liver disease. Also, this is may be due to the role of fibrosis which became severe at the end stage of liver damage which participates in cell-to cell virus transmission.

A more detailed structural analysis of native HCV particles from infected hosts requires higher titres of virus. Unfortunately, low titre of HCV antigen will be found in serum, therefore characterization of native virus particles has been difficult. In the present study, the specific HCV antigen band (27-KDa) was identified. Using preparative gel of SDS-PAGE a large amount of the target HCV antigen was obtained, eluted and characterized using biochemical techniques which were previously illustrated in subjects and methods. The results of the characterization study showed that the reactivity of the serum HCV antigen was lost after exposure to 56 ºC or more. Also, it was lost after acid, alkali and β-mercaptoethanol treatment. On the other hand, it was maintained after periodate. Also, the HCV antigen which was treated with constant concentration of α-chymotrypsin enzyme for 15, 30, 45, and 60 minutes showed a decease in reactivity with the increase in the incubation time with the enzyme. Also the reactivity was completely lost after 60 minutes of incubation indicating a complete digestion of the simple protein antigen. In conclusion, HCV antigen was detected in serum samples of 81% of the patients which were previously infected with HCV using ELISA indicating that HCV may be one of the major factors leading to HCC development. Also, the target antigen was characterized and was found to have simple protein structure with a molecular weight of 27- KDa.

Correspondence to:
Dr/ El-Shahat A. Tosom
Faculty of science (Damietta)
Mansoura University, Egypy
Telephone: 002-050-2280518
Cellular phone: 002-010-3899211
Emails: eatoson@yahoo.com

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Overview on Hepatitis B virus

Engy Yousry Elsayed Ashor

Department of Internal Medicine, Ain Shams University, Cairo, Egypt

Abstract: Hepatitis B is an infectious illness caused by hepatitis B virus (HBV). The disease has caused epidemics in parts of Asia and Africa, and it is endemic in China. About a third of the world's population, more than 2 billion people have been infected with thehepatitis B virus. This includes 350 million chronic carriers of the virus. This paper offers an overview of hepatitis B virus.
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Keywords: Hepatitis B; infectious illness; epidemics; virus

Intoduction:

Hepatitis B is an infectious illness caused by hepatitis B virus (HBV).[1] The disease has caused epidemics in parts of Asia and Africa, and it is endemic in China.[2] About a third of the world's population, more than 2 billion people, have been infected with thehepatitis B virus.[3] This includes 350 million chronic carriers of the virus.[4]

Signs and symptoms: Acute infection with hepatitis B virus is associated with acute viral hepatitis – an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, itching, dark urine, and then progresses to development of jaundice. The illness lasts for a few weeks and then gradually improves in most affected people. A few patients may have more severe liver disease (fulminant hepatic failure), and may die as a result of it. The infection may be entirely asymptomatic and may go unrecognized. [5]Chronic infection with hepatitis B virus may be either asymptomatic or may be associated with chronic hepatitis, leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (2-5% after development of cirrhosis) . Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of Membranous glomerulonephritis.[6]

Transmission: Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include unprotected sexual contact, blood transfusions, re-use of contaminated needles & syringes, and vertical transmission from mother to child during childbirth. Without intervention, a mother who is positive for HBsAg confers a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also positive for HBeAg. HBV can be transmitted between family members within households, possibly by contact of nonintact skin or mucous membrane with secretions or saliva containing HBV.[7] However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor.[8]

The life cycle of hepatitis B virus is complex. Hepatitis B is one of a few known non-retroviral viruses which use reverse transcription as a part of its replication process. The virus gains entry into the cell by binding to an unknown receptor on the surface of the cell and enters it by endocytosis. Because the virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called chaperones. The partially double stranded viral DNA is then made fully double stranded and transformed into covalently closed circular DNA (cccDNA) that serves as a template for transcription of four viral mRNAs. The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and re-cycled to produce even more copies. The long mRNA is then transported back to the cytoplasm where the virion P protein synthesizes DNA via its reverse transcriptase activity. [9]

Serotypes and genotypes: The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight genotypes (A-H) according to overall nucleotide sequence variation of the genome. [10] Most genotypes are now divided into subgenotypes with distinct properties.[11] The genotypes have a distinct

geographical distribution and are used in tracing the evolution and transmission of the virus. Genotype A is associated with better response to interferon. Disease progression appears to be slower in genotype B and C.[10]

The hepatitis B surface antigen (*HBsAg*) is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or *HBcAg*. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies to the hepatitis B core antigen (*anti-HBc IgM*) may be the only serological evidence of disease .[12]

Shortly after the appearance of the HBsAg, another antigen named as the hepatitis B e antigen (*HBeAg*) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true. During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (*anti-HBe*) will arise immediately afterwards. This conversion is usually associated with a dramatic decline in viral replication. If the host is able to clear the infection, eventually the HBsAg will become undetectable and will be followed by IgG antibodies to the hepatitis B surface antigen and core antigen, (*anti-HBs* and *anti HBc IgG*). A person negative for HBsAg but positive for anti-HBs has either cleared an infection or has been vaccinated previously.[12]

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers.[13] Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase levels and inflammation of the liver, as revealed by biopsy. Carriers who have seroconverted to HBeAg negative status, particularly those who acquired the infection as adults, have very little viral multiplication and hence may be at little risk of long-term complications or of transmitting infection to others.[14] PCR tests have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment.[15] At least two laboratory tests over 12 months is fundament to establish a correct diagnosis and the indication for treatment, liver biopsy is recommended either when liver enzymes are abnormal or HBV DNA > 2000 Iu/ml(10000 copies/ml).[16]

Table 1. Chronic hepatitis B is a dynamic infection with five major phases: [16]

	HBs Ag	HB DNA	Liver enzymes	Histology
Immune tolerant	+ ve	High level	Normal	Mild or no necroinflammation
Immune reactive	+ ve	Low level	Increased and or fluctuating	Moderate to severe necroinflammation
Carrier	+ve	Very low level	Normal	Mild or nonecroinflammation
HB E Ag –ve hepatitis	+ve	Fluctuating	Increased often fluctuating	Moderate to severe necroinflammation
HBS Ag –ve	-ve	- ve	Normal	No necroinflammation

All pregnant women, persons need immunosuppressive therapy, persons undergoing renal dialysis, HCV patients, persons with elevated liver enzymes, with multiple sexual partner or history of sexually transmitted diseases, addicts, health care worker. Testing for HBsAg and anti HBs should be performed, and seronegative persons should be vaccinated [16]

Prevention: Hepatitis B is transmitted through body fluids; prevention is thus the avoidance of such transmission: unprotected sexual contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission during child birth. Infants may be vaccinated at birth. Several vaccines have been developed by Maurice Hilleman for the prevention of hepatitis B virus infection. These rely on the use of one of the viral envelope proteins (hepatitis B surface antigen or HBsAg). The vaccine was originally prepared from plasma obtained from patients who had long-standing hepatitis B virus infection.

However, currently, it is made using a synthetic recombinant DNA technology that does not contain blood products. One cannot be infected with hepatitis B from this vaccine.[17] Following vaccination, hepatitis B surface antigen may be detected in serum for several days; this is known as vaccine antigenaemia.[18] The vaccine is administered in either two-, three-, or four-dose schedules into infants and adults, which provides protection for 85–90% of individuals.[19] postvaccination testing should be performed at 9-15 months of age in infants of carrier mothers and 1-2 months after the last dose in other persons. Protection has been observed to last 12 years in individuals who show adequate initial response to the primary course of vaccinations, and that immunity is predicted to last at least 25 years.[20]

Shi, et al showed that besides the WHO recommended joint immunoprophylaxis starting from the newborn, multiple injections of small doses of hepatitis B immune globulin (HBIg, 200–400 IU per month), or oral lamivudine (100 mg per day) in HBV carrier mothers with a high degree of infectiousness ($>10^6$ copies/ml) in late pregnancy (the last three months of pregnancy),[21] effectively and safely prevent HBV intrauterine transmission, which provide new insight into prevention of HBV at the earliest stage.

Treatment: Acute hepatitis B infection does not usually require treatment because most adults clear the infection spontaneously.[22] Early antiviral treatment may only be required in fewer than 1% of patients, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised. [23]

Indication of treatment in chronic HBV infection

A: Combination of two of the following:

1: Increased liver enzymes.

2: HBV DNA >2000 Iu/ml or 10000 copies/ml.

2: Liver biopsy shows moderate to severe necroinfalmmation ≥ A2 and or F2.

B: Cirrhosis with and detectable HBV DNA. [16]

Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. Currently, there are seven medications licensed for treatment of hepatitis B infection: Nucleoside analogues (lamivudine, Telbivudine and Entecavir) Nucleotide analogues (Adefovir, Tenofovir and Emtricitabine) and Pegylated interferon alpha-2a or alpha 2b once weekly. [16]

A: Interferon is an antiviral agent with antiproliferative and immunomodulatory agent that is administered by subcutaneous injection once weekly. However, some individuals are much more likely to respond than others (HB eAg +ve, genotype A or B, low viraemia ($<10^7$copies/ml), ALT > 3 times ULN), high activity score in histology).[24] Response to treatment differs between the genotypes. Interferon treatment may produce an e antigen seroconversion rate of 37% in genotype A but only a 6% seroconversion in type D. Genotype B has similar seroconversion rates to type A while type C seroconverts only in 15% of cases. Sustained e antigen loss after treatment is ~45% in types A and B but only 25–30% in types C and D.[24] Side Effects: Depression, muscle aches, fatigue, and low grade fevers, low white blood cell count, headaches, irritability, and thyroid dysfunction. Underlying autoimmune disorders may also be unmasked. [16]

B) Lamivudine: Is an oral nucleoside analog inhibits hepatitis B viral DNA synthesis. It is approved for use in adults and children and is usually tolerated well. Daily dosing of 100 mg reduces serum HBV-DNA to below detectable levels within 6 weeks. In HBeAg-positive patients, approximately 16% of treated patients seroconverted with the first year. This was associated with significant improvement in liver histology. Long-term treatment induces further HBeAg seroconversion, but overall clinical benefit is undermined by continuous emergence of drug-resistant YMDD mutants. YMDD mutants may cause a flare of hepatitis, resulting in deterioration of liver histology and, occasionally, liver failure. The most common side effects seen during treatment were headache; abdominal discomfort and pain; nausea and vomiting; diarrhea; muscle pain; sore throat; joint pain; fever or chills; and skin rash. [25]

C: Adefovir: Is a nucleotide analogue inhibits DNA polymerase activity and reverse transcriptase. This drug is administered orally on a daily basis and is typically well tolerated. The most common side effects observed were weakness, headache, stomach pain and nausea. very serious hepatitis if you stop taking it, nephrotoxicity, lactic acidosis. [16]

D:Baraclude: Nucleoside analogue reverse transcriptase inhibitor. The recommended dose of

Baraclide is 0.5 mg once daily in nucleoside-naïve adults, and 1 mg once daily in lamivudine-refractory adults. Baraclude should be administered on an empty stomach (at least 2 hours after a meal and at least 2 hours before the next meal). The optimal duration of treatment with Baraclude for patients with chronic hepatitis B infection and the relationship between treatment and long-term outcomes such as cirrhosis and hepatocellular carcinoma are unknown. Dosage adjustment is recommended for patients with a creatinine clearance of less than 50 ml/min, including patients on hemodialysis or continuous ambulatory peritoneal dialysis (CAPD). Entecavir should be administered after hemodialysis. CAPD removed approximately 0.3% of the dose over 7 days. The most common adverse events of moderate to severe intensity among patients treated with Baraclude in clinical trials included: headache (4%), fatigue (3%), diarrhea (1%), and dyspepsia (1%). Therapy with Baraclude is not recommended for HIV/HBV co-infected patients who are not also receiving highly active antiretroviral therapy (HAART). Before initiating Baraclude therapy, HIV antibody testing should be offered to all patients. [16]

D: Tenofovir: Is a nucleotide analogue reverse transcriptase and hepatitis B virus (HBV) polymerase inhibitor (NRTI). [16]

Lamivudine has the lowest barrier to viral resistance followed be Telbivudine, Adefovir has an intermediate efficacy (barrier to resistance). The most potent antiviral with the lightest barrier for resistance mutation are Tenofovir and Entecavir both may be the first line for treatment of CHB and the treatment of choice whenever resistance occurs. [16]

Treatment adjustment in case of resistance to antivirals. [16]

Resistance to	Treatment adjustment
Lamivudine	Switch to Adefovir or Tenofovir
Adefovir	If N236T mutation is present add Lamivudine or Entecavir or Telbivudine or switch to Tenofovir and Emtricitabine If A181V/T mutation is present add Entecavir or switch to Tenofovir
Entecavir	Add Tenofovir
Telbivudine	Add Tenofovir
Tenofovir	Add Lamivudine or Entecavir or Emtricitabine

Treatment of especial patients groups: [16]

1: Children: The majority of children are in an immune tolerant phase and should not be treated.

2: Liver cirrhosis: All cirrhotics should be treated with any detectable viraemia.

3: Liver transplantation: All HBV patients should be treated before transplantation regardless of ALT and HBV DNA levels. HBV DNA must be undetectable at transplantation. After transplantation long term treatment combination with NUCs and anti HBs immunoglobulin (HBIG).

4: Co infection with hepatitis C: Usually one virus dominate, often HCV dominate the treatment with pegyltaed interferon and ribavirin till sustained virological response, HBV can reactivate and thus need to be closely monitored, and eventually treated with NUCs.

5: Co infection with HIV: Treatment target both viruses (Tenofovir lamivudine, Entecavir and Emtricitabine) however (Adefovir and Telbivudine) are agents without activity on HIV replication.

6: Pregnancy: Antiviral treatment is to be avoided until the third trimester of pregnancy. Lamivudine reduce the risk of intra-uterine and perinatal transmission of HBV if given in addition to HB IG and vaccination within twelve hours of birth . This treatment allows a mother to safely breastfeed her child.

7: Immuno suppressed patients: Patients who will receive chemotherapy even for short period should be screened (HB SAg, HB c Ab, HBs Ab) HBV DNA if HB SAg is + ve. Vaccination should be given if all marker are – ve. Lamivudine should be given to (HB SAg +ve) carriers before and 12 months after cessation of chemotherapy, while HB c Ab +ve with –ve S Ag carriers should be followed up twice monthly without antiviral treatment. If reactivation occurs a potent NUC should be started.

8: Patients with chronic renal failure: Lamivudine may be the safest choice, with dose adjustement, in patients with renal failure. For renal transplant recipient the best drugs may be lamivudine or entecavir.

9: Health care workers especially those involved in invasive procedures should be treated with a potent NUC, if HBV viraemia ≥ 2000 IU/ml. [16]

Prognosis: Hepatitis B virus infection may either be acute (self-limiting) or chronic (long-standing). Persons with self-limiting infection clear the infection spontaneously within weeks to months. Children are less likely than adults to clear the infection. More than 95% of people who become infected as adults or older children will stage a full recovery and develop protective immunity to the virus. However, this drops to 30% for younger children, and only 5% of newborns that acquire the infection from their mother at birth will clear the infection[26]. This population has a 40% lifetime risk of death from cirrhosis or hepatocellular carcinoma. Of those infected between the age of one to six, 70% will clear the infection.[27]

Hepatitis D (HDV) can only occur with a concomitant hepatitis B infection, because HDV uses the HBV surface antigen to form a capsid.[28] Co-infection with hepatitis D increases the risk of liver cirrhosis and liver cancer.[29] *Polyarteritis nodosa* is more common in people with hepatitis B infection.

Reactivation: Hepatitis B virus DNA persists in the body after infection and in some people the disease recurs.[30] Although rare, reactivation is seen most often in people with impaired immunity.[31] HBV goes through cycles of replication and non-replication. Approximately 50% of patients experience acute reactivation. Male patients with baseline ALT of 200 UL/L are three times more likely to develop a reactivation than patients with lower levels. Patients who undergo chemotherapy are at risk for HBV reactivation. The current view is that immunosuppressive drugs favor increased HBV replication while inhibiting cytotoxic T cell function in the liver.[32]

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Extraction of Molybdenum (VI) With 4-Adipoyl and 4-Sebacoyl Derivatives of Bis (1-Phenyl-3-Methylpyrazolone-5) In Acid Media

Kalagbor, A. Ihesinachi [1]*, Uzoukwu, B. Augustus[2] and Chukwu, U. John[2]

1. Department of Science Laboratory Technology, Rivers State Polytechnic, Bori, P.M.B. 20, Bori, Rivers State, Nigeria.
ksinachi@yahoo.com
2. Department of Pure and Industrial Chemistry, University of Port Harcourt, P.M.B 5323, Choba,Port Harcourt, Rivers State, Nigeria
uzoukwupob331@yahoo.co.uk, lydiuche@yahoo.com

Abstract: Liquid – liquid extraction of Molybdenum (VI) ions from various aqueous medium have been carried out using chloroform solution of 4-adipoylbis (1-phenyl-3-methylpyrazolone-5), H_2Adp and 4-sebacoylbis (1-phenyl-3-methylpyrazolone-5), H_2SP in acid media (HCl, H_2SO_4 and HNO_3) in the presence and absence of butanol as a synergist. The degree of extraction of Mo (VI) using H_2Adp was found to be in the range of 82 – 95% for HCl concentrations of 10^{-3}M to 10^{-1}M and 90 – 97% for HNO_3 (10^{-3}M to 10^{-1}M) while H_2SO_4 concentrations gave 70% extraction. On the other hand, the degree of extraction of Mo(VI) using H_2SP was comparatively lower in all acid media. Under all acid conditions studied, H_2Adp was found to be a better extractant for Mo(VI) than H_2SP, while optimal extraction was better in HCl followed by HNO_3 and least in H_2SO_4 concentrations. However, introduction of butanol into the organic phase resulted in enhanced extraction of Mo(VI) to above 98% using both ligands in all three acid media for both H_2Adp and H_2SP. Statistical treatment using slope analysis show that the extracted specie is $M_OO_2(SP)_{(o)}$ and $M_OO_2(Adp)_{(o)}$.

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Keywords: Liquid – liquid extraction, chloroform, Molybdenum (VI), acid media

1. Introduction

Extraction of metals using various ligands has been an on going study over the years. Hence the search for more ligands and better conditions for extraction of various metals cannot be over emphasized. In view of this, the ligands 4-adipoylbis (1-phenyl-3-methylpyrazolone-5), (H_2Adp) and 4-sebacoylbis (1-phenyl-3-methylpyrazolone-5), (H_2SP) which were first synthesized and characterized by Okafor and Uzoukwu (1991) having been found to exhibit the ability of effectively trapping toxic metals such as Fe, Cd, Cu, Ni, Mn, U, V and W from water, was considered for this study. Their extensive use in organic solutions as extractants (Bukowsky et al (1992), Uzoukwu and Okafor 1990, Uzoukwu et al 1996, 1998) has been demonstrated. They are β diketones, and like other 4-acylpyrazolones have equally proven to be efficient metal extractants forming stable complexes with some group I, II and many transition metals (Okafor, et al, 1993; Uzoukwu and Adiukwu, 1996; Chukwu and Uzoukwu, 2010). Introduction of various synergists have further improved the efficiency of these ligands in solvent extraction studies (Umetani et al 1990; Atanassova and Dukov, 2006; Bond et al, 1999). Therefore, this present work, aims at extraction of Molybdenum (VI) from concentrations of different acid media (HCl, HNO_3 and H_2SO_4) using 4-adipoylbis (1-phenyl-3-methylpyrazolone-5), H_2Adp and 4-sebacoylbis (1-phenyl-3-methylpyrazolone-5) H_2SP in chloroform. The distribution behavior of the metal ions in the organic phase containing these chelating agents in the presence and absence of butanol will also be studied.

2. Experimental

2.1 Materials

All reagents used were of analytical grade (Merck or BDH). They include HCl, HNO_3, H_2SO_4, Butanol, ammonium molybdate hydrate, Chloroform ($CHCl_3$). Deionized water was used throughout the experiment. Consort C531 pH/conductivity meter, BioBlock UV Spectrophotometer, Mechanical Shaker and extraction bottles were used.

2.2 Analytical Methods

Stock solutions of 0.01M of H_2Adp and H_2SP were prepared by dissolving the appropriate mass of the ligand (H_2Adp and H_2SP) each in Chloroform ($CHCl_3$). These solutions (H_2Adp and H_2SP) were stable for at least one

month. Stock solution (100mgL^{-}) of the metal ion Mo(VI) was equally prepared by dissolving appropriate mass of ammonium molybdate hydrate.

The aqueous phase was made up of 0.1ml aliquot of the metal ion stock solution followed by a solution of acid and deionized water to give 1ml such that the final dilution gave different concentrations of 0.001M to 5M for the acid. An equal volume (1ml) of 0.01M solution of H_2Adp / $CHCl_3$ and H_2SP / $CHCl_3$ was added respectively to make up the organic phase. This mixture was shaken mechanically for 30 minutes at room temperature. A shaking time of 30 minutes was found to be enough for equilibration. The phases were allowed to settle and then separated for analysis of the metal ion in the aqueous raffinate. Extraction in the presence of synergist was prepared using the same procedure. However, the organic phase was prepared using the extractants (H_2Adp and H_2SP) in a $CHCl_3$/BuOH mixture in the ratio 4:1.

The Mo(VI) ions in the aqueous raffinate were determined using established techniques (Jeffrey et al, 1987, Allen et al, 1974). A 0.5ml volume of the Mo(VI) extraction raffinate was introduced into 5ml sample bottle. Subsequently, 1ml of 60% HCl was added to acidify the solution, followed by 0.5ml of 20% NH_4SCN. A 0.2ml volume of freshly prepared 5% Ascorbic acid was added to form a cherry red complex. The solution mixture was made up to 5ml with deionized water. The absorbance of the cherry red complex was read at 470nm against a blank using a BioBlock UV Spectrophotometer. The amount of Mo(VI) in the aqueous solution after extraction was determined by comparing the absorbance of the solution with that of a standard calibration curve. The concentration of metal ion extracted into the organic phase was determined by difference. Distribution ratio, D, was calculated as the ratio of metal ion concentration in organic phase (C_o) to that in the aqueous phase (C) thus, $D = C_o/C$.

3. Results and discussion

3.1 Theoritical considerations

Earlier works of Uzoukwu et al (1998[a]) have shown that these ligands are weak organic acids and therefore behaved as 4-acylbispyrazolone chelating acids in solution. Thus the extraction of the metals species (MoO_2^{2+}) can be represented as follows.

$$MoO_2^{2+} + (b + c)\, H_2L_{(o)} \longrightarrow (MoO_2)L_b\ (HL)_{C(o)} + (2b + c)\, H^+ \quad \ldots\ldots (1)$$

Where H_2L is the 4-acylbispyrazolone

$$K_{ex1} = \frac{[(MoO_2)L_b(HL)_c]_{(o)}[H^+]^{(2b+c)}}{[MoO_2^{2+}]\,[H_2L]_{(o)}^{(b+c)}} \quad \ldots\ldots\ldots\ldots (2)$$

Where K_{ex1} is the extraction constant. Hence the distribution ratio D is given by:

$$\log D_1 = \log K_{ex1} + (b + c) \log [H_2L]_o - (2b + c) \log [H^+] \ldots\ldots\ldots\ldots (3)$$

In the presence of a solvating agent such as butanol (BuOH), the distribution ration becomes:

$$\log D_2 = \log K_{ex2} + (b + c) \log [H_2L]_{(o)} + d \log [BuOH]_{(o)} - (2b + c) \log[H^+] \quad \ldots\ldots\ldots\ldots (4)$$

where $(MoO_2)L_b(HL)c_{(o)}$ and $(MoO_2)L_b(HL)_c(BuOH)_{d(o)}$ represent the extractable metal complexes in the absence and presence of butanol respectively

Determination of the values of b, c, and d for interactions between the ligand and one mole of metal in the equations can be done through evaluation of the partial derivatives of the equations by the method of slope analysis as follows:

$$\frac{d\,[\log D]}{d\,[\log [H^+]\,]} = (2\,b + c) \ldots\ldots\ldots (5)$$

$$\frac{d\,[\log D]}{d\,[\log [H_2L]_o} = b + c \ldots\ldots\ldots (6)$$

$$\frac{d\,[\log D]}{d\,[\log [BuOH]_{(o)}]} = d \ldots\ldots\ldots\ldots (7)$$

$$\frac{d\,[\log D]}{d\,[\log [H^+]\,]} = (a - 1) \ldots\ldots\ldots (8)$$

If an acid is involved as a solvating agent in the extraction of the metal, the expected ion pair complex that would be extracted can be described by the following equation as proposed by Okafor and Uzoukwu (1990).

$$MoO_2^{2+} + (b+c)H_2L + xHA_{(o)} \longrightarrow (H^+)xMoO_2L_b(HL)_c.xA_{(o)}^{-} + (2b+c)H^+ \ldots\ldots\ldots (9)$$

$$K_{ex3} = \frac{[(H^+)_xMoO_2L_b\,(HL)_c.xA^-]_{(o)}\,(H^+)^{2b+c}}{[MoO_2^{2+}]\,[H_2L]_{(o)}^{b+c}[HA]^x} \ldots\ldots\ldots (10)$$

$$D_3 = \frac{[(H^+)xMoO_2L_b(Hl_c).xA^-]_{(o)}}{[MO_2^{2+}]} \ldots\ldots\ldots (11)$$

$$\log D_3 = \log K_{ex3} + (a\text{-}1) \log[M^{2+}] + (b+c) \log[H_2L]_{(o)} + e \log [HA] + (2b+c) \log H^+ \ldots\ldots (12)$$

Determination of the value of e in the equations can be done through evaluation of the partial derivative of the equation by the method of slope analysis as follows:

$$\frac{d[\log D_3]}{d[\log[HA]]} = e \quad \ldots\ldots\ldots\ldots (13)$$

Evaluation of the quantities b, c, d and e will enable the stiochiometry of the extraction process for each of the extraction systems to be elucidated.

3.2 Extraction of Mo(VI) in the presence and absence of BuOH

Presented in Fig. 1 are plots of % extraction of Mo(VI) against acid concentrations of HCl, H_2SO_4 and HNO_3 for extraction using H_2Adp in the presence and absence of BuOH as a synergist. The result shows generally, that below 0.1M acid concentrations close to 98% extraction was achieved by the ligand, both in the presence and absence of BuOH as a solvating agent. However, in the extraction of the metal from H_2SO_4 acid concentrations, a maximum of 70% extraction was achieved at 0.001M H_2SO_4 acid concentration and this falls monotonously to less than 1% extraction at 0.5M H_2SO_4 acid concentration. This trend is similar to what was reported by Koladkar and Dhadke (2002) on the solvent extraction of Sc (III) from H_2SO_4 acid with PIA – 8 in toluene. The distribution ratio of Sc (III) decreased with increasing H_2SO_4 acid concentration while quantitative extraction was obtained at H_2SO_4 concentration range of 0.1 – 0.5M.

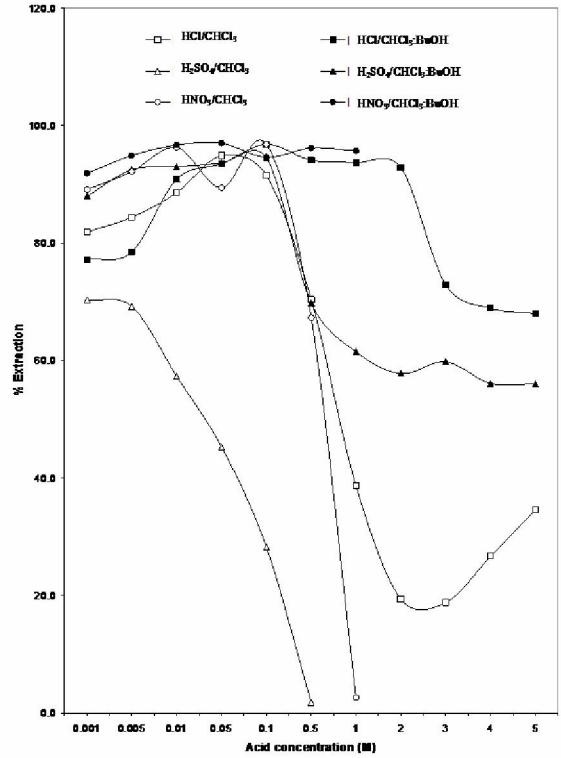

Figure 1: Variation of % Extraction of Mo(VI) with acid concentration for H_2Adp.

The synergist effect of BuOH was very obvious in the extraction of Mo(VI) from various H_2SO_4 concentrations as shown in Fig. 1, with almost 96% extraction of Mo(VI) at 0.01M H_2SO_4 acid concentration. This also fell monotonously but not drastically as was the case in the absence of BuOH in the organic phase. Hence 58 – 68% extraction was achieved even at high acid concentrations of 1M – 5M H_2SO_4.

Extraction from HCl and HNO_3 solutions also showed evidence of the synergistic effect of BuOH in the organic phase. Hence, between 96 – 98% was achieved for extractions using HCl when BuOH was introduced into the organic phase for all concentrations of HCl. However, in the absence of BuOH, this fell drastically to 0.5% at 1M HCl concentration.

For extractions using H_2SP in the presence and absence of BuOH as a synergist, plots of % extraction of Mo(VI) against acid concentrations of HCl, H_2SO_4 and HNO_3 is shown in Fig. 2.

Maximum % extraction was recorded at 80% and 50% for HCl and HNO_3 respectively between acid concentrations of 0.01M and 0.1M while at 0.001M H_2SO_4 concentration maximum % extraction of 38% was achieved, but fell monotonously to 5% at 0.5M acid concentration. Introduction of butanol into the extraction process enhanced the extraction of the metal ion in all the acid media with maximum % extraction of above 90% occurring between 0.5M and 0.05M acid concentrations for all three acids.

The results generally show that the synergist effect is more pronounced for extractions from H_2SO_4 solution more than from HCl and HNO_3 solution and so the trend can be summarized as follows:

$H_2SO_4 > HCl \approx HNO_3$

Decrease in BuOH synergism for Mo(VI) extraction

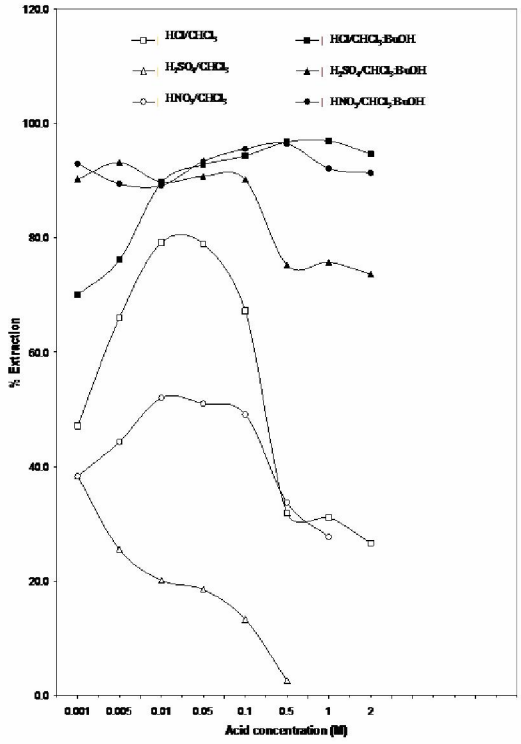

Figure 2: Variation of % Extraction of Mo(VI) with acid concentration for H_2SP

From Figs. 1 and 2 above, the extraction of Mo(VI)

from various acid concentrations for both ligands show that optimal % extraction is minimal at H_2SO_4 concentrations followed by HNO_3 and highest at HCl.
This has been attributed to low $[H^+]$ concentration from the relatively weaker acid, in which case the strong anionic conjugate base from the weak acids tend to be more reactive with the metal ions and masking them at these low acid concentrations.
These results therefore suggest that H_2Adp is a better extractant of the metal ion Mo(VI) than H_2SP. This is probably due to the differences in the chain length and orientation of the carbon atoms in these chelating agents.

3.3 Extraction Mechanism of Mo(VI)

Statistical treatment of data using slope analysis as applied to this work has earlier been discussed as reported by Uzoukwu 1998; Uzoukwu et al 1998[b] and the mechanism of interaction presented in Figs 3 to 5. The mechanism of interaction between Mo(VI) and the ligands for H_2SO_4, HCl and HNO_3 solutions are presented as plots of log D versus log [Acid] in Fig 3.

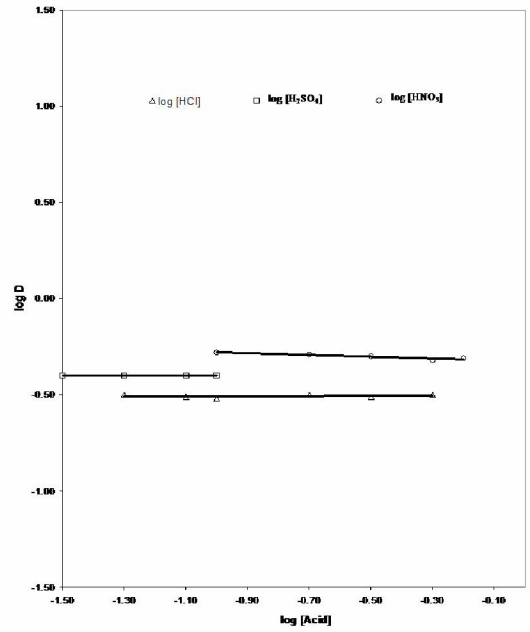

Figure 3: Plot of log D Vs log [Acid] for Mo(VI) with H_2Adp

The plots on Figs 3 show that the slopes are zero; hence applying equation (12), the value of "x" is equal to zero. Equation (8) presented a situation in which the extraction of the metal may take place through ion pair complex formation. However, from the result obtained from the slope analysis, it is therefore evidenced experimentally that the acids were not incorporated in the complex during the extraction process. Therefore no ion pair complex was extracted into the organic phase. This shows that the appropriate equation for the extraction of the non-ion pair complex is that of equation (1).

Figure 4 also shows that the slope obtained from plots of log D versus metal ion concentration is equal to zero as well. Hence, on applying statistical methods the extraction of the non-ion pair complex species implies that 1 mole of $M_OO_2^{2+}$ is involved according to equation 8 where "a - 1=0". This is an indication that 1 mole of the metal ion is involved in the extraction process since the log D plot is independent of changes in the metal ion concentration.

The interaction between the concentrations of the ligand is shown in figure 5 for the extraction of Mo(VI). A slope of one was recorded for all the three acids studied. Since there is no adduct complex formation as shown by the involvement of only 1 mole of Mo(VI) therefore "c" in equation (1) is then equal to zero. Hence from equation (6)

$b + c = 1$
$b = 1$ since $c = o$

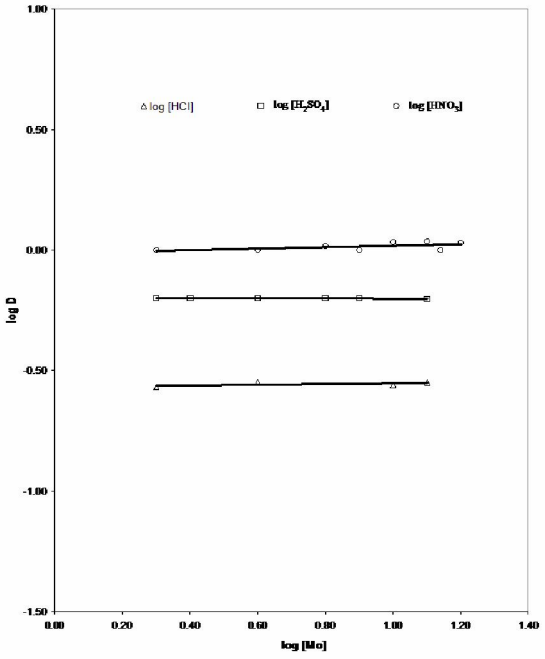

Figure 4: Plot of log D Vs log [Mo] with H_2Adp.

With b = 1 and c = o this is an indication that 1 mole of the ligand was involved in the extraction process. Since it is a quadridentate ligand this implies that 2 moles of protons were released in the process.

Therefore the extraction process can be presented as follows:

$$M_OO_2^{2+} + H_2Adp_{(o)} \longrightarrow M_OO_2(Adp)_{(o)} + 2H^+ \quad \ldots\ldots\ldots\ldots (14)$$

$$K_{ex} = \frac{[M_OO_2\,(Adp)_{(o)}]\,[H^+]}{[M_OO_2^{2+}]\,[H_2Adp]_{(o)}} \quad \ldots\ldots\ldots (15)$$

$$\log D = \log K_{ex} + \log [H_2Adp]_{(o)} - 2 \log [H^+] \quad \ldots\ldots\ldots (16)$$

Similarly, extraction of Mo(VI) using the ligand H_2SP followed the same trend (fig 6).
Therefore the extraction process can equally be presented as follows:

$$M_OO_2^{2+} + H_2SP_{(o)} \longrightarrow M_OO_2(SP)_{(o)} + 2H^+ \quad \ldots\ldots (17)$$

$$K_{ex} = \frac{[M_OO_2\,(SP)_{(o)}]\,[H^+]}{[M_OO_2^{2+}]\,[H_2SP]_{(o)}} \quad \ldots\ldots (18)$$

$$\log D = \log K_{ex} + \log [H_2SP]_{(o)} - 2 \log [H^+] \quad \ldots\ldots\ldots\ldots (19)$$

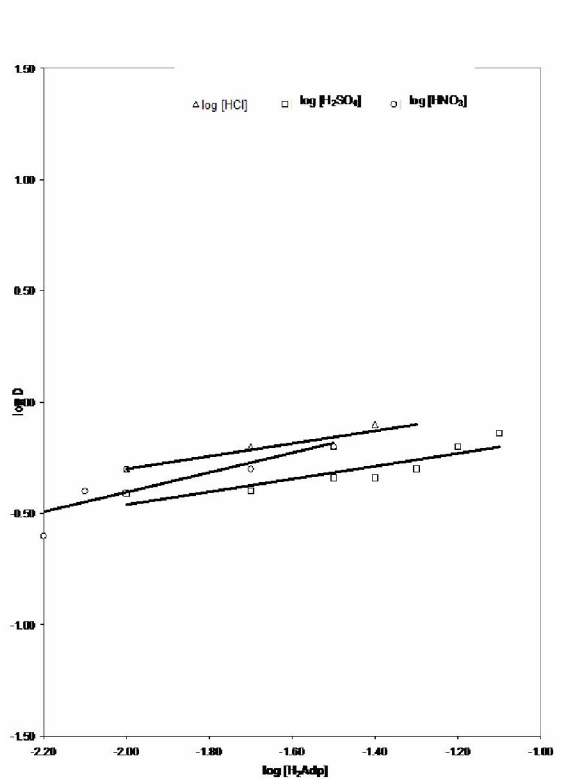

Figure 5: Plot of log D Vs log [H_2Adp] with Mo(VI)

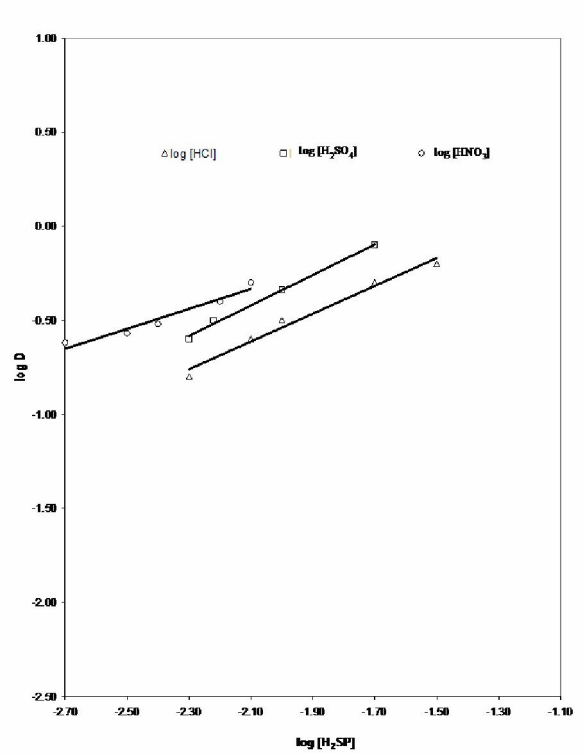

Figure 6: Plot of log D Vs log [H_2Adp] with Mo(VI)

4. Conclusion

Extraction of Mo(VI) ions using 0.01M of the ligand H_2Adp in chloroform from various acidic medium gave optimal % extraction above 90% within 0.01M and 0.5M acid concentrations for HCl and HNO_3 while extractions in H_2SO_4 solutions gave a slightly lower % extraction yield of 70% within the concentration range of 0.001 and 0.005. In the case of the ligand H_2SP, a much lower % extraction yield of 80% for HCl, 50% for HNO_3 and 39% for H_2SO_4 was obtained within the same acid concentrations.

Introduction of butanol as a synergist into the extraction system greatly enhanced the overall optimal % extraction to 98% and 95% for extractions using 0.01M H_2Adp and H_2SP in chloroform respectively, within the acid concentrations of 0.01M and 1.0M in all cases. In addition, increase in acid concentrations above 1.0M has a masking effect on the extraction of Mo(VI) ions likewise very dilute acid concentration below 0.01M. Hence extraction of Mo(VI) using 0.01M H_2Adp and H_2SP in chloroform is best favored within the acid concentration of 0.01M and 1.0M.

Using theoretical considerations and slope analysis, the extracted species were found to be $M_OO_2(Adp)_{(o)}$ and $M_OO_2(SP)_{(o)}$.

Correspondence to:
Ihesinachi A. Kalagbor
Department of Science Laboratory Technology, Rivers State Polytechnic, Bori,
P.M.B. 20, Bori, Nigeria.
Telephone: +234 803 3098983
E-mail: ksinachi@yahoo.com

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Comparative Response of Different Varieties of Maize (*Zea mays* L) to NPK 15:15:15 Compound Fertilizer and Poultry Droppings Applications

Okaka Victor, Alleh Eric, Ogedegbe Felix, Ayodele Emmanuel

Department of Crop Science, Ambrose Alli University, Ekpoma, Nigeria
E-mail: vbokaka@yahoo.com

ABSTRACT: Two similar field experiments were conducted during the early cropping seasons of 2007 and 2008 in the Teaching and Research Farm of Ambrose Alli University, Ekpoma, to evaluate DMSR and TZSR improved varieties as well as a popular local maize for agronomic performance and yield responses to NPK 15:15:15 compound fertilizer and poultry droppings. The three varieties were fertilized with 100,200 and 300kg/ha NPK as well as 6t/ha poultry droppings and a control in a 3 × 5 factorial arrangement fitted into a randomized complete block design replicated three times. The three varieties differed significantly ($P<0.05$) in most of the vegetative parameters monitored including plant height, number of leaves and leaf area, in the order of the local > TZSR >DMSR. The positive and significant ($P<0.05$) response of the vegetative and yield parameters to fertilizer application mostly to 300kg/ha NPK, then poultry droppings and 200kg/ha NPK in that descending order, was independent of variety. The DMSR, the TZSR and the local variety produced tassels and silks in 63 and 73 days, in 66 and76 days, and in 72 and 83 days respectively. The three varieties had similar shelling percentage values (68.5-69.9%). The improved varieties: DMSR and TZSR varieties did not differ significantly, out yielded the local by 18.9% and 10.8% respectively and were found to be adapted to this ecological zone. The DMSR was highest yielding with a 100grain weight of 32.0grams and total grain yield of 4.4t/ha.
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Key words: improved varieties; popular local; NPK; Poultry droppings; adapted; ecological zone

1. Introduction

Maize (*Zea mays* L.) is adapted to a wide range of environments (Ajibefu *et al*., 2002) and it is more extensively distributed over the earth than any other cereal crop(Remison,2005).The strategic plan of IITA in 1987 acknowledged the large potential of maize as human food, livestock feed, and industrial use and that the moist savannas have the greatest potential for increased production in West Africa(IITA,1987). FAO (1995)'s estimate of 1.9 million metric tonnes of maize grains from 1.5 million hectares of land in Nigeria translates to about 1.3 tonnes/ha. This aptly shows that the yield of maize in the country is abysmally low considering the demonstrated and potential yields of 4.5t/ha and above 5.0t/ha respectively (Remison, 2005). Despite the all importance of maize and favourable environmental conditions, the present production trend is not keeping pace with growth in demands for the crop products. This apparent production - demand gap is attributed to the inherent poor fertility status of tropical soils due to high rainfall and consequent leaching (Agboola and Unamma, 1991; Forbes and Watson, 1992). Due to population pressure and decreased land per farmer, cropping is continuously done on the same piece of land even on marginal soils. This situation further exacerbates loss of soil fertility and inadequate supply of plant nutrients with attendant low yields (Ojeniyi and Adegboyega, 2003). Farmers are not ignorant of the obvious implications of continuous cropping and how to alleviate same with the commonly recommended inorganic fertilizers. Most farmers lack the resources needed to adapt to the changing context or to integrate this agronomic practice of fertilizer application which may help them to conserve soil nutrients. For enormous are the challenges of scarcity and exorbitant cost of inorganic fertilizers (Ighalo *et al*.,2008) which place them beyond the reach of the low to medium farmers who form the bulk of farmers in Nigeria. According to Ojeniyi and Adegboyega (2003), the scarcity and high cost of the inorganic fertilizers hamper their adequate use in ameliorating soil deficiencies. There is however a solace in organic manures from the ever expanding livestock farming by-products for soil amendments. According to Giller *et al*. (2006), organic materials are important sources of nutrients and are necessary for the management of soil fertility by small scale farmers. There is the need for an empirical evidence of the comparative effects of the commonly sought inorganic fertilizer N.P.K. and the source able poultry droppings on growth and yield of maize. To this end, this study has the main objective of evaluating three commonly grown maize varieties for

growth and yield response to organic and inorganic fertilizers. The specific objectives are to;

1. Compare three commonly grown maize varieties in Ekpoma and environs, for growth and yield in a derived savannah ecological zone of Edo state, South South Nigeria.
2. Determine the optimum rate of NPK 15:15:15 compound fertilizer that equates with the rate of poultry droppings used for maize in this ecological zone.
3. Determine the desirability of poultry droppings as a viable alternative to inorganic fertilizer for maize

2. Materials and Methods

Two similar experiments were conducted in adjacent fields of the Teaching and Research Farm, Ambrose Alli University Ekpoma, a rain forest-savannah transition zone of Nigeria in the early cropping seasons of 2007 and 2008. The site had been under bush fallow for two years after harvest of cassava in 2005 before the experiment commenced in 2007. The characteristics of 0-15cm layer of the soil in 2007 were; total nitrogen (N): 0.99, phosphorous (ppm): 7.89, potassium (m-equiv/100g): 0.19, calcium (m-equiv/100g):5.00, magnesium (m-equiv/100g):0.43, carbon (%):1.50, pH (0.01mkcl): 5.9 and Effective Cation Exchange Capacity (ECEC): 5.25. The characteristics of the adjacent soil used in 2008 were as follow; total nitrogen (N): 1.01, phosphorous (ppm):8.25, potassium (m-equiv/100g):0.21, calcium (m-equiv/100g): 4.96, magnesium (m-equiv/100g):0.45, carbon (%): 1.65, pH (0.01 mkcl): 5.6 and Effective Cation Exchange Capacity(ECEC): 5.34.

Three varieties of maize; Downy Mildew Streak Resistant cultivar with red kernels(DMSR-R),Tropical Zea mays Streak Resistant cultivar with white kernels(TZSR-W) and a popular local tagged Ekpoma local were used. Five fertilizer rates including the control, NPK 15:15:15 compound fertilizer at 100,200 and 300kg/ha as well as poultry droppings at 6tonnes/ha equivalents were used to fertilize each variety in a 3 × 5 factorial scheme replicated 3 times. Adjacent plots within and between replicates were spaced 1m apart and 1.5m apart respectively. Each plot measured 4.5m×2m in a total land area of 44m×16.5m.

The organic fertilizer; poultry droppings, dried and earlier analysed for percentage organic matter: 59.9, N:4.5, P_20_5: 2.7, K_20:1.6, Ca:2.9 and Mg:0.8 was worked into plots receiving same, a week before plantings were done. Planting was done on 30th May for the 2007 experiment and on 13th June in the 2008 experiment. Spacing was 75cm×25cm which gave an equivalent population density of 53333plants/ha after supply of missing stands and thinning to 1 plant/stand. Weed control was manual, twice; at 3 and 7 weeks after planting (WAP). The inorganic fertilizer, NPK 15:15:15 compound was applied according to treatment rates, at 3WAP immediately after the first weeding operation.

Data were collected on both vegetative and yield parameters. At the onset of tasseling (i.e. end of vegetative phase) in 9WAP, data were collected on plant height, stem girth, number of leaves and leaf area from five randomly selected plants in the four central rows. Number of days from planting to when half of the plants in a plot tasselled and developed silk was counted for 50% tasseling and 50% silking respectively. The crops were left dry in the field, before harvest. The moisture content was adjusted to the standard 12.5% by oven drying at 80°C for 72hrs. Yield and components of yield data including 100 grain weight, grain yield and shelling percentage were determined on plants from the net plots. All data were subjected to analysis of variance applicable to randomized complete block design (Gomez and Gomez, 1984). Significant means were compared using least significant difference (LSD) at p=0.05. Data for both years were not significantly different (t at 0.05) and thus pooled for presentation.

3. Results and Discussion

The vegetative parameters of maize including plant height, stem girth, number of leaves and leaf area responded positively and significantly ($P<0.05$) to NPK 15:15:15 compound fertilizer and poultry droppings application, independent of variety. The parameters increased with increased rate of NPK fertilizer application up to the highest rate of 300kg/ha used in the study (Table 1). This is in consonance with the earlier observation made by Allan (1984) that an increase in supply of nutrient from a level that is low, commensurately increases growth. The effects of 6t/ha poultry droppings and 200kg/ha NPK application were comparable on plant height, stem girth and number of leaves. Whereas, 6t/ha poultry droppings application compared with 300kg/ha NPK 15:15:15 compound fertilizer on the leaf area of the maize plants. For instance, maize plant height in the unfertilized plots increased 16.7%, 39.5%, 58.0% and 39.6% in response to the application of 100kg/ha NPK, 200kg/haNPK, 300kg/haNPK and 6t/ha poultry droppings respectively. While the leaf area of maize plants in the unfertilized plots increased 43.3%, 61.0%,75.1% and 71.1% due to the application of 100kg/ha NPK, 200 kg/ha NPK, 300 kg/ha NPK and 6t/ha poultry droppings respectively (Table1). Varietal differences in the plant architecture of the three varieties apparently manifested in the plant height, number and size of the leaves. In the consideration of compatibility with other crops in mixtures, the DMSR would be the choice of farmers because of the relatively low number and size of leaves compared to the local and TZSR varieties.

The three varieties differed significantly (P<0.05) in days to 50% taselling and silking with the DMSR producing tassels and silks earliest while the local variety was latest in both parameters. The DMSR tasselled on the average, 3 and 9 days before the TZSR and the local variety respectively while the TZSR tasselled 6 days earlier than the local variety. Similarly, the DMSR produced silk about 3 and 10 days earlier than the TZSR and the local variety respectively while the TZSR produced silk about 8 days before the local variety (Table 2). Earliness in maturity as a major breeding objective has been a major distinguishing feature of improved varieties of crops from the local varieties.

The non application of fertilizer significantly (P<0.05) delayed tasselling and silking in the three varieties. Tasselling in the 100 and 200 kg/ha NPK, 6t/ha PD as well as 300kg/ha NPK supplied plants was delayed by about 4 days, 5 days and 7 days respectively due to non application of fertilizer. The effects of 100 and 200 kg/ha NPK application on commencement of tasselling were the same while the 300kg/haNPK fertilized plants tasselled earliest. However, silks were developed in the order of plants in the 300 kg/ha NPK plots, 6t/ha PD and 200kg/ha NPK plots, 100kg/ha NPK plots before the control(Table 2). The earliness in tasselling and silking in response to fertilizer application in this study is a negation of the assumption made by Okaka(1992) that fertilizer application may have the tendency of prolonging vegetative growth phase in annuals. It is logical to attribute the prompt development of tassels and silks occasioned by adequate nutrients from fertilizers applied, to enhancement of ample environment for optimal physiological activities of the plants.

Shelling percentage of the maize plants was not significantly (P >0.05) affected by variety. The three varieties had almost similar shelling percentages (68.5% – 69.9%) as shown in Table 3. Most of the crop varieties in vogue are improved and best locals which evolved either by natural or artificial selection for desirable traits. Improved yield has always been the most compelling objective in the development of any plant variety. Relative proportion of seeds(grains) in a maize cob is a trait that determines the yield. Shelling percentage increased slightly (P >0.05) with 100 and 200 kg/ha NPK applications but significantly (P<0.05) with 300kg/ha NPK and 6t/haPD applications. Plants supplied 300kh/ha NPK had the highest shelling percentage though comparable with the plants fertilized with 6t/ha PD. Apparently, adequacy in nutrient supply occasioned by the applications of 300kg/ha NPK and 6t/ha poultry droppings enhanced grain filling irrespective of variety.

The improved varieties and the local variety differed significantly (P<0.05) in grain yield. Though comparable (LSD at 0.05), the DMSR variety was higher yielding than the TZSR variety. Grain yield also differed significantly (P<0.05) among the different fertilizer treatment effects in the order of 300kg/ha > 6t/ha PD= 200kg/ha >100kg/ha > control.

The results of this experiment have shown that response of maize vegetative and yield parameters to 100kg/ha and 200kg/ha NPK 15:15; 15 compound fertilizer was significant (P<0.05) while response to 300kg/ha NPK 15:15; 15 compound fertilizer and 6t/ha poultry droppings was highly significant (P<0.01).

4. Conclusion and Recommendation

The findings in this study show that there is considerable variation in the growth and yield of different varieties of maize in this ecological zone. The yield of the local can be substantially improved by adopting a more yielding variety as DMSR in this study. It is very obvious that the use of fertilizers in this ecological zone is inevitable since most available lands for cropping rarely fallow long enough to recover the nutrients lost to previous croppings. This is evident in the response of the maize varieties to even the lowest rate of fertilizer (100kg/ha NPK) used in the study.

It is worthy of note that yields from even the local variety are far below the potentials because of poor agronomic practices such as non application of fertilizer. Both vegetative and yield components increased consistently with increasing rates of NPK fertilizer up to the highest rate of 300kg/ha used in this study. This exposes the inherent limitations in adopting the blanket recommendation of 300kg/ha of NPK 15:15:15 compound fertilizer for maize cultivation in Nigeria. DMSR is a viable variety for use in this ecological zone and NPK 15:15:15 compound fertilizer at the highest rate used in this study or a higher rate of poultry droppings is recommended.

Further studies are however necessary to:

1. Evaluate all available maize varieties including the locals, the downy mildew and streak resistant varieties for yield response to fertilizers(organic and inorganic), and
2. Increase the rates of NPK and poultry droppings and determine the optimal rates.

Table 1: Effects of NPK 15:15:15 Compound Fertilizer and Poultry Droppings Application on the Vegetative Characters of Different Varieties of Maize

	Variety	0	100	200	300	PD	Mean	Se±
	DMSR	73.0	93.0	138.0	158.6	134.8	119.5.c	
	TZSR	114.7	134.9	147.5	165.1	142.5	140.9b	4.00
	LOCAL	147.4	163.2	182.0	205.9	190.3	177.8a	
	MEAN	111.7d	130.4c	155.8b	176.5a	155.9b		
Plant Height (CM)	SE±			5.16				
	DMSR	5.4	5.8	6.1	7.1	6.4	6.2a	
	TZSR	5.4	5.7	6.2	6.2	5.6	5.8a	0.23
	LOCAL	5.0	6.1	6.2	7.3	6.2	6.2a	
	MEAN	5.3c	5.9b	6.2b	6.9a	6.1b		
Stem Girth (CM)	SE±			0.30				
	DMSR	7.7	9.0	10.3	11.0	10.3	9.7c	
	TZSR	10.0	10.3	10.8	11.2	10.6	10.6b	0.14
	LOCAL	12.7	13.0	14.1	14.4	14.1	13.7a	
	MEAN	10.1d	10.8c	11.7b	12.2a	11.7b		
Number of Leaves	SE±			0.19				
	DMSR	221.7	284.1	359.7	432.1	447.6	349.0c	
	TZSR	317.5	531.2	541.6	597.5	541.3	505.8b	14.91
	LOCAL	399.5	530.0	609.7	613.9	617.1	554.0a	
	MEAN	312.9d	448.4c	503.7b	547.8a	535.3a		
Leaf Area	SE±		19.26					

Table 2: Effects of NPK 15:15:15 Compound Fertilizer and Poultry Droppings Application on 50% Tasseling & Silking of Different Varieties of Maize

NPK (Kg/ha) & Poultry Droppings 6 (t/ha)

50% Tasseling

Variety	0	100	200	300	PD	Mean	Se±
DMSR	68.0	62.7	62.0	60.3	62.3	63.1c	
TZSR	70.3	66.3	66.0	63.0	65.5	66.2b	0.41
LOCAL	74.3	73.3	71.7	70.0	71.3	72.1a	
MEAN	70.9a	67.4b	66.6b	64.4c	66.4b		
SE±			0.53				

50% Silking

Variety	0	100	200	300	PD	Mean	Se±
DMSR	77.7	72.3	71.1	70.7	72.0	72.8c	
TZSR	79.3	76.3	75.0	73.0	74.3	75.6b	0.42
LOCAL	87.7	84.0	82.3	79.7	82.0	83.1a	
MEAN	81.6a	77.5b	76.3c	74.5d	76.1c		
SE±			0.55				

Table 3: Effects of NPK 15:15:15 Compound Fertilizer and Poultry Droppings Application on the Component of Yield & Parameters of Different Varieties of Maize

NPK (Kg/ha) & Poultry Droppings 6 (t/ha)

100 - Grain Weight (g)

Variety	0	100	200	300	PD	Mean	Se±
DMSR	30.8	31.8	31.7	33.9	21.9	32.0a	
TZSR	30.4	31.2	30.5	32.7	32.0	31.4a	0.55
LOCAL	28.6	29.4	30.2	32.0	32.1	30.5b	
MEAN	29.9b	30.8b	30.8.b	32.9a	32.0a		
SE±			0.71				

Shelling Percentage (%)

Variety	0	100	200	300	PD	Mean	Se±
DMSR	65.1	67.3	63.3	75.6	71.1	68.5a	
TZSR	65.9	68.7	67.7	75.0	69.9	69.4a	1.39
LOCAL	67.1	67.9	68.8	73.1	72.9	69.9a	
	66.0b	68.0b	66.6b	74.6a	71.3a		
SE±			1.78				

Grain Yield (tons/ha)

Variety	0	100	200	300	PD	Mean	Se±
DMSR	3.3	4.2	4.5	5.7	4.3	4.4a	
TZSR	3.0	4.0	3.8	5.2	4.3	4.1a	0.13
LOCAL	3.0	3.3	3.8	4.2	4.3	3.7b	
MEAN	3.1d	3.8c	4.0b	5.0a	4.3b		
SE±			0.16				

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Directorate of Teaching and Research Farm, Ambrose Alli University Ekpoma, International Institute for Tropical Agriculture (IITA), Ibadan.

Correspondence to:
Okaka, Victor
Department of Crop Science,
Faculty of Agriculture,
Ambrose Alli University: 01186-10-8491-3946
Cellular phone: 08056177254
08063299449
Email: vbokaka@yahoo.com

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Phytosanitary Protection in Horticultural Seed Production: A Bridge to National Seed Demand

Ihejirika, Gabriel Onyenegecha, Ibeawuchi, Izuchukwu Innocent, Obiefuna, Julius Chiedozie and Ofor, Marian Onomerhievurhoyen

Department of Crop Science and Technology
Federal University of Technology, Owerri
P.M.B. 1526 Owerri, Imo State, Nigeria
ihegab@yahoo.com

Abstract: The problem of quantifying seed losses and their effect on agricultural production and food availability is of primary importance to meet national seed demand. Through that, rational control measures can be developed and applied and resources can be better allocated as well. Seeds being living things, respire by absorbing oxygen and giving off carbon dioxide and water vapour, producing heat at the same time and these phenomena play a major role in its preservation as if not properly taken care off, could cause the seeds to stick together, coagulate as a mass, creating blockage in the store. Seed deterioration is due to a number of interrelated factors like physical e.g. temperature, humidity, water; Biological like microflora (mould, bacteria , fungi, yeast etc) or arthropods (insect, mites); Vertebrates (rodents, birds) or technical (conditions, methods, duration of storage) as well as state of seeds (broken, impurities, residues etc). Infestation in the field, during transportation, storage premises, sacks and containers as well as putting contaminated seeds in store and unhygienic store are inimical to phytosanitary protection and cause losses in seed viability. A reduction in these losses would lead to production of high quality and quantitatively valued seeds to meet national seed derived.

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Keywords: Phytosanitary, protection, horticultural, seed, national demand.

1. Introduction:

The food products which man has to preserve in other to feed himself are of animal or plant origin. The former-milk product, meat, fish etc require expensive freezing and sterilizing facilities. The later consist of perishable commodities such as roots, tubers, fruits and vegetables, which are not stored for long periods of time, and durable commodities such as cereals and legumes which are preserved for several months and sometimes even for a number of years (Nwufo, 2004; Babatola, (2000).

Seeds consist of the embryo and the seed coat. In many spermatophyta, the seed also contains a special tissue in which the food reserves are stored. The embryo, resulting from the proliferation of the egg is the analog of the future plant in horticultural seeds, the organs for propagating the plant species are normally protected against adverse factors. Thus, their water content may reach an excessively low percentage (well below 4%) thereby entail a very slow metabolism. They are preserved from external attack by their seed coast and by the production of bactericidal and fungicidal substances. (Hall, 1971; Chirapp, 1988)

Seeds, being living organisms, respires by absorbing oxygen and giving off carbon dioxide and water vapour, producing heat at the sometime. These phenomena play a major role in the preservation of horticultural seeds to meet the national seed demand. The seed at any point in its storage life should have ideal seed reserve. These reserves are stored by the plant to be subsequently used inter alia in order to develop, after hydrolysis, vegetative organism when activity is resumed. Examples are albumen starch, bean and pea parotids, groundnut lipids etc.

Horticultural seeds in bulk behave just like a fluid; it runs or can be sucked up, the flour depending on the form, size and moisture content of the seeds and how clean it is. Damp or heating may cause a number of seeds to stick together and coagulate as a mass, creating blockage in the store (bags, baskets, silo pipes etc). The natural angle of repose of a heap of grain for example is around 30^0 (Multon, 1982).

Between the grains in a heap there is a substantial volume of air (around 40% of the overall volume) which circulates slowly due to convection currents. When it become necessary to dry or cool grain, or even to replace the air by gases (such as nitrogen or carbon dioxide) forced ventilation is employed, using blowers provided for this purpose in the silos.

2. Problems of Horticultural Seed Preservation

The main problems connected with the preservation of horticultural seeds in tropical climates are the result of the high temperature which prevails throughout the year typical of these regions and the effect of temperature depends however, very much on ambient humidity, which varies considerably according to region and the time of year (Sharples, 1990).

In order to meet National seed demand, horticultural seeds should be mostly preserved in dry regions than in wet region, since the maximum threshold of 13% moisture content in the seed is attained at harvest time, whilst in wet region drying facilities are essential. Rainfall is one of the principal uncertainties with which farmers have to contend. In a dry climate, the inadequate rainfall may hamper horticultural seed production. To guard against such an eventuality, governments should make provision for strategic stocks. Normal, well-distributed rainfall sometimes leads to bumper harvest. The capacity of existing storage facilities is then sometimes inadequate to accommodate them. In wet climates, it may also happen that excessive rainfall will make harvesting difficult resulting in the rotting of the seeds in the filed.

Horticultural seed deterioration is due to a number of interrelated factors which can be modified by man either by aggravating their effects, through negligence, ignorance or error, or by limiting or nullifying their impact by prudent measures and good store hygiene. There is a whole range of physical, chemical and biological phenomena which remain constant and with which it is essential to be controlled so as to meet the natural seed demand of horticultural seeds.

These include:

1. Physical: Temperature, humidity, water, gas.
2. Biological: Microflora (mould, bacteria, yeast, etc) arthropods (insects mites) vertebrates (rodents, birds).
3. Technical: Storage (conditions, method, duration, etc), state of the seed (whether it is broken, impurities, residues etc) (Appert, 1987; Anslem, 1981).

Insects may be vector of bacteria and pathogenic protozoa and thus carry typhoid and dysentery. They may also give rise to allergies due to the impurities contained in the grain, for which they are responsible, or their presence may even give rise to mould and mites.

3. Deterioration of Horticultural Seeds

When horticultural seeds are attacked, their quality are impaired either as a result of the embryo being consumed or merely damaged or as a result of the food reserves being partly or completely eaten. *Coleopteran* of genera *Tribolium, Orgzaephilus, Callosobruchus, Lepidoptera* such as *Ephestia cautella* and mites such as *Acarus siro* show a preference for the germ, which leads to a reduction in germination capacity. Other pests such as weevils of genus *Silophilus* or *Silotroga* sp. Moths attack only the starchy part of the grain, thus enabling it to germinate, although the plantlet will not have sufficient reserves to develop normally.

Infestation of stocks comes either from outside or inside the premises where the goods are stored. It is important to be able to specify where it originates, in order to be able to choose wisely the control measures to be employed and to prevent, by means of appropriate measures, the incident from recurring (Poulet and Hubbert, 1982).

i Infestation in the fields

Certain speicies only (but these are the most dangerous and may be in hidden forms) start to lay their eggs on or in the seeds as soon as it ripens, prior to harvesting, or during the period between harvesting and storage, which may be fairly long if drying of the seeds is necessary.

The weevils *Sitophilus oryzae* and *S. zeamis*, the adults of which fly, lay their eggs on seeds before and during the storage period. The same applies to the groundnut beetle, and to *bruchids* in legumes and *alucitidae* in maize.

ii Infestation during Transportation

A batch of pest free seed, transported from the field, village or collection centre to the slore or silo by carts, trucks or wagons may be contaminated by insects hidden in nooks and crannies, gaps between the boards, folds in covers or debris from previous loads that has not been swept out properly.

iii Infestation by insects flying in from outside

There is of course no need to fear this type of infestation in hermetically sealed stores like silos, but only in warehouse, open stores and transit facilities.

iv Infestation from the storage premises, sacks and containers

If the containers used for the horticultural seeds have not been thoroughly cleaned out, or if the stores have not been maintained since the previous crop was removed, i.e if cracks have not been blocked up, doors and windows do not close properly, or there is waste, packaging material or dust lying about, there is a greater risk of generalized infestation of the incoming grain. Certain highly resistant long-lived species such as *Trogodermae* and *Silavnidae*; and *Cryptolestes* or *Tribolium* (which are fond of broken seed or dust), are commonly found in poorly maintained storage facilities (Hall, 1980).

v Infestation caused by putting contaminated seeds into store

The whole contents of a healthy store may be infested by introducing a batch of contaminated seed; such seed should either be rejected or disinfected prior to storage.

4. Store hygiene and Phytosanitary protection:

It can never be stressed too often that perfect maintenance and scrupulous cleanliness of the container bags, stores granaries, and silos intended to accommodate the seed are the best guarantees of excellent preservation, provided of course, that the goods in question have been properly dried and treaded beforehand. If the seed becomes damp again during storage, this can only encourage insects to multiply rapidly (ISTA, 1987; Nwufo, 2004).

5. Losses in terms of the viability of seeds

Horticultural seeds preserved for sowing the following year are the subject of particular care in view of their considerable potential value. In order to preserve their germination capacity, so as to meet the national seed demand, care must be taken to protect them not only from the insects which attack the germ, but also from excessively wide variations in height, humidity or temperature.

Horticultural seed loses may have serious repercussions on the amounts of food available the following year for the family and sometimes even nationally. In order to meet national seed demand, horticultural seed should be preserved in such a way that there should be reduction in its nutritional as well as qualitative losses. Qualitative losses are partially subjective in that they are assessed according to consumer taste and criteria used by local traders.

Normally they are judged on the basis of appearance, size, shape, small, flavour etc. The presence of foreign bodies and various impurities inevitably reduces the value of the goods. While it is possible to eliminate these undesirable elements, contaminants are in completely different matter. These consist of the solids excreted by insects, pathogenic organisms spread by rats, *mycotoxins*, pesticides residues etc. In the case of oilseeds attacked by insects, the level of free fatty acids increases, causing the goods to go rancid.

Nutritional loss represents a reduction in the food value of the horticultural seeds as a result of a lowering of its protein, hydrocarbon and vitamin content. Pests prefer to consume certain parts of the seed, thus rodents and caterpillars go for the germ, thereby destroying a high proportion of proteins and vitamins. Pulse beetles significantly affect the protein levels, while weevils, which eat into the endosperm, reduce the hydrocarbon content of cereals. Vitamin losses are due to pests which feed on the bran or to cryptogamic infection (Hall, 1971; Teng, 1980).

6. Loss of goodwill

In other to meet national seed demand, horticultural seeds should be preserved so that loss of goodwill would be avoided. Loss of goodwill is not immediately quantifiable but crucial all the same, that is why producers must endeavor to offer for sale seeds which meet the quality standards and agree to comply with control measures. As products intended for export have to meet increasingly stringent standards, their reputation for quality will have considerable repercussions for the country's economy (Appert, 1987).

Conclusion

Horticultural seeds are susceptible to deterioration and heavy losses at high temperature and relative humidity and losses include poor harvesting, handling and poor storage condition as well as pest and disease infection. These reduce the quality and quantitative value of the seeds. These losses should be reduced to a minimum to meet the national seed demand.

Correspondences to:

1. Dr. G.O. Ihejirika
   Department of Crop Science and Technology,
   Federal University of Technology, Owerri
   P.M.B. 1526, Owerri, Imo State, Nigeria.
   E-mail: ihegab@yahoo.com;

2. Dr. Ofor, Marian Onomerhievurhoyen
   Department of Crop Science and Technology,
   Federal University of Technology, Owerri
   P.M.B. 1526, Owerri, Imo State, Nigeria.
   E-mail: mariofor2002@yahoo.com

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On the Chromosomes of two Cyprinid Fishes of the Subfamily Schizothoracinae from Kashmir.

[*1]Farooq Ahmad Ganai, [1]Abdul Rahman Yousuf, [2]Narinder Kumar Tripathi, [1]Ummer Rashid Zargar

[1] Limnology and Fisheries Laboratory, Centre of Research for Development, University of Kashmir, Hazratbal Srinagar-190 006 (India). Email: farooqmd84@gmail.com

[2]Animal Cytogenetics Laboratory, Deptt. Of Zoology, University of Jammu.

[*]Corresponding author: Farooq Ahmad Ganai. Email: farooqmd84@gmail.com.

Abstract: Karyotypic study of two *Schizothorax* species viz. *Schizothorax plagiostomus* and *Schizothorax curvifrons* belonging to family Cyprinidae, from River Jhelum Kashmir, was carried out. Conventional KCl-acetomethanol air-drying protocol was followed for the chromosomal preparation. The diploid chromosome number in *S.plagiostomus* was 96 with a chromosomal formula of 24m+18Sm+54t and fundamental number (NF) =138. Diploid chromosome number in *S.curvifrons* was 94 with Karyotypic formula 26m+20Sm+20St=28t and fundamental arm number (NF) =140. The evolutionary significance of polyploidy and the role of chromosomal rearrangements was discussed. Both these fishes are new to cytological literature.

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Key words: *Schizothorax plagiostomus*, *Schizothorax curvifrons*, River Jhelum, Karyotype.

Introduction

Cytogenetic studies in recent years gained a considerable importance concerning species characterization, evolution and systematic (Gold *et al*., 1990; Barat *et al*., 2002). The cytogenetical studies in fishes are limited to just about 10% of the total fishes known taxonomically all over the world (Barat *et al*., 1996). Fish chromosome data have great importance concerning evolution, systematics, aquaculture and mutagenesis (Al-Sabti, 1991). Chromosomal studies in fishes have not been as successful as those in other vertebrates because of relatively small size and large number of chromosomes found in many fish species and the limitations of the techniques employed (Klinkhardt, 1991). The air-drying technique, originally developed for mammalian organisms, is the most common procedure used for chromosome preparation in fish.

The increasing importance of chromosomal studies and the lack of data on fish karyotypes in Kashmir prompted us to examine the chromosomal content of *Schizothorax plagiostomus* and *Schizothorax curvifrons*. Both these fishes belong to subfamily Schizothoracinae, a widely distributed group in mountain streams, rivers and lakes around Himalayan Karakorum and Hindukush Ranges, The Tibet Plateau and Central Asia. These two fishes inhabit the River Jhelum, Lidder stream, Sindh Nallah of Kashmir Valley (Kullander *et al*., 1999).

In this study, cytogenetic analysis of *S.plagiostomus* and *S.curvifrons* from River Jhelum was carried out with air-drying technique to determine their basic karyological structure. Both these species are new to cytological literature.

Materials and Methods

Ten live specimens (five each for *S.plagiostomus and S.curvifrons*) were collected from River Jhelum, near Chattabal Downtown Srinagar. The initial identification was made on the basis of morphology (Kullander *et al*., 1999).

Chromosome and Karyotype analysis

All the samples were injected intraperitoneally with 0.05% Colchicine (Sigma, US) 1ml/100gm of body weight and kept alive for 2-3 hrs in fully aerated aquaria. Anterior kidney tissue was processed for chromosome preparation following conventional KCl-acetomethanol-air-drying protocols (Khuda-Bukhsh and Barat, 1987). The slides were stained with 2% Giemsa stain in phosphate buffer (PH 6.8). Leica DM LS2 trinoccular microscope fitted with a camera and 100x×10x oil immersion lens combination was used to scan the cells and take the photographs. Fifty to sixty

well spread metaphase complements were obtained for each species. The chromosomes of 5 well spread metaphase complements for each species were individually measured from photomicrographs with precision dial callipers and their centromeric indices and arm ratios were determined in order to ascribe the morphology as suggested by Levan et al. (1964). Using chromosomal indicators (Table II and Table III) an ideogram (Figure 3 and Figure 4) was prepared for each species in MS Excel 2007 software.

Results

Schizothorax plagiostomus: Chromosome number counts from 60 cells from five individuals varied between 94-100 (Table I). The modal value of 2n=96 was seen in 60% of the cells examined. The diploid metaphase complements consisted of 96 chromosomes measuring between 2-8µm.Detailed karyotype analysis revealed that the 96 chromosomes comprised of 24 metacentric, 18 submetacentric and 54 telocentric chromosomes. The number of chromosome arms was determined as (NF) =138 and the chromosomal formula can be expressed as 29=24m+18Sm+54t (Figure 1).

Schizothorax curvifrons: The somatic metaphase complements contained 94 chromosomes in 40 out of 50 cells studied (Table I). Therefore, the diploid chromosome number in this species was ascertained to be 94 and the karyotype (Figure 2) consisted of 2n=26m+20Sm+20St+28t with the fundamental arm number (NF) of 140. The size of the chromosome varied between 10.4-1µm. Cells not showing modal counts were probably caused by loss during preparation or by chromosomes being obscured by surrounding cell nuclei. ‘

Discussion

Fishes are the most speciose vertebrate group, with an estimated 24618 recognised species; more than half the total number of living vertebrate species (Nelson, 1994) and exhibit a bewildering range of diversity in their ecology, morphology, life history, behaviour and physiology (Comber and Smith, 2004). Despite their diversity, they have remained cytologically neglected as standard karyotypes are reported for less than 10% of the total extant species of fish (Gold *et al.* 1990).

Both the species of Schizothorax analysed cytologically in the present study revealed a high number of chromosomes ranging from 94-96. . Species with high numbers are considered to have resulted through polyploidy from ancestral 2n= 48 or 50 (Rishi *et al.*, 1998). Large-scale genomic expansions or whole-genome duplication events have been documented in early vertebrate evolution(Friedman and Hughes, 2001; Ohno.1970; Wang and Gu,2000), near the base of the phylogenetic tree of teleost fishes (Christoffels *et al.*, 2004; Meyer and Schartl, 1999; Robinson-Rechavi *et al*., 2001;Wittbrodt *et al*., 1998), and near the basal roots of several major teleostean clades [such as salmonids (Allendorf and Thorgaard, 1984), catastomids (Ferris, 1984; Uyeno and Smith,1972), acipenserids (Vasil’ev,1999) and some cyprinids (Larhammer and Risinger,1994)]. Such genomic enlargements have been hypothesised as key factors that enable or even drive diversification in various vertebrate groups (Holland *et al*., 1994; Meyer and Malaga-trillo, 1999; Navarro and Barton, 2003a,b; Ohno,1970; Stephens,1951). Chromosome counts in nearly all cyprinid polyploids occur in multiples or combinations of the most common karyotype (48-50) and tetraploids (96, 98 or 100) and hexaploids (148-150) have arisen through hybridisation (Dowling and Secor, 1997). Our results suggest that these fishes are tetraploid. This is well illustrated by a number of species of fish belonging to diverse orders. Buth *et al*., (1991) noted 52 such taxa most of which belong to cyprinidae identified through karyological analysis (Dowling and Secor, 1997) and such forms are ancestral polyploids (Ohno *et al.*, 1969). Polyploidy in fishes has been associated with traits including large body size, fast growth rate, long life and ecological adaptability (Uyeno and Smith, 1972; Schultz, 1980). Since *Schizothorax* fishes are hill stream fishes, it may be that polyploidy may have resulted on account of cold temperature of their habitat. The use of thermal shocks to eggs for induction of polyploidy (Chourrout, 1988) provides support to the above assertion. The role of polyploidy in evolution and survival of fish is very important because it prevents from natural selection pressure (Oellerman and Skelton, 1990).

Schizothorax plagiostomus revealed a diploid number of 29=96 (24m+18Sm+54t) and NF=138 while as *S.curvifrons* showed a diploid number of 29=94 (26m+20Sm+20St+28t) and NF=140. Decrease in the 2n and NF may be attributed to the Robertsonian arrangements and pericentric inversion (Choudhury *et al*., 1982).

The chromosomes of *S.curvifrons* were categorized into four groups (Fif.2) viz. Metacentric, submetacentric, subtelocentric and telocentric but none of the chromosomes could be recognised as subtelocentric in *S.plagiostomus* because cyprinid fishes are characterised by presence of relatively small chromosomes with their centromeric positions ranging gradually from median to nearly terminal, making it difficult to assign some chromosomes to particular

chromosomal categories and thus making correct identification of individual chromosomes nearly impossible (Rab and Collares-Pereira, 1995). And the karyological study of teleost fish presents technical difficulties which are not encountered in the study of other vertebrates and these difficulties are due to small size and high number of chromosomes (Cucchi and Baruffaldi, 1990). Further, differential arm contraction can alter a chromosome classification in a karyotypic formula (Joswiak *et al.*, 1980).

The overall dissimilarity in the 2n, Karyotypic configuration and NF points to the role of almost all types of chromosomal rearrangements in the karyological evolution of these two fishes. Both these fishes are new to the cytological literature.

Table I: Showing percentage frequency of the metaphases.

Species	No. Of chromosomes	No. Of cells	Frequency % of chromosomes	Modal diploid No.
Schizothorax plagiostomus	94	5	8.33	
	96	36	60	**96**
	98	12	20	
	100	7	11.66	
Schizothorax curvifrons	92	4	8	
	94	40	80	**94**
	96	6	12	

Table II: Chromosome morphometry of *Schizothorax plagiostomus* (m=metacentric; Sm= sub-metacentric; St= sub-telocentric; t=telocentric).

Pair No.	Length of short arm (µm) 'S'	Length of long arm (µm) 'L'	Total length(µm) 'L+S'	Arm ratio (L/S)	Centromeric index	Category
1	3	5	8	1.6	37.5	m
2	4	4	8	1	50	m
3	3	4	7	1.3	42.8	m
4	3	3	6	1	50	m
5	3	3	6	1	50	m
6	3	3	6	1	50	m
7	2	3	5	1.5	40	m
8	2	3	5	1.5	40	m
9	2	3	5	1.5	40	m
10	2	3	5	1.5	40	m
11	2.5	2.5	5	1	50	m
12	2	2	4	1	50	m
13	2	5	7	2.5	28.5	Sm
14	2	5	7	2.5	28.5	Sm
15	2	5	7	2.5	28.5	Sm

16	2	4	6	2	33.3	Sm
17	1	3	4	3	25	Sm
18	1	2.5	3.5	2.5	28.5	Sm
19	1	2.5	3.5	2.5	28.5	Sm
20	1	2	3	2	33.3	Sm
21	1	2	3	2	33.3	Sm
22	0	4	4	∞	0	t
23	0	4	4	∞	0	t
24	0	4	4	∞	0	t
25	0	4	4	∞	0	t
26	0	4	4	∞	0	t
27	0	4	4	∞	0	t
28	0	4	4	∞	0	t
29	0	4	4	∞	0	t
30	0	3	3	∞	0	t
31	0	3	3	∞	0	t
32	0	3	3	∞	0	t
33	0	3	3	∞	0	t
34	0	3	3	∞	0	t
35	0	3	3	∞	0	t
36	0	3	3	∞	0	t
37	0	3	3	∞	0	t
38	0	3	3	∞	0	t
39	0	3	3	∞	0	t
40	0	3	3	∞	0	t
41	0	3	3	∞	0	t
42	0	3	3	∞	0	t
43	0	2.5	2.5	∞	0	t
44	0	2.5	2.5	∞	0	t
45	0	2	2	∞	0	t
46	0	2	2	∞	0	t
47	0	2	2	∞	0	t
48	0	2	2	∞	0	t

Table III: Chromosome morphometry of *Schizothorax curvifrons* (m=metacentric; Sm= sub-metacentric; St= sub-telocentric; t=telocentric).

Pair No.	**Length of short arm (µm) 'S'**	**Length of long arm (µm) 'L'**	**Total length(µm) L+S**	**Arm ratio (L/S)**	**Centromeric index**	**Category**
1	5.2	5.2	10.4	1	50	m
2	5	5	10	1	50	m
3	5	5	10	1	50	m
4	4	4.5	9.5	1.12	42.1	m
5	4	4.5	9.5	1.12	42.1	m
6	3	3.5	6.5	1.16	46.1	m
7	3	3.5	6.5	1.16	46.1	m
8	3	3	6	1	50	m

9	2.5	2.5	5	1	50	m
10	2.3	2.3	4.6	1	50	m
11	2	2	4	1	50	m
12	2	2	4	1	50	m
13	2	2	4	1	50	m
14	3	5.5	8.5	1.83	35.2	Sm
15	2.9	5.4	8.3	1.86	34.9	Sm
16	2.5	5.3	7.8	2.12	32.0	Sm
17	2.2	5	7.7	2.27	28.5	Sm
18	2	5	7	2.50	28.5	Sm
19	1.8	4.1	5.9	2.27	30.5	Sm
20	1.5	3.8	5.3	2.53	28.3	Sm
21	1.2	3.3	4.5	2.75	26.6	Sm
22	1.2	3.3	4.5	2.75	26.6	Sm
23	1	2.8	3.8	2.80	26.3	Sm
24	1.3	4.8	6.1	3.69	21.3	St
25	1	4.5	5.5	4.5	18.1	St
26	1	4.2	5.2	4.2	19.2	St
27	1	4	5	4	20	St
28	1	3.8	4.8	3.8	20.8	St
29	1	3.8	4.8	3.8	20.8	St
30	1	3.7	4.8	3.8	20.8	St
31	1	3.6	4.6	3.6	21.7	St
32	1	3.6	4.6	3.6	21.7	St
33	1	3.6	4.6	3.6	21.7	St
34	0	6	6	∞	0	t
35	0	6	6	∞	0	t
36	0	6	6	∞	0	t
37	0	5	5	∞	0	t
38	0	5	5	∞	0	t
39	0	5	5	∞	0	t
40	0	5	5	∞	0	t
41	0	5	5	∞	0	t
42	0	5	5	∞	0	t
43	0	4	4	∞	0	t
44	0	4	4	∞	0	t
45	0	4	4	∞	0	t
46	0	3	3	∞	0	t
47	0	1	1	∞	0	t

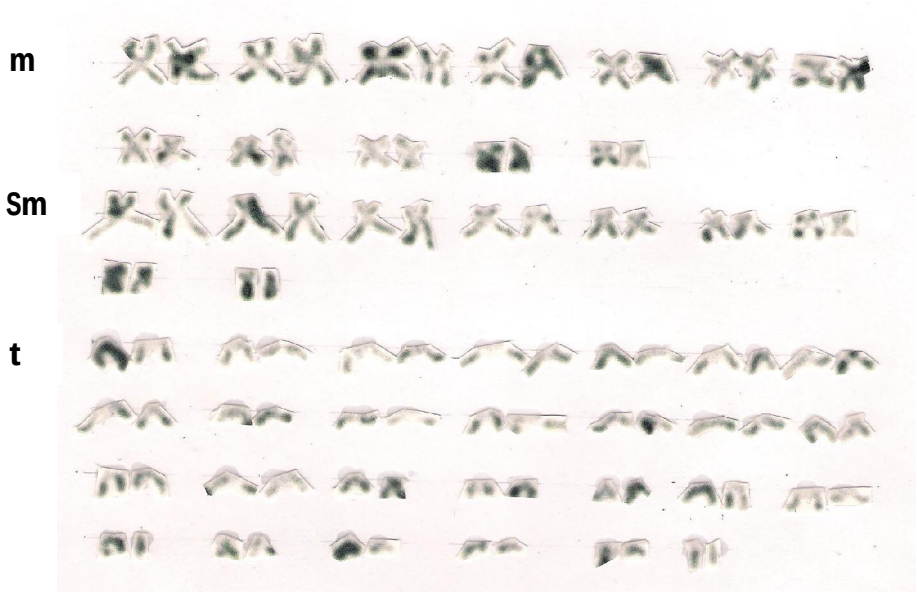

Figure 1: Karyotype of *Schizothorax plagiostomus*

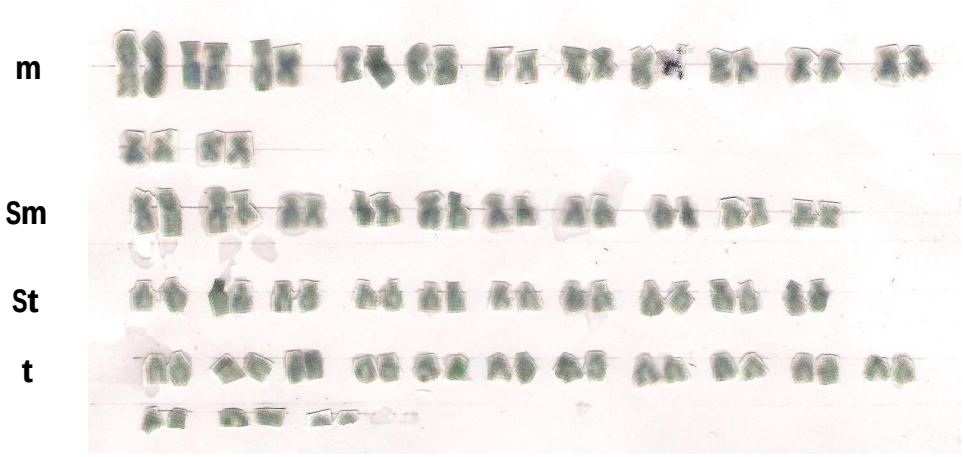

Figure 2: Karyotype of *Schizothorax curvifrons*

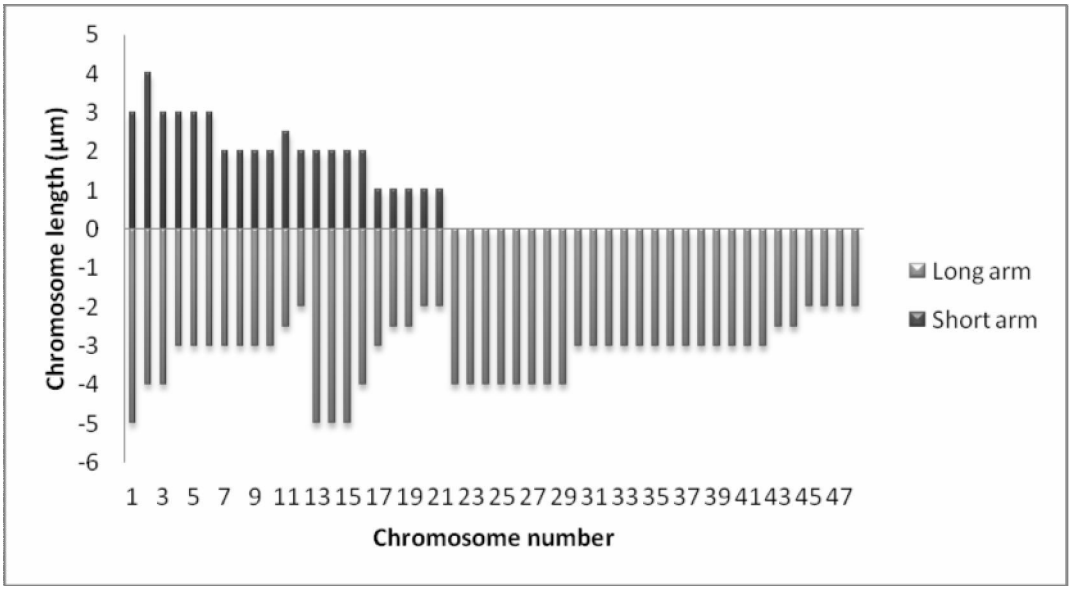

Figure 3: Haploid ideogram of *Schizothorax plagiostomus*.

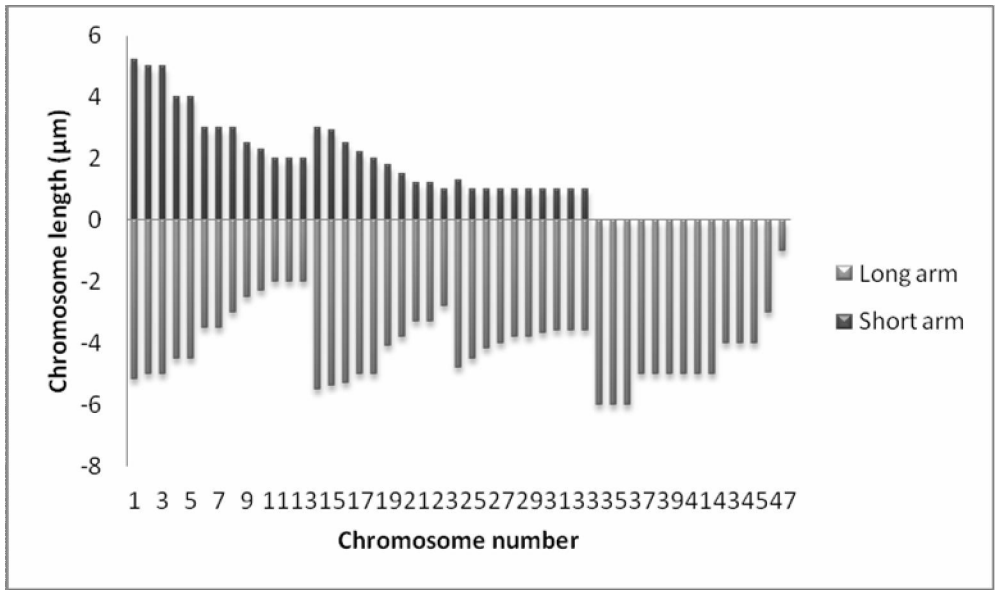

Figure 4: Haploid ideogram of *Schizothorax curvifrons.*

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Biotechnological potential of bacterial flora from Cheend juice: Alcoholic beverage from Bastar, India

Shukla P*, Vishwakarma, P and Gawri S

G.D. Rungta College of Science and Technology, Bhilai 490024, Chhattisgarh, India
prashant19782000@gmail.com

Abstract: Cheend is an alcoholic beverage of tribal people of Bastar region of Chhattisgarh State in India. It is extracted from *Phoenix dactylefera*. From a sample of Cheend juice seven different bacteria were isolated which were of four genera: four of Bacillus, one each of Paenibacillus, Micrococcus and Streptococcus. All the seven bacteria produced ethanol, PHA and EPS in varying capacity.
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Key words: Bacteria; ethanol; PHA; EPS; Cheend

Introduction

Cheend juice is one of the alcoholic beverages of tribal people of Chhattisgarh state in India. Cheend juice is extracted from a palm *Phoenix dactylefera*. The juice of this palm is used as an alcoholic beverage by the tribal people of Chhattisgarh since ages. Cheend is extracted from the inflorescence of the palm. The young inflorescence is cut and an earthen pot is tied below the cut to collect the juice. The juice is collected very slowly as it oozes from the cut into the pot. The fresh juice is sweet in taste and as it is kept microbial activity within the juice converts the sugars in the juice into acids and alcohols. Therefore after sometime the taste of the juice turns from sour to bitter. Cheend is of much practical importance to the tribal people of the state of Chhattisgarh as the juice collected from it provides a means of economic benefit to the people owning the palm.

Beer was brewed by Babylonians and also exported to Egypt around 3000 BC (Abegaz, 2007). Boza from Turkey (Hancioglu and Karapinar, 1997), borde and tej from Ethiopia (Abegaz, 2007, Bahiru *et. al*. 2001), sobia from Saudi Arabia (Gassem, 2002), Fermented milk product from Fulani (a tribe) of Burkina Faso (Savadogo *et. al.* 2004), pulque a traditional Mexican alcoholic beverage (Escalante *et. al.* 2004), suusac from Kenya (Lore *et. al*. 2005), bhaati jaanr from Eastern India, hamei and marcha from Sikkim and Manipur (Tamang and Thapa, 2006, Tamang *et. al*. 2007) are just few fermented food products and beverages. Many others, which are also used, may not have found themselves in the literature. Cheend is one such product.

Almost all the traditional fermented beverages have microbial activity within them which makes these drinks alcoholic. These drinks harbor many types of bacteria and fungi. Many different uses of these microorganisms have been found in the modern day fields which are found in abundant in these traditional drinks.

The purpose of this study was of to isolate bacteria from juice of Cheend along with their characterization and probable identification along with the testing of biotechnological potential of the isolated bacteria like production of PHA, EPS along with ethanol production.

Material and Methods

Cheend juice was brought to the laboratory from Garhbengal, Narayanpur, Dist. Narayanpur (C.G), India. The sample was inoculated onto the NAM media plate. Morphologically distinct colonies were pure cultured on NAM slants. The pure cultured colonies were then characterized morphologically, physiologically and biochemically. NAM was used for obtaining colonies from Cheend juice and maintaining pure cultures.

Morphological tests performed were Gram's test and endospore test. Physiological tests performed were growth at different temperatures, pH and NaCl concentrations. Different biochemical tests were performed for probabilistic identification. Ethanol production was tested on various substrates. Production of PHA and exopolysaccharides were also tested in the bacteria isolated from Cheend juice.

Qualitative estimation of ethanol:

0.5 ml H_2SO_4 and 1 ml $K_2Cr_2O_7$ is taken in a test tube. 1 ml-distilled solution is added to the chromate solution. Blue green color indicated the presence of ethanol in the distilled liquid.

Quantitative estimation of ethanol:

Quantitative estimation of ethanol from the distilled liquid is done the method given by Caputi Jr. *et. al.* (1968).

0.5 ml H_2SO_4 and 1 ml $K_2Cr_2O_7$ are taken into a test tube and a series is made with ethanol. Along with 0.5 ml H_2SO_4 and 1 ml $K_2Cr_2O_7$ 3 ml of distilled liquid is taken for the test sample.

Production of PHA and EPS:

For production of PHA the seven bacteria were grown on specialized medium as given by Lee and Choi (1999). PHA production was tested at 37°C. Same sample was used for extraction of exopolysaccharides.

Extraction of PHA and EPS:

PHA was extracted from the bacterial cells using sodium hypochlorite method (Law & Slepecky, 1960, Matsuyama *et al* 1999, Garcia *et al* 2001) and EPS by method given as in Welman *et al*, 2003. The following procedure was employed for extraction of PHA and exopolysaccharides from a single sample.

10 ml broth containing bacterial cultured was centrifuged for 20 minutes at 1500 rpm. The bacterial pellet was taken for PHA extraction and the supernatant for extraction of exopolysaccharides. Supernatant was again centrifuged for 20 minutes at 1500 rpm to separate remaining cells. 5 ml supernatant was taken in fresh tube and 3 times volume ethanol was added to it and was incubated for 1 hour to precipitate the exopolysaccharides. The bacterial pellet was resuspended in 5 ml distilled water and PHA was extracted from bacterial cells using sodium hypochlorite method.

Results and discussions:

From the sample of Cheend juice seven different bacteria were isolated on NAM and were named A to G. On the basis of various tests performed on the bacterium four different genera were identified. A, D, E and G were identified as bacteria of *Bacillus* genera, while B is of *Paenibacillus*, C is of *Micrococcus* and F belongs to *Streptococcus*.

Results of various testes performed on the bacteria isolated from Cheend juice are given in different tables. Results of morphological testes are presented in Table 1, physiological tests in Table 2, ethanol production in Table 3 and PHA and EPS production in Table 4.

All the bacteria produced highest ethanol in either apple juice, radish juice of mango pulp (Table 3). Species of Micrococcus i.e. C gave highest PHA production while one of the Bacillus species gave highest EPS production (Table 4).

It can be said that Cheend juice has diversity of bacteria and as it has been shown all the bacteria isolated were able to produce ethanol, PHA and EPS which also is an indication that bacteria present in Cheend juice would have biotechnological potential and can be utilized as such.

Table 1: Results of Morphological tests performed on the bacterial strains

So.No	TEST	A	B	C	D	E	F	G
1	Colour	Creamy	creamy	Brown Yellow	Off white	creamy	creamy	Creamy
2	Margin	Entire	Wavy	Entire	Entire	Entire	Entire	Wavy
3	Elevation	Elevated	Elevated	Elevated	None	None	Elevated	None
4	Pigment	None	None	Present	None	None	None	None
5	Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Transperent	Opaque
6	Surface	Smooth	Rough	Smooth	mucelaginous	mucelaginous	Smooth	Rough
Cell Morphology								
1	Gram Stain	Positive	Positive	Positive	Positive	Positive	Positive	Positive
2	Cell Shape	Rods	Rods	Coccus	Rods	Rods	Coccus	Rods
3	Arrangement	Single	Single arrange in L shape	Arrange in chain	Single arrange in L & V shape	Single group and chain	Bunch	Single
4	Endospores	Present	Present	Absent	Present	Present	Absent	Present
5	Motility	Non motile	Motile.	Non motile	Non motile	Motile.	Non Motile	Non motile

Table 2: Results of Physiological tests performed on the bacterial strains

S.No	TEST	A	B	C	D	E	F	G
A	Growth in different temperature							
1	37°C	+	+	+	+	+	+	+
2	40°C	+	+	+	+	+	+	+
3	45°C	+	+	+	+	+	+	+
4	50°C	+	+	+	+	+	+	+
5	55°C	+	+	+	+	+	+	+
6	60°C	+	+	+	+	+	+	+
7	65°C	+	+	+	+	-	-	+
8	70°C	+	-	-	-	-	-	-
B	Growth in different pH (O.D)							
1	2	0.01	0	0	0.02	0.01	0	0.02
2	3	0.02	0.01	0.01	0.04	0.01	0.02	0.02
3	4	0.03	0.03	0.03	0.04	0.02	0.03	0.03
4	5	0.06	0.05	0.04	0.09	0.05	0.04	0.06
5	6	0.04	0.05	0.07	0.1	0.07	0.05	0.07
6	7	0.05	0.06	0.08	0.08	0.06	0.05	0.07
7	8	0.07	0.03	0.05	0.07	0.05	0.06	0.05
8	9	0.04	0.01	0.03	0.06	0.05	0.02	0.03
9	10	0.01	0	0.01	0.05	0.02	0.01	0.01
C	Growth in different NaCl concentration							
1	2%	+	+	+	+	+	+	+
2	4%	+	+	+	+	+	+	+
3	6%	+	+	+	+	+	+	+
4	8%	-	-	-	-	-	-	-

Table 3. Ethanol production by bacteria

S.No	Sources	Percentage of ethanol production (%)						
		A	B	C	D	E	F	G
1	Plum	6.16	5.33	3	5.16	5.5	3.16	3.33
2	Pomegranate	3	5	3.83	3.33	3.83	4.33	4
3	Fig	4	4.5	4.5	4.33	4.5	4.83	4.5
4	Apple	8.33	5.33	7.83	8.33	7.16	7.66	7.16
5	Banana	4.66	4.33	6	5.33	6.66	6	6.33
6	Pearl millet	3.83	3.83	4.33	4	4.83	4.16	4.5
7	Beet	5.83	6	6.83	6.16	7.33	6.16	6.33
8	Carrot	7.5	7.66	7.66	7.83	7.66	7.83	6.83
9	Corn	4.16	4.66	5.66	4.16	4.83	4.83	4
10	Date	6.33	5.66	6.5	6.5	6	6.33	6.66
11	Radish	5.33	5.33	5.66	3.33	6	5.66	5
12	Gorgon Nut	7.16	6.83	5.83	5.66	5.83	6.83	7.83
13	Mango	6.33	7.16	7.83	6.83	7	7.16	6.66
14	Pear	6.16	7	6	6.83	5.83	6	7.33
15	Pine apple	4.66	5	6.33	6	6	6.33	6.33
16	Potato	7.33	7.16	5.16	5.33	5.33	7.66	7.33
17	Rice	6	6	7	6.33	5.33	6.5	6
18	Water chest nut	3.66	4.5	4.66	3.5	3.33	5.5	5
19	Sugar	5.33	5.83	5	5.16	5.33	5.66	5.5
20	Sweet lemon	3.83	4.33	4.83	4.66	2.83	4.83	3.83
21	Wheat	4.5	4.5	4.33	5.16	5	4.16	5.16

Table 4: PHA and EPS production

S.No.	Bacteria	PHA production (g/l)	EPS production (g/l)
1	A	0.03	0.005
2	B	0.01	0.01
3	C	0.1	0.01
4	D	0.02	0.02
5	E	0.02	0.06
6	F	0.01	0.02
7	G	0.012	0.01

Correspondance to:
Dr. Prashant Shukla
GD Rungta College of Science and Technology
Bhilai-490024
Chhattisgarh, India
prashant19782000@gmail.com
Mob. 919300468815

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Serum Trace Element Levels In Sickle Cell Disease Patients In An Urban City In Nigeria

[*1]Idonije B.O, [2]Iribhogbe O.I, [3]Okogun G.R.A

[1]Department of Chemical Pathology, [2]Department of Pharmacology and Therapeutics and [3]Department of Medical Microbiology, College of Medicine, Ambrose Alli University Ekpoma.
*oignis@yahoo.com

ABSTRACT: Assessment of serum trace element levels was carried out in a total of eighty (80) subjects comprising of forty (40) sickle cell disease patients attending the sickle cell centre, Benin City and forty (40) age and sex matched apparently healthy control subjects. Blood samples collected from participants were analyzed for trace elements using atomic absorption spectrophotometer. The mean serum level of magnesium, zinc, manganese, copper, selenium and chromium in sickle cell disease patients were 11.03±1.77mg/L, 120.85±10.29µg/dL, 68.30±3.63µg/dL, 68.54±10.49µg/L, 60.98±7.29µg/L and 62.90±5.97µg/L respectively. Serum magnesium, zinc and selenium levels were significantly lower ($p<0.05$) while serum manganese levels were significantly higher ($p<0.05$) in sickle cell disease patients when compared with apparently healthy control. Serum trace metal levels was not age or sex dependent, as similar pattern of serum trace metals was observed in both male and female sickle cell disease patients. Conclusively, assessment of trace element levels is vital in the management of sickle cell disease. Supplementation with deficient trace elements may reduce the severity of symptoms and complications associated with sickle cell disease, thereby improving the chances of survival in sickle cell disease.

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Key Words: Sickle cell disease, Serum Trace Metals, Oxidative Stress.

INTRODUCTION

Sickle cell disease is an inherited blood disorder that affects red blood cells. People with sickle cell disease have red blood cells that contain mostly hemoglobin S, an abnormal type of hemoglobin. In certain situation, these red cells become sickle and have difficulty in passing through blood vessels (Platt, 2000; Platt et al., 2004). Although sickle cell disease is present from birth, symptoms are rare before the age of 3 to 6months since a large percentage of the erythrocyte hemoglobin is of the fetal type (Hb F). As more Hb S replaces Hb F in the subject, the main symptoms; episode of anemia, pains and infections and associated crisis become manifested due to irreversible sickling of the erythrocytes when Hb S molecules polymerizes invariably leading to vaso-occlusion in the small capillaries (Durosinmi et al., 1993). Trace elements are essential inorganic molecules found in minute quantities of milligram or microgram per kilogram of body weight. Trace elements include zinc, copper, selenium, manganese, chromium, magnesium, fluorine, cobalt, iron and iodine. Some such as lead, cadmium, arsenic, aluminium and nickel are classified as pharmacologically beneficial and toxic hence monitoring of dosage is required (Burtis et al., 2008).

People with sickle cell disease suffer from many micronutrient deficiency but preliminary research on dietary habits show that food and nutrient intake by sickle cell patients meet or exceeds recommendation and is not significantly different from healthy controls. This suggests that higher rates of nutrient deficiency may be due to increased needs of many nutrients in sickle cell patients. (Tagney et al., 1989). The global use of micronutrients in health care delivery system has taken central stage due to the realization of their importance in disease management. Sickle cell disease is among the disease plaguing a sizeable population of the developing world and the cost implication of its management is very high. Sickle cell disease is characterized by anemia and immunological disturbances. Free radicals are generated in sickle cell disease; hence a balance between minerals and antioxidants is imperative in maintaining red cell membrane integrity and function (Okpuzor and Okochi, 2009). Protection of red cell membrane from free radical mediated oxidative stress is crucial to the management of sickle cell disease. Minerals such as copper, zinc, iron, chromium, magnesium, selenium, vanadium as well as vitamins like vitamin

A, C, E, folate and vitamin B complex have been found to relieve oxidative stress associated with red cell membranes (Reed et al., 1987).

MATRIALS AND METHODS

Study Design

This is a case control study with sequential recruitment of study participants with sickle cell disease and those without the disease (apparently healthy) with genotype AA or AS who served as control.

Study Subjects

A total of 40 sickle cell disease patients and 40 apparently healthy controls were recruited for this study from the sickle cell centre, Benin City after obtaining ethical clearance from an ethical review board and appropriate informed consent from the subjects as well as their parents/guardian. The recruited participants were appropriately age and sex matched.

Sample Collection/Analysis

Blood samples (5mls) were collected by venepuncture from each subject into a plain container. The labeled samples were spun in a bucket centrifuge at a speed of 2500rps to separate serum from red cells. The serum obtained was stored in a chest freezer at a temperature of -20^0C. Serum trace elements (copper, zinc, chromium, magnesium and selenium) levels were determined by atomic absorption spectrophotometer technique as described by Kaneko (1999).

Data Analysis

Data obtained was analyzed using SPSS version 17 statistical soft ware package. Results were expressed as mean ± SD and a P value of <0.05 was considered significant.

RESULTS

As shown in Table 1, there was a reduction in serum Mg, Zn, Se and Cr levels. This reduction was however, significant (P<0.05) for serum Mg (11.03±1.77mg/L), Zn (120.85±10.29 µg/dl) and Se (60.98±7.29µg/dl) levels when compared with control. This was also the case in male and female sickle cell disease patients. However, in male sickle cell disease patients the reduction in the serum zinc concentration was not significant (P>0.05) when compared with control (Table 3). The serum manganese concentration (68.3±3.63 µg/dl) was significantly elevated (P<0.05) in the sickle cell patients when compared with apparently healthy control (63.10±5.70µg/dl). This profile was also observed in male and female sickle cell disease patients (Table 2 and 3). However, among the different sickle cell age groups examined there was no statistically significant difference in serum trace metal levels when compared with apparently healthy control within the same age group.

Table 1: Serum Trace Element Levels in Sickle Cell Disease Patients

Trace Elements	Patients N = 40	Controls N = 40
Mg (mg/L)	11.03±1.77*	12.35±0.89
Zn (µg/dl)	120.85±10.29*	127.10±14.25
Mn (µg/dl)	68.3±3.63*	63.10±5.70
Cu (µg/dl)	68.54±10.49	67.45±3.37
Se (µg/dl)	60.98±7.29*	65.75±5.49
Cr (µg/dl)	62.90±5.97	64.43±6.15

Values are expressed as Mean ± SD, P<0.05 is considered significant compared with control

Table 2: Serum Trace Element Levels in Female Sickle Cell Disease Patients

Trace Elements	Patients N = 16	Controls N = 16
Mg (mg/L)	11.43±0.81*	12.54±1.00
Zn (µg/dl)	116.88±10.18*	129.69±13.64
Mn (µg/dl)	68.30±3.74*	62.38±4.50
Cu (µg/dl)	67.56±5.24	67.31±3.59
Se (µg/dl)	61.38±8.67	65.94±4.68
Cr (µg/dl)	61.88±7.18	63.25±7.33

Values are expressed as Mean ± SD, P<0.05 is considered significant compared with control

Table 3: Serum Trace Element Levels in Male Sickle Cell Disease Patients

Trace Elements	Patients N = 24	Controls N = 24
Mg (mg/L)	11.18±0.74*	12.22±0.80
Zn (µg/dl)	123.5±9.67	125.69±14.68
Mn (µg/dl)	68.25±3.63*	63.42±6.45
Cu (µg/dl)	69.04±3.28	67.54±3.30
Se (µg/dl)	60.75±6.40*	65.63±6.06
Cr (µg/dl)	63.58±5.05	65.21±5.24

Values are expressed as Mean ± SD, P<0.05 is considered significant compared with control

Table 4: Age Distribution of Serum Trace Element Levels in Sickle Cell Disease Patients

Trace Elements	Patients/Age Groups (years)			Control/Age Groups (years)		
	1-15, N=18	16-25, N= 12	≥ 26, N= 10	1-15, N= 20	16-25,N= 17	≥ 26, N =3
Mg (mg/L)	11.36±0.94	11.32±0.87	11.08±0.59	12.24±0.97	12.35±1.04	13.07±1.11
Zn (µg/dl)	120.11±5.41	123.33±3.47	119.2±2.17	127.65±3.24	126.06±6.78	126.33±9.71
Mn (µg/dl)	67.78±2.13	68.58±4.15	68.9±3.21	61.20±2.49	64.88±5.23	64.33±3.48
Cu (µg/dl)	68.89±5.25	67.08±2.47	69.3±5.78	66.95±6.27	67.82±4.57	68.67±4.47
Se (µg/dl)	60.11±3.17	61.67±2.65	61.70±3.21	64.70±4.15	66.59±3.23	68.00±4.67
Cr (µg/dl)	62.33±6.42	62.08±4.19	64.90±7.13	62.30±3.25	65.88±4.29	70.30±6.78

Values are expressed as Mean ± 2SD, P<0.05 is considered significant compared with control

DISCUSSION

The deficiencies of essential trace elements some of which are important in red blood cell maintenance, body growth and development have been observed in sickle cell disease (Durosinmi et al., 1993; Okpuzor and Okochi, 2009). A significantly low serum magnesium, zinc and selenium concentration was obtained from the general comparison of sickle cell disease patients with control subjects. In a study conducted by Defrancheschi et al., (1997), low concentration of red blood cell magnesium have been noted in patients with sickle cell disease, this in turn is thought to contribute to red blood cell dehydration and a concomitant increase in the symptoms of sickle cell disease. Significantly low serum magnesium obtained in this study may also contribute to the low red cell magnesium level which is in agreement with the study of Defrancheschi et al., (1997). The significantly low serum zinc level is in agreement with the report of Prasad and Cossack, (1993) and Prasad, (2002) who related zinc deficiency in sickle cell disease to manifestations such as growth retardation, hypogonadism in males, hyperammonemia, abnormal dark adaptation and cell mediated immune disorder. Zinc deficiency can also be the result of the adverse effect of hydroxyurea which increase zinc excretion as reported by Silliman et al., (1993). The significantly low serum selenium level is in agreement with the report of Durosinmi et al., (1993). Selenium plays an important role as a cofactor for the reduction of antioxidant enzyme such as glutathione peroxidase, an enzyme which helps react with potentially harmful oxidizing agents in substances like hemoglobin. High levels of glutathione function in the blood are associated with longevity. Deficiency of selenium can thus be attributed to the mortality in sickle cell disease. The reason for the significantly elevated serum manganese level in sickle cell disease patients is not yet known. Our findings demonstrate a similar trace element profile in both male and female sickle cell disease patients when compared with sex matched controls. This suggests that the serum pattern of trace element is not sex dependent.

CONCLUSION

Although, not much discoveries have been made on the relevance of trace elements in sickle cell disease, it can be concluded from our findings that deficiency of essential trace elements important in maintenance of erythrocyte stability and in proper growth and development, occur in sickle cell disease. Hence, dietary supplementation with essential trace elements may be used as adjuvant in sickle cell therapy.

Correspondence to:
Idonije B.O
Department of Chemical Pathology, College of Medicine,
Ambrose Alli University, Ekpoma, Edo State, Nigeria.
E mail:oignis@yahoo.com

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Rice Husk Extract is Potentially Effective as a Phytopesticide against Root-/Soil-borne Fungal Pathogens of Cowpea

A.S. Killani[1, 2], R.C. Abaidoo [1*] and A.K. Akintokun [2]

1.International Institute of Tropical Agriculture PMB 5320, Ibadan,Oyo State, Nigeria
2. Department of Microbiology, College of Natural Sciences, University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria
E mail: killani405@yahoo.com, skillani@cgiar.org; rabaidoo@cgiar.org ron_akintokun@yahoo.com

Abstact: Phytopesticide produced from rice husk extract (RHE) was evaluated, in the laboratory and in the glasshouse as a potential biocontrol agent for controlling root- and soil-borne fungal pathogens isolated from field-grown cowpea in the northern Guinea savanna of Nigeria. The pathogenicity test was carried out in the glasshouse on the fungal species isolated from infected plants in cowpea field trials conducted in 2006 and 2007 cropping seasons. Five root- and soil-borne fungal pathogens: *Fusarium verticilloides*, *F.equiseti*, *F.solani*, *F. oxysporum* and *Rhizoctonia solani*, were the major highly virulent fungal pathogens which caused severe problems including damping off, root rot, reduction in nodulation, vascular wilt/discoloration, chlorosis, necrotic lesions, leaf blight, complete defoliation, seedling mortality, and death in cowpea. Plants from the glasshouse experiments (on the microbial antagonism study) were examined for disease incidence and severity symptoms. *In–vitro* and *in–vivo* studies revealed that RHE significantly (P<0.05) inhibited all the five fungal pathogens at 1.5% concentration. However, at 1% concentration of RHE did not inhibit mycelia radial growths of *F. verticilloides*, *F. equiseti* and *F. oxysporum* after 7 days incubation *in-vitro*. The RHE was phytotoxic on cowpea seedlings at 2% concentration. The RHE can thus be regarded as a potential bioprotectant as an alternative to chemical pesticides which are known to be environmentally unsafe for the management of common root- and soil–borne fungal pathogens of cowpea.
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1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is highly susceptible to a number of root-/soil-borne fungal diseases, causing great losses in yield and seed quality (Lichtenzveig et al. 2006). A critical appraisal of why farmers in sub-Saharan Africa particularly, in Nigeria are becoming skeptical about growing cowpea unlike other legumes showed that the number one reason was the increasing yield losses that root-/soil-borne fungal pathogens have been causing over the years. Despite the technological changes in cowpea research across the globe which had resulted in yield increases over-time, diseases and pests have been identified as major production constraints (Singh et al. 1989), limiting high productivity and accounting for more than 80% yield losses in cowpea (Komarwa et al. 2002). In Africa, diseases and pests are often responsible for 100% losses of cowpea yield if not controlled. Yield losses to pests in northern Nigeria of 78% and 80% in southern Nigeria have been reported (Booker, 1965).

Cowpea is attacked by more than 35 major diseases caused by viruses, bacteria, fungi and nematodes (Emechebe and Soyinka, 1985). Lichtenzveig et al. (2006) reported that root-/soil-borne fungal pathogens are causal agents of legume diseases of increasing economic importance, such as root rots, seedling damping-off, and vascular wilts. As agricultural production intensified over the years, farmers became increasingly dependent on application of agrochemicals as a method of crop protection and conservation without considering safer and environmentally friendly biological control agents (Newsham et al. 1995). The increasing trend in environmental awareness has prompted efforts towards finding environmentally and toxicologically safe and efficacious integrated disease management options (Mukerji and Ciancio, 2007). Likewise, the increasing incidence of resistance by pests to pesticides and environmental impact associated with the use of agrochemicals for crop protection contributed immensely to the search for safer and environmentally friendly pest control measures. In this respect, natural products are considered to be potential sources of developing biodegradable pesticides.

Plants are known to produce a variety of secondary metabolites, which are bioactive and thus may have inhibitory effects on bacteria, fungi, insects and other microorganisms (Odebode et al. 2004). The objective of this study was to evaluate the effect of (RHE) as a phytopesticide against root-/soil-borne fungal pathogens isolated from cowpea in northern Guinea savanna of Nigeria

2. Materials and Methods

2.1 Laboratory Experiments

2.1.1 Isolation and Identification of Root-/soil-borne Fungal Pathogens

Root-/soil-borne fungi were isolated in the laboratory from naturally infected roots/stems of cowpea plants and their rhizosphere soil collected from the experimental plot site. Infected plant tissues were surface sterilized in 3% sodium hypochlorite (NaClO) for 3 min, rinsed in three changes of sterile distilled water and then blotted dry with a sterile paper towel pad. Approximately 2mm × 7mm tissue sections were cut from the advancing portion of the lesion of surface sterilized tissue using a sterile scalpel blade. The sections were plated on specific *Fusarium* spp. medium [Peptone Pentachloronitro-benzene Agar (PPA) modified by Nash and Snyder (1962). The PPA contained the following: Difco agar powder (15 g l^{-1}), peptone (15 g l^{-1}), KH_2PO_4 (1 g l^{-1}), $MgSO_4.7H_2O$ (0·5 g l^{-1}). The medium was autoclaved at 120°C for 20 min. The medium was then amended with Chloramphenicol (0·05 g l^{-1}), Pentachloronitrobenzene (0·75 g l^{-1}), Chlorotetra-cycline (0·5% in water) (10 ml l^{-1}) as suggested by Ros et al. (2005).

For *R. solani*, full strength Difco Potato Dextrose Agar (PDA), prepared according to manufacturer's specifications was employed. The plates were incubated at 28°C in an incubator (Model Gallenkamp Cooled Incubator) for 7 days. Young active growth of different fungal mycelia from each isolate on the plated tissue was sub-cultured onto PDA and *Fusarium* spp. isolates were purified using single spore technique on PPA. The pour plate method was used for the isolation of fungi from the soil. Fungal isolates were characterized and identified based on their colonial morphology and microscopic characteristics using different identification keys and methods developed by Domsch et al. (1980); Nelson et al. (1983); and Summerell et al. (1993). The phytopesticide **(**concentrated RHE) was obtained from Gernplasm unit, IITA, Ibadan.

2.1.2 *In-vitro* Phytopesticide and Pathogens Antagonistic Interaction

One litre of PDA (39 g/l) was prepared in media bottles and dispensed at varying volumes of 99 ml, 98.5 ml, 97.5 ml, 95 ml and 100 ml (control) into 250 ml sterile media bottles. Bottles and its contents were then sterilized in the autoclave at temperatures of 121°C for 15 min at 1.2 bar. The medium was allowed to cool to 45°C. Thereafter, 1 ml, 1.5 ml, 2 ml, 2.5 ml, and 5 ml of RHE were aseptically measured with a 5 ml sterile syringe into the sterilized medium to represent concentration of 1%, 1.5%, 2%, 2.5% and 5% (v/v). Each bottle was rolled in the palm to allow a homogenous mixture of medium and the extract. Fifteen millilitres of this mixture was poured aseptically into 9 cm sterile disposable Petri dishes and allowed to solidify at room temperature inside the laminar flow. With a sterile 5 mm cork borer, mycelia discs of young actively growing cultures of each pathogen were cut separately and inoculated right at the centre of the already prepared plates containing the mixtures and the control plates. There were three replicates for each pathogen, both on the cultured plates with RHE and the control. The plates were incubated at 28°C for 9 days and periodically observed for antagonist-pathogen interactions. Laboratory data were collected at 3, 6, and 9 days. The mycelial growth diameter (cm) of each pathogen was measured and the percentage growth inhibition was calculated according to Awuah (1989) and Odebode et al. (2004) as follows:

Percentage of growth inhibition = $(D_c - D_t) / D_c \times 100$.

Where D_c = Diameter of pathogen in the control plates, and D_t = Diameter of the pathogen in the treatment plates

2.2 Glasshouse Experiments

These experiment were conducted in the glasshouse of IITA, Ibadan, Nigeria (7°30'N, 3°5'E) using 3 kg (dry weight) of sterilized sub–soil and acid washed ocean sand (1:1) with the following physico–chemical properties: soil texture: sand, 80%, silt, 10%, clay, 10%, pH, 6.0, organic C, 0.39%, N, 0.036%, Ca, 1.546 cmol+/kg, Mg, 0.38 cmol+/kg, K, 0.08 cmol+/kg, Na, 0.81 cmol+/kg, Exch. Acidity, 0.00 cmol+/kg, ECEC, 2.82 cmol+/kg, Zn, 0.41 ppm, Cu, 1.43 ppm, Mn, 95.84 ppm, and Fe, 53.77 ppm.

2.2.1 Pathogenicity Test

This *in-vivo* assay was conducted in the glasshouse employing the method developed by Koch (1891) and modified by Ros et al. (2005). Twelve root-/soil-borne fungal isolates were randomly selected based on their degree of occurrence and virulence in the field from the 22 fungi isolated from diseased roots/stems of cowpea and soil collected from their rhizosphere in the NGS agroecology for the glasshouse pathogenicity test. The objective was to select the most virulent strains among several isolates. Isolates R101A, R105E, R126F, S106B, S103C, S102D, S126F, S117J, S109I, R110J, R113K, and S112L, were sub-cultured on Difco PDA and incubated at 28°C for 7days. Spores of each fungal isolate were harvested and suspended in sterile distilled water. The fungal spores' suspension was re-adjusted and standardized spectrophotometrically to approximately 10^7–10^8 spores/mL (CFU mL^{-1}) with SDW (Optical Density (OD) of 1.0–1.3 at 600 nm using Spectro- UV-VIS AUTO UV 2602 Labo. Med. Inc.).

For non-spore formers (*R. solani*), mycelia bits were use as inoclum. The counting of the mycelia bits was done and re-adjusted with haemocytometer to 3.3 x 10^6

mycelia bits/ml. Each inoculum suspension was immediately inoculated to each pot containing a mixture of sterile sub-soil and acid washed ocean sand (1:1) at planting to reach the pathogenic level around 10^4 CFU g^{-1} inside sterile 3 kg plastic pots. Seeds of healthy cowpea genotype (IT90K-277-2) were surface sterilized in 1% sodium hypochlorite for 3 min to remove surface contaminants. The seeds were rinsed immediatelly in three changes of distilled sterile water, and allowed to air dry in the laminar flow. Four seeds were simultaneously planted in the 50 ml of fungal inocula and RHE mixture at a depth of approximately 1 cm. The control pots were inoculated with 50 ml of sterile distilled water. The development of signs and symptoms of pathogenicity were observed weekly and records were taken. Re–isolation was carried out to confirm isolates identity at 8 weeks after planting (WAP).

2.2.2 Effect of Interaction between Phytopesticide (RHE) and Fungal Pathogens on Cowpea.

Materials used were two clean cowpea genotypes, one susceptible IT90K-277-2 (Gen 1) and one resistant IT97K-340-1(Gen 2). The two genotypes were selected based on their natural field reactions to root-/soil-borne fungal pathogens. Seeds were surface sterilized with 1% sodium hypochlorite for 3 min to remove surface contaminants and rinsed immediately in three changes of distilled sterile water. The water holding capacity of the mixture of sterile sub-soil and sand were determined. Four seeds of each cowpea genotypes were then planted. Experiments were in completely randomized designs, and in three replicates.

The quantitative estimation of the pathogenic fungi used in this study i.e. *Fusarium* spp. spores suspension ($3{\cdot}3 \times 10^5$ CFU ml^{-1}) and *R. solani* (3.3×10^6 mycelia bit / ml) of the root/soil-borne fungal pathogen inocula were inoculated simultaneously with 1.5% concentration of RHE to reach the soil pathogenic level of around 10^4 CFU g^{-1} (Ros et al. 2005). Plants were watered daily for 2 weeks and thereafter twice every other day until harvesting. The data for glasshouse *in-vivo* antagonistic experiment were collected weekly from 3 WAP for a period of 5 weeks (8 WAP).

2.3 Assessment of Disease Occurrence

Disease incidence (DI) for each root/soil borne fungal pathogen at 8 weeks after planting (8 WAP) was calculated using the equation proposed by Cooke (2006) as follows:

$$DI = \frac{\text{No. of infected plant units}}{\text{Total no. of plant units assessed}} \times 100$$

The disease severity (DS) was assessed according to alternative rating scale index proposed by Ros et al. (2005) as follows: 1- all leaves green (plant without symptoms), 2- 25-49 % lower leaves yellow (very slight browning of hypocotyls), 3- 50-75 % lower leaves dead and some upper leaves yellow (some wilting of plant), 4- 75-99 % lower leaves dead and upper leaves wilted (wilting of entire plant) and 5- 100% plant dead. Nodule number, nodule dry weight (wt), biomass dry weight, biomass nitrogen uptake and phosphorus accumulation were also determined using method developed by Olsen et al. (1954) and IITA (1982).

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2.4 Statistical Data Analysis

All statistical analyses were performed using General Linear Modeling (GLM) procedure with Duncan Multiple Range Test (DMRT) using SAS® (2009) System for Windows Version 9.1 software, SAS Institute, Cary, North California, USA, to compare different states with respect to disease incidence and severity. In all the observational laboratory, and greenhouse bioassay experiments, dependent variables were subjected to analysis of variance (ANOVA). The least square means (LSM) test at 0.05 level of significance was used to compare treatment means for each measured parameter. Standard error (SE) and Coefficient of variation (CV in %) were also computed.

3. Results

3.1 Pathogenicity test

In-vitro tests on the 12 selected fungal isolates revealed that only five root-/soil-borne fungi were virulent, *F. verticilloides* (R105E), *F. equiseti* (R126F), *R. solani* (S102D), *F. solani* (R108H) and *F. oxysporum* (R103C). Isolates R105E and R108H were significantly (P<0.05) different from the rest of the isolates. Isolate R103C was significantly (P<0.05) different compared with the control (C100M). The isolates, R103C, R105E, and R108H, were highly virulent compared with the others and control. Cowpea plant height, total biomass and nodulation were greatly affected by the root-/soil-borne fungal pathogens, although there were no significant (P>0.05) differences in nodule number, and nodule dry weight between the treatments compared with the control (Table 1). However, root formation and architecture were affected by the devastating effect of the fungal pathogens, particularly by isolates R103C, R105E, and R108H. The root and shoot biomass dry weight showed significant (P<0.05) differences between the treatments and the control (Table 1).

3.2 Phytopesticide and Pathogens Antagonistic Experiment

The minimum *in-vitro* inhibitory concentration of RHE on the root-/soil-borne fungal pathogens of cowpea was 1.5 % after 9 days incubation at 28°C (Plate 1A-E). All the five fungal pathogens, *F. verticilloides*, *F. equiseti*, *F. solani*, *F. oxysporum*, and *R. solani*, were

significantly (P<0.05) inhibited at 1.5% RHE concentration. The mycelial radial growths of the five fungal pathogens were completely inhibited compared with the control. However, at 1% RHE concentration, the mycelial radial growths of *F. verticilloides*, (R103C), *F. solani* (R108H) and *R. solani* (S102D) were not inhibited completely, whereas the mycelial radial growth of *F. equiseti* (R126F) and *F. oxysporum* were completely inhibited, compared with the control (Plate 1A-E).

3.3 Chemical Composition of Phytopesticide

Chemical analysis of diluted RHE used in this study showed that it contains: PO_4, 9.33 ppm; NH_4, 62.15 ppm: NO_3, 42.87 ppm; Pb, 0.16 ppm Cd, 0.04 ppm; Cr, 0.017 ppm; Co, 0.24 ppm; Ni, 1.24 ppm; Mg, 0.06 ppm; K, 2.007 ppm; Na, 1.28 ppm; Mn, 0.02 ppm; Fe, 1.41 ppm; Cu, 0.011 ppm; Zn, 0.12 ppm; Ca- hardness, 3.108 mg/l; Mg- hardness, 0.246 mg/l; Total- Hardness, 3.355 mg/l; Electrical conductivity, 15 uS; and has pH of 3.2. Pyroligeneous acid concentration of the diluted RHE was not determined due to lack of facilities. However, Yoshida et al. (2000) reported that the concentrated RHE contains 0.25 g/liter of pyroligeneous acid.

3.4 Effect of Interaction between Phytopesticide (RHE) and Fungal Pathogens on Cowpea.

The *in-vivo* antagonistic study between the five fungal pathogens and 1.5% RHE concentration showed greater reduction in the development of disease signs and symptoms. There were significant (P<0.05) effect of genotypes, treatment, and genotypes × treatment interactions in the number of dead plants compared with the control. However, no significant (P<0.05) effects of genotypes, treatments and genotypes × treatment interactions were observed in the number of infected plants, disease incidence, and severity scores compared with the control (Table 2).

The plant heights at 8 WAP showed significant (P<0.05) difference in both genotypes. In genotype 1 RHE+P5 had highest mean value of 21.09 cm followed by RHE+P1, with 17.09 cm, and RHE+P2 with 8.50cm compared to the control with 11.63 However, in genotype 2 the control had the highest plant height at 8 WAP with high level of significant (P<0.05) difference compared to the treatments (Table 2).

Root dry weight, and shoot dry weight showed significant (P<0.05) differences between treatments compared with the controls. Regarding nodulation, RHE+ *F. oxysporum* was significantly (P<0.05) different from the rest of the treatments and the controls but there were no significant (P<0.05) differences between positive control (+), RHE+ *F. verticilloides*, and RHE+ *R. solani* compared to other treatments (Table 2). Moreover, RHE+ *F. verticilloides* and RHE+ *F. oxysporum* were significantly (P<0.05) higher in shoot biomass production than the rest of the treatments. Percentage N in shoot biomass was significantly (P<0.05) higher among many of the treatments relative to the control. However, percentage biomass phosphorus showed significant (P<0.05) difference between genotypes but not significant (P<0.05) differences between the treatments and treatment × genotype interactions (Table 2) were recorded.

4.0 Discussion

Biological control of plant diseases is one of the viable alternatives in sustainable agriculture because it is safe and environmentally friendly (Newsham et al. 1995). However, little work has been done on the potential use of phytopesticides such as RHE as biocontrol agent against cowpea fungal pathogens. The present preliminary findings suggest that natural products from the RHE have high potential for the control of root/soil-borne cowpea fungal pathogens with little or no environmental hazard.This in agreement with the reported work of Abiala et al. (2010) on the use of rice husk extract in the laboratory to control mycelial growth of *Mycosphaerella fijiensis* causing black sigatoka diseases of banana and plantains. Yoshida et al. (2000) also reported that RHE contains pyroligeneous acid which is bioactive secondary metabolite that has inhibitory effects on fungi.

The RHE used in this study completely inhibited the mycelial growth of the five root/soil-borne fungal pathogens *F. verticilloides, F. equiseti, R solani, F. solani* and *F. oxysporum in-vitro* and *in-vivo*. This is in agreement with the result of Peluola (2005) that Neem extract at lower concentration inhibited some fungal pathogens of cowpea in both laboratory and greenhouse experiments. Odebode et al. (2004), had previously confirmed this observation but only for an *in–vitro* experiment. The basic chemical analysis of RHE carried out in this study showed that it is an acidic compound with pH 3.2 which agreed with the report by Yoshida et al. (2000) that RHE contains pyroligeneous acids which completely inhibited the growth of fungal mycelia of *Thanatephorus cucumeris* (MAFF305844) at the concentration of 1/80 in and *F. solani* (MAFF306358) in 1/20 concentration.

The results of the bioassay tests both *in-vitro* in the laboratory and *in-vivo* in the glasshouse suggested that the RHE is a source of naturally occurring bioactive compounds that have antifungal properties which inhibited radial mycelial growth of the five root-/soil-borne cowpea fungal pathogens used in this work.Odebode et al. (2004) reported similar result from the bioassay test carried out on the two annonaceous plants *Isolona cualifora* Verdc and *Cleistochlamys krikii* Benth (Oliv); the crude extract and pure compounds isolated from both plants inhibited both bacterial and fungal pathogens tested.

Colonization of cowpea root by the five root/soil-borne fungal pathogens, i.e., *F. verticilloides*, *F. equiseti*, *R solani*, *F. solani* and *F. oxysporum* were completely inhibited by RHE at 1. 5% leading to reduction in diseases incidence and severity as well as increased total plant biomass accumulation. Our observations are in agreement with the reports of Gharib et al. (2008) that confirmed the effectiveness of extract of aqueous compost in controlling fungi diseases leading to increases in fresh and dry weight of *Majorana hortensis*. In this study, nodule dry weight in of the RHE treatment plants significantly increased compared to that of controls. This might be the result of RHE effectiveness in the control of root borne pathogens. The percentage nitrogen (%N) in biomass in both cowpea genotypes was also affected by the fungal pathogens; nitrogen uptake was higher in IT90K-227-2 compared to IT90K-340-1 however percentage phosphorus (%P) accumulation in dry biomass was not significantly affected.

Killani (2010) observed a linear relationship between the *in-vitro* laboratory experiments and *in-vivo* glasshouse experiments when phytopesticide was used against *F.verticilloides*, *F.equiseti*, *R. solani*, *F.solani* and *F. oxysporum.* Therefore, phytopesticides tested in this present study is a promising potential biocontrol agents as well as bioprotectants against major root-/soil-borne fungal pathogens isolated from the northern Guinea savannan (NGS) cowpea based cropping system.

Comment [r2]: Steve, we need to work on this paragraph. The discussion here does not correspond with what is in the table 2. Moreover, you also mixing up the genotype names. You need to pay close attention to detail. Please be patient and let us work through this when I arrive in Ibadan first week in January 2011.

Table 1**.** Pathogenicity of Root and Soil-borne Fungal Pathogens on Cowpea Variety IT 90K-227-2 *in-vivo*

Isolate Identity	**Isolates Code**	**Plant Height 8WAP (cm)**	**Nodule Number**	**Nodule Dry Weight (mg)**	**Root Dry Weight (g)**	**Shoot Dry Weight (g)**
F. equiseti	R101A	17.13ab	2b	1.00b	1.07cd	2.80c
F. oxysporum	S106B	17.63ab	3b	4.00b	0.94d	3.38bc
F. oxysporum	R103C	7.75c	0b	0.00b	0.04e	0.16d
R. solani	S102D	15.25b	3b	5.00b	1.31a	3.00bc
F. verticilloides	R105E	0.00d	0b	0.00b	0.00e	0.00d
F. equiseti	R126F	16.38b	3b	3.00b	1.25ab	3.51b
F. oxysporum	S117G	16.63ab	3b	2.00b	1.12bc	3.25bc
F. solani	R108H	0.00d	0b	0.00b	0.00e	0.00d
F. oxysporum	S109I	17.25ab	2b	2.00b	1.27ba	3.09bc
Fusarium spp	R110J	17.75ab	4b	24.00b	1.07dc	2.76c
R. solani	S113K	17.75ab	2b	4.00b	1.17abc	3.08bc
F. oxysporum	S112L	18.00ab	1b	2.00b	1.14abc	3.27bc
Control	C100M	19.88a	24a	13.50a	1.15abc	4.49a
CV (%)		7.80	79.07	121.71	10.05	13.00

*Means within column followed with same letter are not significantly different at 95% confidence interval (P<0.05) analyzed using GLM procedure with DMRT test, CV (%) = Coefficient of variation in percentage; 8WAP = 8 Weeks After Planting.

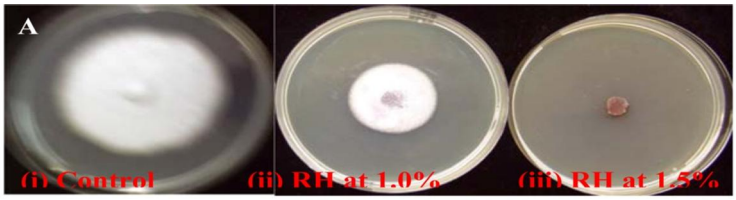

Plate (a) *F. verticilloides* (P1)

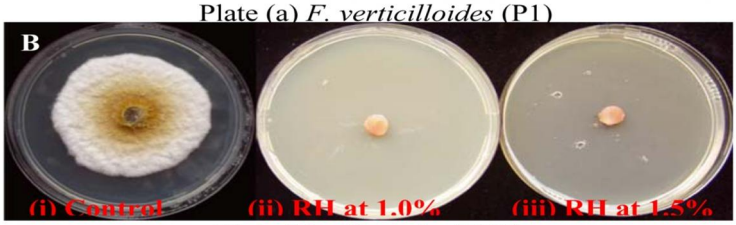

Plate (b). *F. equiseti* (P2)

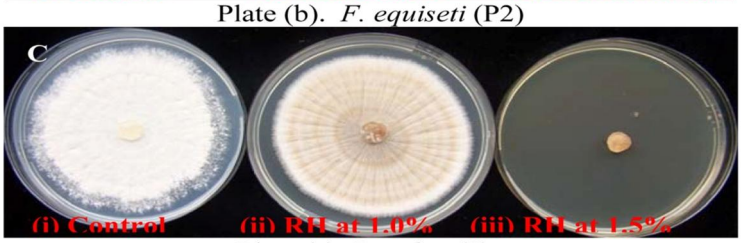

Plate (c). *R. solani*(3)

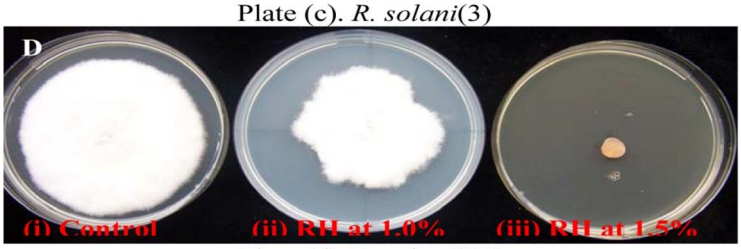

Plate (d) *F. solani*(P4)

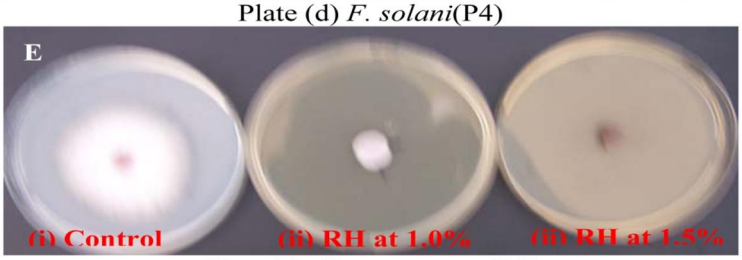

Plate (e). *F. oxysporum* (P5)

Plate 1A-E. Inhibitory effect of RHE on the mycelial growth of A = ***F. verticilloides,*** B = ***F. equiseti,*** C = ***R. solani***, D = ***F. solani,*** E = ***F. oxysporum*** at two concentration after 7 days of incubation at 28°C ± 2°C

Table 3: Effect of interaction between phytopesticide (RHE), five fungal pathogens on cowpea *in-vivo*

Treat	gen	no dpl	no ipl (%)	dis incs (%)	dis sev (%)	plt ht (cm)	rt dwt (g)	sh dwt (g)	N (%)	P (%)
RHE+P1	1	0.33	0.00	0.00	0.00	17.76	2.04	6.27	1.09	0.13
RHE+P2	1	2.67	0.00	0.00	0.00	8.50	1.10	3.19	0.77	0.14
RHE+P3	1	1.00	0.00	0.00	0.00	14.89	1.63	4.63	1.29	0.10
RHE+P4	1	2.00	0.00	0.00	0.00	13.13	0.76	3.70	1.81	0.10
RHE+P5	1	0.33	0.00	0.00	0.00	21.09	2.35	6.52	1.13	0.10
Control	1	0.00	0.00	0.00	0.00	11.63	0.63	2.04	4.71	0.08
Means		1.06	0.00	0.00	0.00	14.50	1.42	4.39	1.80	0.11
RHE+P1	2	0.33	0.00	0.00	0.00	13.88	1.71	4.39	1.16	0.14
RHE+P2	2	0.33	0.00	0.00	0.00	13.88	1.83	5.13	1.30	0.12
RHE+P3	2	0.00	0.00	0.00	0.00	18.40	1.80	5.62	1.46	0.10
RHE+P4	2	0.00	0.00	0.00	0.00	15.95	1.35	4.93	1.36	0.13
RHE+P5	2	0.67	0.00	0.00	0.00	14.90	1.91	4.94	1.58	0.13
Control	2	0.00	0.00	0.00	0.00	20.65	3.26	5.51	1.36	0.15
Means		0.22	0.00	0.00	0.00	16.28	1.98	5.09	1.37	0.13
S.E.										
Genotype		0.13	0.00	0.00	0.00	0.38	0.10	0.23	0.07	0.005
Treatment		0.24	0.00	0.00	0.00	0.67	0.19	0.44	0.13	0.009
Genotype*treatment		0.35	0.00	0.00	0.00	0.94	0.27	0.62	0.18	0.013
F value										
Genotype		***	ns	ns	ns	**	***	**	***	**
Treatment		**	ns	ns	ns	***	**	*	***	ns
Genotype*treatment		**	ns	ns	ns	***	***	***	***	*

Treat = Treatment, S.E. = standard error, ns = non significant at (P≤0.05), * = significant at (P≤0.05), ** = significant at (P≤0.01), ***= significant at (P≤0.001), gen = genotype; 1 = IT90k-277-2; 2 = IT97K-340-1, nodpl = number of dead plants; noipl = number of infected plants; disincs = disease incidence score; dissev = disease severity score; pltht = plant height; rtdwt = root dry weight; shdwt = shoot dry weight; N% = percentage nitrogen uptake in dry shoot; P % = percentage phosphorus accumulation in dry shoot; RHE = rice husk extract, P1 = *F. verticilloides*, P2 = *F. equiseti*, P3 = *R. solani*, P4 = *F. solani*, P5 = *F. oxysporum*.

Conclusion

Application of phytopesticide (RHE) in this study for the control of root/soil-borne fungal pathogens of cowpea is a promising control strategy for the management of cowpea fungal pathogens in the field. The RHE also exhibited great phytoprotectant capability due to its good *in- vitro* and *in-vivo* inhibitory performance in the control and the reduction of the root/soil-borne fungal cowpea pathogens.

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Corresponding to

Killani, A. Steven (PhD)
Soil Microbiology Laboratory
International Institute of Tropical Agriculture
PMB 5320 Oyo Road, Ibadan,
Oyo State, Nigeria.
Fax (234 2)241 2221
Tel: (+234 2) 75174721 Ext.2681,
Mobile: 08023240049, 08054068763
E- Mails: killani405@yahoo.com

International Mailing Address:
IITA Limited, Carolyn House,
26 Dingwall Road, Croydon CR9 3EE, UK

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Comment [r3]: Check spelling

Spatial distribution and habitat preferences of selected large mammalian species in the Nech Sar National Park (NSNP), Ethiopia

Aramde Fetene[1], Girma Mengesha[2] and Tsegaye Bekele[3]

[1]Debre Markos University, Department of Natural Resources Management, P.O Box 269 email: aramdefetene@yahoo.com

[2]Wondo Genet College of Forestry & Natural resource, Department of wildlife & Ecotourism Management, P.O.Box 128, Shshemene, Ethiopia

[3]Associate Professor, Hawasa University, Planning and Programming Office, P.O. Box 05, Hawassa, Ethiopia, e-mail:bekele57@yahoo.com (corresponding author)

ABSTRACT. A study on spatial distribution and habitat preferences of five large mammal species was conducted in the Nech Sar National Park (NSNP) for one year from January 2007-January 2008. The spatial distribution and habitat preference information is useful to propose appropriate patrol strategy for the management and conservation of the species with regard to attracting tourist and management of the park. The objective of this study was to determine the spatial distribution and habitat preference of five large mammals (Defassa waterbuck, Swayne's Hartebeest, Greater Kudu, Lesser Kudu and Black and White Colobus). For the purpose of this study, the park was divided in to three management zones and nine patrolling teams composed of six individuals were involved in the data collection. Each individual was assigned to a certain management zone to monitor the status and distribution of large mammals and the impact of human activities on the Park on daily basis. The patrolling team was equipped with Garmin Etrix Venture GPS receiver and Communication Radio and point sampling technique was used to collect the necessary information. The data was summarized and all spatial data were recorded and analyzed using GIS Software (DNRgarmin and ArcGIS9.1). DNRgarmin was used to transfer data from GPS receiver to computer. ArcGIS9.1 was used to analyze the spatial distribution of the wild animals, habitat association and human activities. Comparison of the mean on the observation of different wild animals in the NSNP was carried out using SPSS17. The results of the study showed that there were a total of 3340 observations of the five large mammals on 29013 km track movements in the NSNP. Observation in this sense does not mean the number of individuals, but the frequency of wild animals seen during the inspection. In this regard, Greater Kudu has shown a significant wider distribution in the three zones of NSNP ($P>0.05$), with high ecological amplitude and high tolerance range to different habitat factors. The other four large mammals were concentrated in a particular association of different habitats. Herds of Swayne's Hartebeest were restricted only to the Nech Sar Plain, Lesser Kudu, to west of the plain, on the mountain near to the hot spring, Defassa waterbuck on a hill of wooded grasslands near to Kulfo river and, the Black and white Colobus in the riverine forests of Kulfo and Sermele river valleys. Large numbers of peoples were observed in the Arba Minch forest and Lake Chamo collecting fuel wood and harvesting fish, respectively. The results of the study are important tools for the park managers, researchers and tourists, since it revealed clear species spatial distributions and habitat preferences.

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Key words: habitat preferences, large mammals, monitoring, Nech Sar National Park, spatial distribution

INTRODUCTION

The distribution of an organism is primarily dependent upon the suitability of the environment for its survival, growth, and reproduction. Therefore, knowledge of ecology, physiology and systematic of the concerned organisms is essential. Plants and animals follow definite types of distribution such as continuous distribution, discontinuous or disjunctive distribution, and very restricted distribution in small areas (endemic) (Odum, 1971). Animals vary widely in their tolerance to environmental conditions. Some can survive in a variety of habitats, whereas others perish when removed from their natural surroundings.

However, when this natural factor is disturbed by the intervention of mankind that pushes the animals to exist outside of their range of tolerance, this condition leads them to dwindle to the point of extinction. Researchers study animal distribution to understand the spread of animal-borne diseases, to acquire knowledge about the preservation of rare species that may have special needs, and to be informed about the changing geographical conditions, and our environmental history and its future. To understand these issues, a study needs to identify the specific climate, feed habits, and geographic features that different animals require, and what areas provide the best (Encarta, 2008; Mwangi and Western, 1997). Habitat for mammals and other organisms is disappearing quickly from the Earth's surface due to human interferences (Patterson *et al*., 2003). This condition particularly affects the distribution patterns of large mammals as they wander in search of preferred habitats which are found in patchy habitats of protected area. As a result, some of the rare and endangered mammalian species have shifted their original range and occur in a few habitats in some countries. But there is a lack of information on where they frequently occur and on their migratory corridor within the habitat.

Therefore, understanding of habitat preference and spatial distribution of such large herbivores mammals is basic tool for the management of protected areas and it is also a prerequisite to determine the stocking density for introduction and re-introduction of animals to and from particular habitat (Dekker *et al*, 1996). For instance, mammals like Swayne's Hartebeest (*Alcellaphus buselaphus swaynei)* which is a highly threatened subspecies is restricted to Ethiopia (Duckworth *et al*, 1992). Knowledge of habitat requirement and distribution along the environmental gradient is essential not only for the species survival but also for the sustainable management and conservation of protected areas. Based on this understanding, this study was conducted to identify the key priority habitats of five large mammals and their distribution in the Nech Sar National Park (NSNP) which may be used as an input for the planning and sustainable management of this unique conservation area.

MATERIALS AND METHODS

The Study Area

The study was carried out for one year from January 2007 to January 2008 in three management zones in the NSNP. The Park was established in 1974 in the scenic part of the Rift Valley floor between two lakes namely Abaya and Chamo. It is found at a distance of 500 km south of Addis Ababa and covers about an area of 514 km^2 of which 78 km^2 is covered with water bodies. NSNP is "a mosaic of forest, open woodland, grassland and fresh water habitat" (Duckworth *et al*, 1992). It is located between $5^0$51'-$6^0$05'N Latitude and $37^0$32'-$37^0$48'E Longitude in the Southern Nations, Nationalities and Peoples Regional State (SNNPRS) at the center of Ethiopian Rift valley with an altitudinal range of 1,108-1,650 m.a.s.l (Bolton, 1970) (Figure 1).

The Park is bounded to the east by the Amaro Mountains, to the west the town of Arba Minch and to the north and south by lakes Abaya and Chamo, respectively. In the far eastern part of the park, hot springs bubble to the surface, while numerous natural springs known as Arba Minch (meaning 'forty springs') are found in the western most extreme of the Park (Tamrat, 2001). There are, two main river systems that flow through the park forming riverine forests and woodlands. Sermele River crosses north-south at the eastern part of the park along the grassy plains and *Acacia* woodlands and meets with Miyo River. The Kulfo River flows through the north of Arba Minch and then cuts across the neck of the narrow land and ends in a swamp on the shore of Lake Chamo (Tamrat, 2001).

The park contains more than 90 mammal species and it supports more than 350 species of birds and acts as the destination of many Palaearctic and intra-Africa migrants (Duckworth *et al*, 1992). It is classified as one of the sixty-nine Important Bird Areas in Ethiopia. Large mammals currently present in the study area are the common Zebra, Swayne's Hartebeest, Grant's gazelle, Greater kudu, Guenther's dik dik, Anabus baboon, Black and white colobus, Hippopotamus, Spotted hyaena, Mountain reedbuck, Black-backed jackal, Side striped jackal, Golden jackal, Defassa waterbuck, Bushbuck, Klipspringer, Warthog and Bush pig. Leopard and lions are also occasionally seen. Lake Chamo supports a high density of very large crocodiles (many individuals in excess of 5m in length) with a particular concentration of them at the beach known as the 'Crocodile Market', the largest hippo population in Ethiopia, and abundant fish including Nile perch (Whitaker, 2007).

The Arba Minch ground water forests, Kulfo and Sermele riverine forests are found within the vicinity of NSNP (Duckworth *et al.*, 1992). The ground water forests and the Sermele valley forests are located in the western and eastern part of the park, respectively, whereas, the Kulfo riverine forest is located in between the two forests but lose to the ground water forests.

METHODS

A three patrol zones were formed by dividing the park for ease of management. Boundaries of zones

were made based on the understanding of the conservation requirements of NSNP's Principal Ecosystem Components (FZS and IBC, 2006). The patrolling teams have worked in a shift round base and the patrol zones were the eastern circuits (Figure 2) which included the hot spring and Nech Sar plain and Sermele Valley riverine forest (zone 1), the western circuits included the Chamo Letto area, Arba Minch forest and Kulfo riverine forests (zone 2), and the central circuit included the Lake Chamo and the hilly areas of the park (zone 3).

Nine patrolling teams, each composed of six wildlife scouts were formed and the total number of scouts involved in this activity was 63 including one team leader assigned to each team. Nine Garmin Etrix Venture GPS receivers were handed over to each team leaders with proper setups (such as projection with WGS 84 UTM Zone 37N, recording unit to be in meter, time to count in 24 hours format, recording of track points automatically every 20 seconds) to mark way points and track for the proposed target sites of daily monitoring activities. Daily monitoring data sheets, binocular and radio for communication were also assigned to a team to collect all necessary information Training was given for the patrolling team on how to operate the GPS receiver and data recording in the field. Both direct and indirect wild animals counting systems were employed according to their appropriateness. This includes direct wild animal observation and recording indirectly based on their droppings, spoors, caracas, nesting sites and sounds.

To make a cross check on the impact of human activity in the Nech Sar national Park an independent controlling mechanism was devised other than the regular patrol strategy. On the selected five entrance gates to the park from the Arba Minch town, five additional technicians other than the regular wildlife scouts, were assigned to record information on the people entering and coming out from the park including the items which they collected from the park area and it was without the actual contact to illegal intruders. This extra observation was carried out for the sake of comparison with the regular patrol activity and to design best strategies for monitoring wildlife and human activity in the Nech Sar National Park. This cross checking study was made for three months (December, 2007, January, 2008 and February, 2008) at the last week of each month. The five entrance gates where cross check study made are locally known as 'Green land', 'Moter Sefer', 'Dorze Sefer', 'Konter Sefer' and 'Kulfo Bridge'.

Data analysis

The records from regular patrol data of the GPS receiver was downloaded to the computer using DNRgarmin software and saved in Arcview shape file projected both in points and lines. For the geo-referenced points from the patrol data, the associated information recorded on the data sheet were: number of observations, way points (X&Y) coordinates, altitude, date, team number, type of observation, sighting distance, total animal number including the sex and age structure, human activity, habitat type and the wild animals activities during the recording time, data with respect to all human activities and wild animals' movements were analyzed. Data base was also established for track movements and the associated information during the study period. For comparison and to check the effectiveness of regular patrol activity, the data collected from the independent monitoring activities, from each park entrance gate were also entered into a computer, separate database was established and analysed accordingly.

The spatial distribution and habitat association of the target large mammals were analyzed using ArcGIS 9.1 and mean comparison of the observed values were made using one-way analysis of variance (ANOVA) and Tukey's test (95% confidence interval) was used to separate means of significantly different parameters with SPSS 17 software.

RESULTS

Spatial distribution of wild animals and human activity

The result of the study showed that 29013 km, track movements (Figure 3) and a record of 3340 of wildlife and 3078 human activities observations in the NSNP (Figure 4; a,b, c,d,e & f) . Observation in this sense does not mean the number of individuals, but the frequency of wild animals and human activities seen during the study period.

The different mammalian species had different tolerance rate to different habitat factors and ecological amplitude. In this regard, only Greater kudu showed wider distribution range in the NSNP and the others: Defassa waterbuck, Swayne's Hartebeest, Lesser Kudu, and Black and White Colobus were restricted to particular habitat types (Figure 4).

Comparison with the regular patrol activity and crosschecking with independent monitoring activity, showed significant difference on the recording of information about human activity in the Nech Sar National Park. The results from independent

monitoring activity carried out at the last weeks of the three months (December, 2007, January, 2008 and February, 2008), on the selected five entrance gates to the Park indicated that 3078 people have entered in to the forest and collected forest products for fuel wood, poles, split wood, grass, fruit, and charcoal. When comparing this result, with the data from regular patrol activities which were obtained at the same period by nine patrolling team, only 131 people were recorded for the last weeks of the three months (December, 2007, January, 2008 and February, 2008) which is by far less than the independent monitoring where 3078 people were recorded. This variation in the two monitoring activities showed a good indicator for the managers to change patrolling strategies to secure the park resources from an increased human population pressure. This result also indicated that regular patrol activity is important to collect up-to-date information about the wild life but less effective to monitor human activity as the illegal intruders enter to the park in opposite position and time to the wildlife scouts. Therefore, it is equally important to make in independent monitoring activity at a regular interval.

Habitat preference and frequency distribution

A total of 3340 observations were made for the five large mammals in NSNP during the study periods. The frequency of distribution of these wild animals varied depending on the type of animal considered and the highest frequency was recorded for the Black and White Colobus (27%) followed by Swayne's Hartebeest 9.7% and the lowest record for Lesser Kudu 1.2% (Figure 5). The frequency for the Lesser Kudu is the lowest, because it is a habitat specific. Moreover, the animal is shy and could not be detected easily. As a result, the probability of being detected by the team members was low.

With respect to habitat selection, high frequency was observed for the natural forests 10.6% and lower for wetlands. This might be because the animals used the dense forest not only as source of food but also as cover from strong sun light and predation. The frequency of observation of animals in the wetlands is minimum (0.6%) because they were observed in this site only when they needed to drink water. However, each wild animal has its own habitat preference. Result from habitat-wildlife cross tabulation showed that Defasa waterbuck was frequently recorded in the open woodland (118), Greater Kudu in the open woodland (174), Black and White Colobus in the natural forest (966) and Riverine forest (680), Lesser kudu in the open woodland (56) and non in natural forest, riverine forest and wet lands, Swayne's Hartebeest, in grass lands (559) (Table 1). The goodness-of-fit test showed significance differences in habitat selection of the five large mammals in the seven habitat types of NSNP ($N = 3340$, $\chi 2 = 1595.03$, $df = 6$, $P<0.01$).

The result showed that there existed a significance difference between the different wild animals with respect to different habitats types both in terms of age and sex structure ($p<0.05$). Large number of animals was recorded in the natural forest which might be due to the availability of sufficient food and occurrence of minimum prey-predator interaction. A remarkable wild animal number was also recorded at riverine forest which might be due to the availability of water for the animals. Male adults were recorded most often in the natural forest and female adults in the grass lands which might be because female animals are responsible for raring their offspring and protect them from predator and hence will survive in open area to detect predator from distant. Sub adult, juvenile and calf, were also recoded repeatedly in the grass land that might be related to the aforementioned reasons, prey-predator interaction (Table 2).

When observations were compared along different months in different seasons of a year, the mammals showed variation in spatial distributions (Table 3). Accordingly, the highest observations were recorded during the wet season (April, May, June, July, August and September) for all the mammals. Highest observations for Defasa waterbuck June(54), Greater Kudu, May(100), Swayne's Hartebeest, May (166), Black and White Colobus ,November (209) and for the Lesser kudu, July(16) was recorded (Table 3).

When observations in the different habitats was compared along the different months of the year (Table 4), the result showed significance difference in the occurrences of different mammals during the study period ($p<0.05$). The goodness-of-fit test also indicated significance differences in the observation of the five large mammals in the NSNP at different months and habitat types ($N = 3340$, $\chi 2 = 2628.77$, $df = 4$, $P<0.01$).

Table1. Observation * Habitat types Cross tabulation in NSNP

Obrvations	Habitat types							Total
	Open woodland	Grass land	Natural forest	Riverine forest	Wet land	Shrub land	Wooded grass- land	
Defasa waterbuck	118	35	24	31	8	15	112	**343**
Greater Kudu	174	140	8	5	21	11	122	**481**
Black and white Colobus	36	4	966	680	9	76	23	**1794**
Lesser kudu	56	1	0	0	0	1	21	**79**
Swayne's Hartebeest	34	559	3	2	0	3	42	**643**
Total	**418**	**739**	**1001**	**718**	**38**	**106**	**320**	**3340**

Table 2. (Mean ±SE) Wild animal observation along different habitat types of NSNP

Habitat Type	Observations							
	Obs. of the five wild animal types	**Total animal**	**Male adult**	**Female adult**	**Sub adult**	**Juvenile**	**Calf**	**Unidentified**
Bush land	2.30±0.060[a]	3.67±0.176[a]	1.41±0.096[a]	2.86±0.190	1.36±0.135[a]	1.53±0.133[a]	1.64±0.388[a]	3.38±0.269[a]
Grass land	4.23±0.051[d]	5.43±0.253[b]	1.51±0.064[a]	3.30±0.186	2.49±0.187[b]	3.09±0.196[c]	2.69±0.328[b]	7.11±0.391[b]
Natural forest	2.95±0.011[b]	7.34±0.143[c]	2.28±0.082[b]	2.98±0.087	1.95±0.113[b]	2.18±0.088[b]	1.93±0.168[a]	6.68±0.117[b]
Riverine forest	2.91±0.016[b]	7.80±0.147[c]	2.26±0.101[b]	3.25±0.125	2.05±0.131[b]	2.21±0.082[b]	1.82±0.102[a]	6.98±0.157[b]
Wet land	2.03±0.110[a]	4.74±0.674[a]	1.50±0.251[a]	2.57±0.272	2.00±0.408[b]	1.25±0.250[a]	1.50±0.289[a]	6.54±1.643[b]
wood land	2.68±0.081[b]	5.66±0.348[b]	1.82±0.186[a]	2.95±0.284	1.43±0.163[a]	1.83±0.202[a]	1.67±0.289[a]	5.76±0.448[b]
Wooded grass land	2.27±0.076[a]	4.01±0.229[a]	1.59±0.093[a]	2.77±0.182	1.56±0.133[a]	1.83±0.196[a]	1.53±0.165[a]	4.13±0.424[a]
Sig.	000***	.000***	.000***	.154[ns]	.000***	.000***	.016*	.000***

Tukey HSD, abcd= means along the column followed by the same letter of superscript is not significantly different (p> 0.05), SE = standard error, *** = p<0.001, ** = p<0.01, * = p<0.05 and ns = non significant.

Table 3. Monthly observations of five large mammal species occurrences in the NSNP

Month	observation					Total
	Defassa waterbuck	Greater Kudu	Black and White Colobus	Lesser kudu	Swayne's Hartebeest	
January	11	14	114	3	9	151
February	21	15	121	4	15	176
March	6	5	27	4	29	71
April	41	37	111	15	87	291
May	46	100	141	6	166	459
June	54	92	193	11	117	467
July	52	81	239	16	78	466
August	31	27	188	4	31	281
September	26	28	117	4	40	215
October	13	29	171	2	12	227
November	24	24	209	6	33	296
December	18	29	163	4	26	240
Total	**343**	**481**	**1794**	**79**	**643**	**3340**

Table 4. (Mean±SE) observations for five large mammals along different months of the year in NSNP

	Observations							
Month	Types of animal Obs.	Total *animal*	Male adult	Female adult	Sub adult	Juvenile	Calf	Unidentified
Jan.	2.90±0.065[a]	7.78±0.409[b]	1.87±0.240	3.38±0.389[a]	2.13±0.290	2.17±0.187	1.33±0.167[a]	8.11±0.446[a]
Feb.	2.87±0.072[a]	7.22±0.326[b]	1.73±0.173	3.32±0.525[a]	1.82±0.246	1.75±0.190	2.64±0.691[b]	7.98±0.369[a]
Mar.	3.63±0.156[b]	6.25±0.723[b]	2.21±0.323	3.95±0.778[a]	2.00±0.405	2.88±0.453	1.33±0.333[a]	5.65±0.667[b]
Apr.	3.32±0.095[b]	4.91±0.306[a]	1.68±0.089	3.36±0.218[a]	1.67±0.242	2.16±0.171	1.77±0.228[a]	4.71±0.330[b]
May	3.32±0.066[b]	5.24±0.237[a]	1.66±0.070	3.29±0.180[a]	1.97±0.174	2.13±0.166	2.10±0.237[b]	5.45±0.313[b]
June	3.03±0.067[a]	5.53±0.253[a]	1.81±0.089	3.23±0.187[a]	1.82±0.176	2.32±0.140	2.26±0.259[b]	6.42±0.330[a]
July	2.94±0.062[a]	5.56±0.247[a]	1.88±0.105	2.92±0.120[b]	1.86±0.142	2.32±0.155	1.96±0.217[b]	5.91±0.310[b]
Aug.	2.92±0.059[a]	6.36±0.260[b]	1.74±0.089	2.63±0.117[b]	1.85±0.156	2.12±0.162	1.53±0.165[a]	6.44±0.349[a]
Sept.	3.02±0.080[a]	6.51±0.325[b]	1.77±0.169	2.64±0.201[b]	1.96±0.227	2.02±0.161	2.50±0.567[b]	6.85±0.417[a]
Oct.	2.87±0.050[a]	6.84±0.291[b]	2.20±0.172	2.45±0.157[b]	1.95±0.235	2.19±0.177	1.76±0.185[a]	6.63±0.312[a]
Nov.	3.00±0.054[a]	7.09±0.413[b]	2.08±0.295	3.25±0.432[a]	2.43±0.309	2.07±0.181	2.36±0.341[b]	6.86±0.247[a]
Dec.	2.96±0.060[a]	6.06±0.258[b]	1.61±0.105	2.43±0.144[b]	1.75±0.229	1.94±0.130	1.30±0.153[a]	6.93±0.338[a]
Sig.	.000***	.000***	.109	.002**	.661	.420	.081*	.000***

Tukey HSD, ab= means down the column followed by the same letter of superscript do not differ significantly (p > 0.05), SE = standard error, *** = p<0.001, ** = p<0.01, * = p<0.05 and ns = non significant.

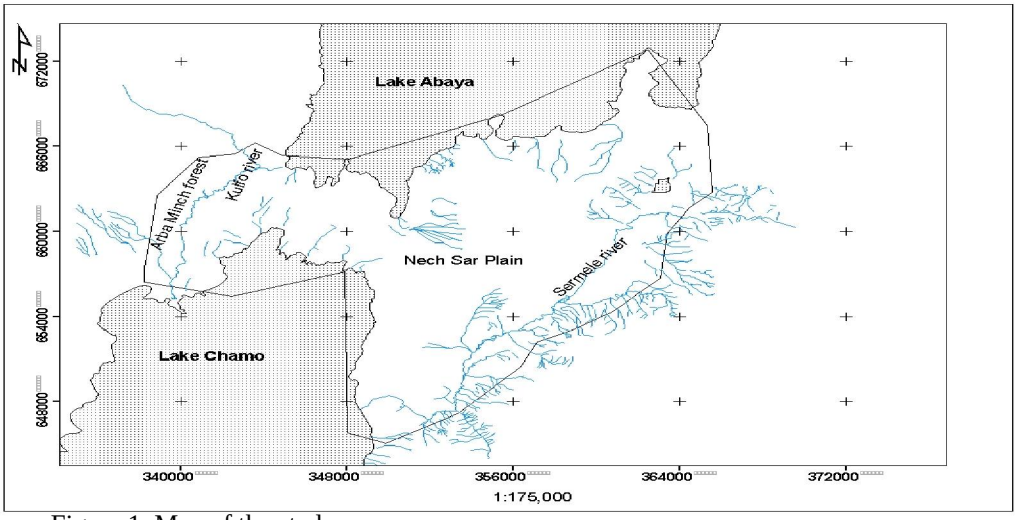


Figure 1. Map of the study area

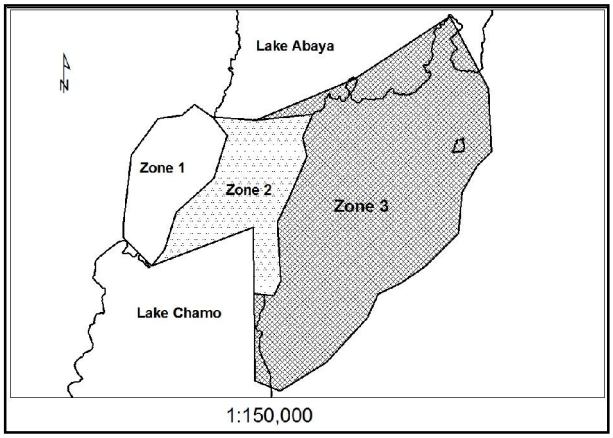


Figure 2. Map of the management zone

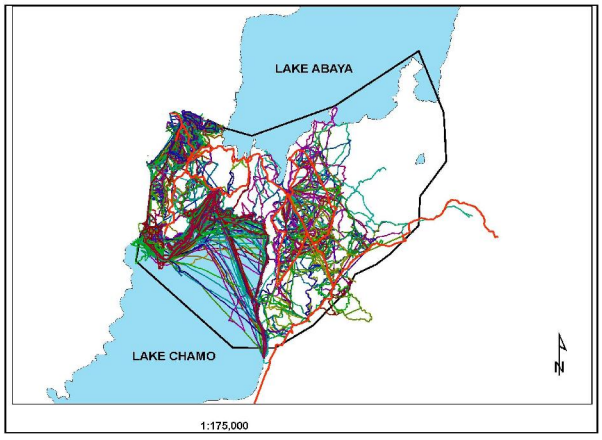
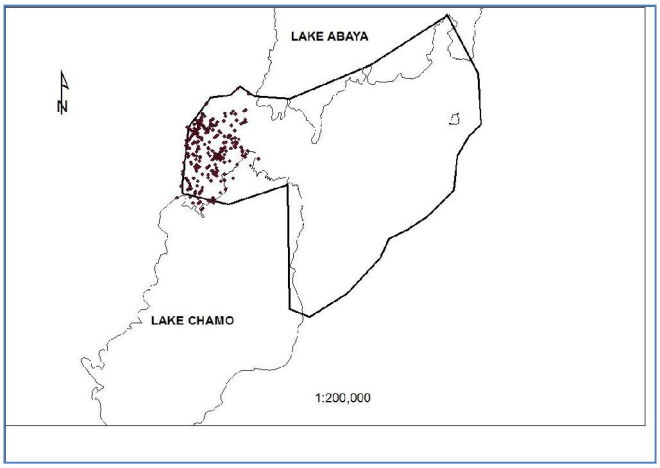
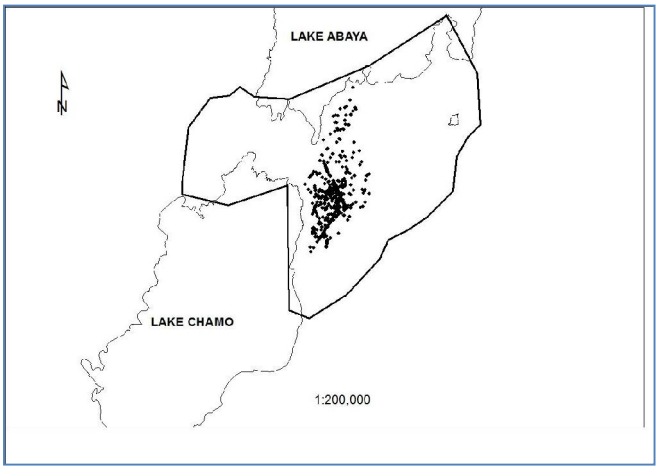


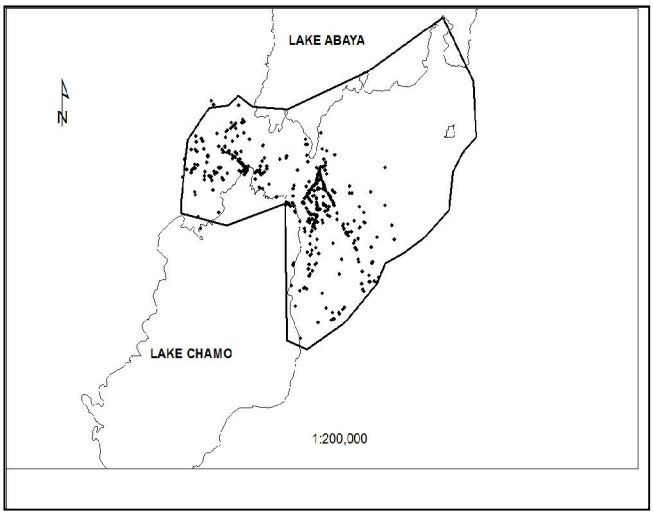
Figure 3. Patrol track movements in the study area during the study period



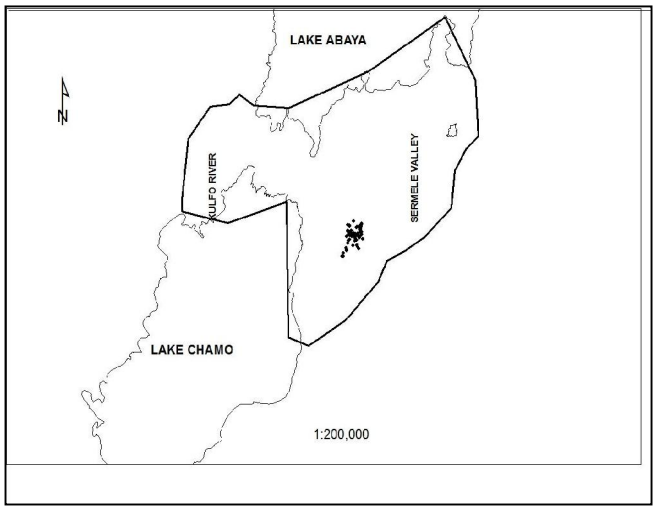
a) Distribution of Defassa waterbuck



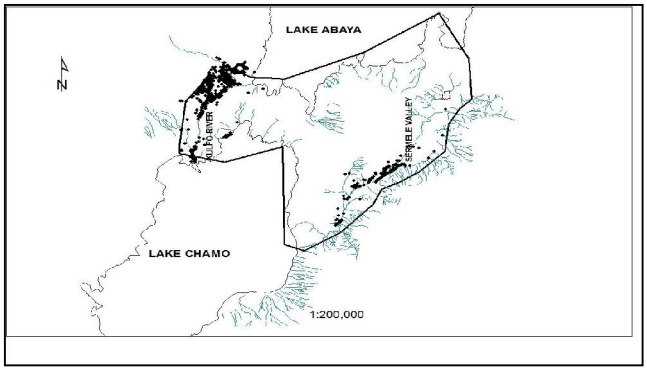
b) Distribution of Swayne's Hartebeest



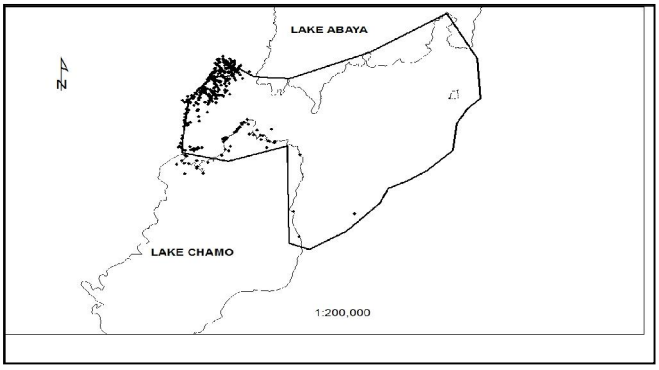
(c) Distribution of Greater kudu



(d) Distribution of Lesser kudu



(e) Distribution of Black and White Colobus



(f) Human activity

Figure 4. Distribution of different wild animals and human activities in the NSNP

DISCUSSION

A total of 481 observations for grater kudu were recorded during the study period. The greater kudu ***(Tragelaphus strepsiceros)*** most often occurred in open woodland (36.2%), grasslands (29.1%) and wooded grasslands (25.4%), less often in wetland (4.4%). The least often record for greater kudu was in closed forests (1.7%) and in riverine forests (1.03%). This result is similar to the most frequent use of woodland thickets and the least frequent use of closed forest by the mammal (Eden, 2006; Stuart and Stuart, 2000, Girma Mengesha and Afework Bekele, 2008). In contrast to the study by Skinner & Smithers (1990), the greater kudu was also observed to frequently use the open wooded grasslands of Nech Sar Plain. This might be due to the availability of more browse in the open woodland plains. In the terrestrial ecosystem of NSNP, greater kudu was observed to use a wide range of habitats. This could be related to the presence of preferred food plants in these habitats, its ability to eat a greater variety of woody plant species that provide browse and get cover and protection (Vaughan *et al*, 2000; Eden, 2006; Gray *et al*., 2007). During the study period, greater kudu was observed from single individual animal to a herd of 17 individuals foraging together indicating the kudu's social behavior. The result was supported by other studies that revealed its gregariousness in the range 4-20 individuals, with females and their offspring forming cohesive social units and males associating in transient bachelor groups (Skinner & Smithers, 1990; Estes, 1997 cited in Eden, 2006).

Observation of greater kudu showed also habitat variation under different months and seasons. Out of the total observation for the 12 months for greater kudu which was 481, large observation was observed during May (100), June (92) and July (81). The reason could be the availability of enough food or grass at this time that kudus might get within their vicinity and can easily be observed by the monitoring team. It was often least observed during January (14), February (15) and March (5) due to the critical food shortage period of the dry season. As a result kudus wander to find food and distributed everywhere. Hence, significance difference was detected ($P<0.05$) in observing greater kudu along different months of the year within the study period.

A total of 79 observations were recorded for lesser kudu (*Tragelaphus imberbis)* during the study period which is the least observations made in relation to the frequency of the other four mammals (Greater kudu, Swayne's Hartebeest, Black and white Colobus monkey and Defasa waterbuck). Lesser kudu frequently occurred in the open wood- land of a specific area (70.9%), less frequent in the wooded grasslands (26.6%) and the least observed in shrub lands and open grasslands (2.5%). This animal was hardly observed in dense forest, Riverine forests and water logged areas. This finding is not in agreement with Dorst and Dondelt (1990) that describes the frequent occurrences of the mammal in thickets and scrublands. The animals were also the least observed among the mammals. This could be related to its behavior i.e. the animals are too shy, alert and they are adapted to a particular area that protect them from predator. Because of this reason they are largely nocturnal, feeding during late evening and early morning and hiding by day in cover (Stuart and Stuart, 2000). They also showed a remarkable social group to a particular habitat type where adult males associate with females only when breeding. Lesser kudu can stay for long time without water which they use as protection mechanism to avoid detection by predators during search for water (Anonymous, 2009).They are also active in hearing and smelling as well as better runner and jumper than greater kudu which contributed to the least observation of the animals. There temporal distribution is almost similar along the months of the year in NSNP. Therefore, there was no significance difference in observing lesser kudu at different seasons and months of the year. The population status was limited to particular sites which might be due to their habitat specificity where locating mating partner is very low in comparison with other animals and resulted in low reproductive capacity per individual, lesser kudu produces only one calf per reproduction occasion (Anonymous, 2009).

A total of 343 observations were observed for Defasa waterbuck (*Kobus ellipsiprymnus defassa*) during the study period. The highest frequency distribution for Defasa waterbuck was recorded during June and July which was 54 and 52 observations, respectively (Table 3). These months are wet seasons where green brows are abundant in the study area for the animals to feed. Defassa waterbuck was frequently recorded in the open wood land (118 observations) which accounts about 34.4 % of the total observation in the study area for the study period (Table 1). Waterbucks are predominantly grazers (Taylor and Lyman, 1969), but they have been observed to include some browse in their diet, especially during the dry season when grasses become higher in structural components and lower in protein (Spinage, 1982). The Defasa waterbuck was restricted to a particular area and showed ecological separation from other species, this is in line with the findings of Mwangi and Western (1997) in Lake Nakuru National Park, Kenya. This could possibly be because they were competitively displaced by other species in feeding and

habitat selection and they may have low ecological amplitude as well as low tolerance range.

A total of 1794 observations of the Black and White Colobus(*Colobus Gureza)* were made during the study period and it was predominantly observed in the Arba Minch forest, Kulfo and Sermele Riverine forests. This is in line with the study of American Zoo and Aquarium Association (2000) which indicated that the Black-and-White Colobus monkey is successful in a variety of habitats and most of the time they are forest dwellers including montane and gallery forests. The same source indicated that, although the Black and White Colobus come down to the ground, they are dependent on trees and are the most arboreal of all African monkeys. Their dominant food choices are strictly leaves from different trees of deciduous forest and spend most of their time in treetops, preferring to eat the tender young leaves found there. The Colobus monkey is at great risk in the NSNP from habitat destruction particularly at the Sermele Valley and also hunted for its beautiful fur, where its skin has been used to make dance costumes, hats and capes leading to its population reduction.

Swayne's Hartebeest (SHB) (*Alcellaphus buselaphus swaynei)* is one of the endangered endemic wild animals of Ethiopia. At present Swayne's Hartebeest are found only in few localities in Ethiopia such as, Senkele SHB Sanctuary, NSNP and Mazie Wildlife Area. A total of 643 observations were made and all of them were recorded in the Nech Sar plain, none of them was recorded in the forests and wetlands. The total number of individuals in the NSNP during the study period was 35 and, they were frequently observed only in the plain. Studies have showed that, 90 individuals were transferred to Nech Sar National Park and 120 individuals were transferred to Awash National Park in 1974 to help ensure their survival (Lealem, 1974). According to Duckworth *et al*, (1992),only 40 individuals of the species were recorded in NSNP in 1992. 35 individuals in this study indicated that the population is declining to the point of extinction. Large number of SHB populations existed in the Senkele Hartebeest Sanctuary during 1976-1988, ranging from 448-2379 individuals (Nobuko, 2004). However, in 2008, the total number of Swayne's Hartebeest counted was in 283 and 351 individuals during the wet and dry seasons respectively (Tewodros Kumssa and Afework Bekele, 2008). Swayne's Hartebeest lives in an open area, light bush, sometimes in tall Savannah woodland. They are social animals and are normally seen in herds of 4-15, up to 30 (EPA, 2004). SHBs is in danger of extinction at present than any other time in the past and is classified as "Critically endangered" (IUCN, 2002). Its range in all over four Ethiopia is threatened by habitat loss to agricultural expansion and livestock overgrazing (EPA, 2004). The same factors, particularly competition with domestic animals for food, space and degradation of their habitat by overgrazing has contributed to its decline in the NSNP. A total of 7587 heads of cattle and goats was recorded from the households in the nearby villages and illegal hunting by the nearby agro-pastoralists (Yisehak *et al.*, 2006) that have destroyed the grassland habitats of SHB. Several studies proposed to create a buffer zone to integrate conservation and rural development; the idea, however, has not been followed by any action for the sustainable management of this endangered animal (Nobuko, 2004).

The five large mammalian species of the NSNP had different ranges of tolerances to different environmental variables. Hence some had wide range of tolerances and the other low range of tolerances. Thus management and conservation strategy for the mammals needs the consideration of their habitat selection. This study has shown that the Greater kudu had a wide range of tolerance as compared to Lesser kudu and Swayne' Hartebeest that had a narrow range of tolerances. In addition to wide range of tolerance abilities, to a wider habitat conditions, the Grater kudu used verities of plant species as alternative food sources. On the other hand, lesser kudu, Defassa waterbuck and Swayne' Hartebeest have high fidelity and low ecological amplitude. The distribution of the endemic Swayne's hartebeest is particularly declining in the NSNP as a result of its narrow range of tolerances to environmental conditions and human activities. Therefore, proper management is urgently need in order to save this critically endangered species from local extinction

The Black and White Colobus Monkey selected riverine and large trees as best habitats. The study has indicated that the distraction of the large Acacia trees in the Sermele Valley for crop production is one of the reasons for the decline in the population number of Black and White Colobus. This could lead the animal to higher extinction risk in the area. Therefore, the riverine woodland habitat where the animals dwell should be managed properly to save the animals.

The habitats of NSNP where these mammals occurred are mosaic of grassland, open woodland, plain land, dense woodland and riverine woodlands. This indicates the importance of NSNP in harboring various species of mammals and provides opportunity to select preferred habitat types for the species. Currently, the situation of this national park is very much discouraging due to the competing claims over its natural resources. Thus, there is strong need for urgent actions that could rehabilitate the Park. The eastern part of the Nech Sar plain where the endangered Swayne'

Hartebeest and lesser kudu occur and the Sermele valley which is the best habitat of Gureza Colobus is commonly shared by the domestic animals and crop production. In the same manner, the independent monitoring data in the western part of the park revealed that the Arba Minch ground water forest is at a greater risk from increased wood collection for Arba Minch town. Therefore, to maintain the sustainable management of NSNP and the wild animals in it, the existing patrol strategy should be strengthened with full material, finance and training capacities.

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Acute Toxicity Of Nile Tilapia (*Oreochromis niloticus*) Juveniles Exposed To Aqueous And Ethanolic Extracts Of *Ipomoea aquatica* Leaf

*****Simeon O. Ayoola[1], Kuton M.P[1], Idowu A.A[2] and Adelekun, A.B[1]

[1]Department of Marine Sciences, University of Lagos, Akoka, Yaba, Lagos State, Nigeria

[2]Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta, Ogun State.

*****Email:soaayoola@yahoo.com, sayoola@unilag.edu.ng, Tel: +234(80)34650102

ABSTRACT: The differential acute toxicity of aqueous and ethanolic extracts of *Ipomoea aquatica* leaf on Nile Tilapia, *Oreochromis niloticus* were carried out under laboratory conditions. The LC_{50} after 96hr of exposure for aqueous and ethanolic extracts of *Ipomoea aquatica* were 2.659g/L and 0.196g/L respectively. These values showed that ethanolic extract of *Ipomoea aquatica* was more toxic than its aqueous extract. Signs of agitated behaviours, respiratory distress and abnormal nervous behaviors including eventual deaths were observed in exposed fish. Control fish neither died nor exhibited any unusual behaviour. The randomized analysis of variance (ANOVA) showed that there were significant differences ($P<0.05$) in the quantal response (mortality) of *O. niloticus* to aqueous and ethanolic extracts of *I. aquatica* at 24hrs, 48hrs, 72hrs and 96hrs of exposure period. It was investigated that leaf of *Ipomoea aquatica* has piscicidal property and can be put into use in the control and management of fish ponds to eradicate predators by farmers.

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Keywords: Acute toxicity, *Ipomoea aquatica, Oreochromis niloticus*

INTRODUCTION

Agrochemical, such as pesticides especially chlorinated hydrocarbons are routinely employed as part of the integrated farming practice to protect crops and animals from insects, weeds and diseases. Widespread use of pesticide on farm is now a worldwide phenomenon (Omitoyin *et al*., 2006). The use of chlorinated hydrocarbon such as DDT, dieldrin and Lindane as pesticides has been documented. Pesticides currently in use are biocides that have high mammalian toxicity and necessitate considerable precautions in their application.

The aquatic ecosystem as a greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Omitoyin *et al*., 2006) . Pesticides become readily available in the food chain and subsequent bioaccumualate in both aquatic and terrestrial flora and fauna, with possible unquantifiable disastrous consequences on the ecosystem (Odiete, 2009). Due to the residual effects of pesticides, important organ like kidney, liver, gills, stomach, brain, muscle and genital organs are damaged in fish exposed to pesticide (Odiete, 2009).

Many plants contain chemicals which have traditionally been used to harvest fish in almost all parts of the world. Fish farmers in Nigeria have persistently and indiscriminately abused these natural plant piscicides (*Derris elliptia*) by using much higher concentrations than necessary, causing mass mortality of fish in ponds, contaminating the freshwater bodies and affecting non target organisms [1]. The physical and chemical changes in aqueous environment often cause some physiological changes in fish, thus, the water quality of an aquatic body is very crucial because it determines the productivity and other parameters necessary for fish survival. Many countries including Nigeria have legislated against the use of chemical poisons in aquatic systems and instead have policies favouring the use of natural bio–degradable alternatives to remove unwanted fish species in aquatic systems (Olufayo, 2009).

Unwanted fishes may enter aquaculture farms through water supplies or along with seed brought into the fish farm. Occasional draining of the pond and fishing are usually inadequate to control and eradicate unwanted fishes. Screening is the standard

method, but it does not stop the entry of predatory fishes in larval form. In ponds where water enters through pipes, screens may significantly restrict the flow of water (Bardach *et al*., 1972). The best way of ensuring total eradication of unwanted fishes is through the use of fish toxicants (piscicide) in the pond water (Chakroff, 1976). The use of piscicides as a tool in pond management during pond preparation to get rid of predators before fish stocking is an important tool Harwood and sytsma(2003) . Ideally, ponds should be sundried and the pond bottom cracked dried to help get rid of fish predators. However, this practice is not always possible particularly during the wet season. Moreover, farmers who are always in a hurry to prepare their ponds always resort to the use of inorganic fish toxicants (Cagaman, 1995). In view of this, farmers resort to nonconventional and unregistered fish toxicants such as agro-pesticides and sodium cyanide because they are fast acting and readily available in the market. However, these chemicals may have negative effects on the environment and farmers and health (Ayoola and Ajani, 2007). Hence, there is a need to explore other environment and health-friendly fish toxicants such as botanical plants with piscicidal activity. Plants are virtually inexhaustible source of structurally diverse biologically active substances (Istvan, 2000). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties. Unlike synthetic chemical pesticides which leave harmful residues in the aquatic environment (Koesoemadinata, 1980), botanical insecticides are believed to be more environments friendly because they are easily biodegraded and leave no residues in the environment. Since some of these pesticidal compounds present in plants were also toxic to fishes, botanical pesticides can be used as piscicide to eradicate unwanted fishes in the pond. Many plants from different families have been applied for catching fish all over the world. Examples of these plants are of the genera *Derris*, *Tephrosia* and *Lonchocarpus* of the family L*eguminosae*. The toxic parts of plants employed as fish poisons include roots, seeds, fruits, bark, latex or leaves(Olufayo,2009).

Ipomoea aquatica is a member of the Morning Glory Family, Convolvulaceae, which contains 500 species. *I. aquatica* and *I. fistula* are the only aquatic species in the genus, which also includes the sweet potato (*Ipomoea batatas* L.) (Cook, 1990). *I. aquatica* has been cultivated for its edible shoots and medicinal properties but sometimes, it is considered to be a serious weed.

Oreochromis niloticus is a member of the family cichlidae. It is considered as one of the organism suitable for toxicity test. This study intends to assess the piscicidal activity of extracts from *Ipomoea aquatica* on *Oreochromis niloticus* as indicator to eradicate predators in pond. This study is to investigate the acute toxicity of *Ipomoea aquatica* on juveniles of *Oreochromis niloticus* and to determine the lethal concentration (LC_{50}) of aqueous and ethanolic extracts of *Ipomoea aquatica* on Nile Tilapia (*Oreochromis niloticus).*

MATERIALS AND METHOD

Four hundred juveniles of *Oreochromis niloticus* were used as test organism for the toxicity test because of its suitability. The Tilapia juveniles were bought from a fish farm in Badagry, Lagos State. The juveniles were transported in two aerated polythene bags to the laboratory in the early hours of the morning (11:00 am). The water to be used for stocking of the juveniles was dechlorinated by exposing it to sun for a period of 48 hours. The Nile Tilapia juveniles were kept in a rectangular glass tank and allowed to acclimatize to laboratory conditions for a period of 14 days in an already dechlorinated tap water. The stock tank had cosmo 10,000 air pump with voltage 220-240v. The juveniles were fed twice daily using coppen commercial supplementary feed (42% protein content). The water was change daily to prevent accumulation of toxic waste metabolite. Experimentation was carried out under ambient laboratory conditions (temp.27 ± 3^0C), Feeding of the juveniles stop a day before the bioassay test.

The fresh leaves of *I. aquatica* were collected along the Oge creek, University of Lagos Akoka, Lagos State. The plant was identified to nearest taxonomical level. The extraction of *I. aquatica* was done using Ohaustriple 700-800 series weighing balance. The fresh leaves of (1 kg) were collected and washed well to remove any adhering foreign particles and soil materials. The washed leaves were oven dried at 48^0C for 36 hours to prevent enzyme action. After drying, it was coarsely powdered and later soaked in 1 litre of clean water for 72 hours. The solution was filtered through a muslin cloth to separate aqueous extract from residue. The aqueous solution was then kept in a black plastic container at room temperature, until the time of use.

1kg of coarsely powdered, well dried fresh leaves of *I. aquatica* was put in a soxhlet extractor with 250ml of 98% absolute ethanol as the extracting

solvent.the set up was placed on a heating mantle and heated for 3-4 hours.

The solvent extract was transferred to a rotary evaporator, Buchii, type 661 (with vacuum pump). The solvent was recovered with the concentration of the extract of the dried leaves reduced up to 25% volume. The extract was then transferred from the rotary evaporating flask to 100ml beaker and further concentrated in a hot-air oven at 80^0C. The extract was a dark-brown pasty substance. The extract was collected and stored at room temperature in a black plastic material that will not allow light penetration. Salinity was measured by using a hand refractometer. pH was measured by the use of Hanna instrument pH 211-micro processor pH meters. Dissolved oxygen (DO) was measured with DO meter (model EUTECH DO 600); water temperature was determined by simple mercury in glass thermometer, calibrated in centigrade (^{o}C).

BIOASSAY PROCEDURES

The preliminary tests were carried out at first to determine suitable range of concentration for the bioassay experiment. The concentration ranges chosen for the aqueous and ethanolic extract of *Ipomoea aquatica* after preliminary test were: 0.5g/L, 1.5gL, 2.5g/L, 3.5g/L, 4.5g/L and 0.11g/L, 0.21gL, 0.43g/L, 0.53g/L, 1.07g/L respectively. These concentrations were carefully measured out to make up 8 litres of solution in 5 boiassays containers. Another bioassay container with 8 litres of water, free of the extract, served as control. In each of the container, 10 juveniles (8.7±0.3) cm were introduced. Care was taken to minimize the stress on the fish by using a hand net to collect and drop the fish carefully into the rectangular plastic tanks. The Tilapia juveniles exposed to different concentration of aqueous and ethanolic extract of *Ipomoea aquatica* were monitored for mortality at 24, 48, 72 and 96 hours.

STATISTICAL ANALYSIS

The quantal response (mortality) was analysed by Probit analysis (Finney, 1980). The logdose values for LC_5, LC_{50} and LC_{95} were obtained and tabulated. Graph of Probit values were against logdose values were plotted using the line of best fit for a straight curve. The following indices of toxicity and their 95% confidence unit derived from a computer statistical programme SPSS 10.5 were:

LC_{95} value (lethal concentration that causes the death/mortality of 95% of the exposed population).

LC_{50} value (lethal concentration that causes the death/mortality of 50% of the exposed population).

LC_5 (lethal concentration that causes the death/mortality of 5% of the exposed population).

One Way Analysis of Variance (ANOVA) and comparison of means by Student Newman (SNK) test were used to test for statistical differences in the result of 96-hrs toxicity tests.

RESULTS

The test organisms showed distress in behaviour on introduction into the bioassay tanks. There were changes in the frequency of movement of the fish subjected to different concentrations of *I. aquatica.* Behavioral changes such as uncoordinated movements, somersaulting, excess secretion of mucus, erratic swimming and increase in operculum ventilation, respiratory distress, strong spasm, paralysis, and prior to the death, paleness of fish were observed during the exposure of fish to *I. aquatica.* The colour of the skin of fish exposed to the toxicant changed from normal darkly pigmentation in the dorsal and the lateral part.

The death of the fish is confirmed by its floating on its side or failure to respond to stimulus even when touch with a forcep. The number of such fishes were recorded and removed from the test media to prevent contamination of the whole media. The numbers of dead fishes were computed as percentage (%) mortality per period of exposure. This process was replicated to eliminate bias that may result due to handling, differences in size and weight and other intrinsic physiological imbalance in the test organisms. The mean values obtained for the physico-chemical parameter of the test media throughout the period of the experiment are presented in tables 1 and 2 for aqueous and ethanolic extract respectively.

Table 1: Mean physico-chemical parameters of the test concentrations *(Ipomoea aquatica*) on *Oreochromis niloticus* using aqueous extracts

Concentration	Physico – chemical Parameters			
g/L	Do (mg/l)	Salinity($^0/_{00}$)	pH	Temp 0C
0.0	5.9±0.1	0	7.0	26.0±0.6
0.5	5.8±0.1	0	7.0	27.0±0.1
1.5	5.8±03	0	7.0±0.1	27.0±0.1
2.5	4.4±0.1	0	6.9±0.2	27.1±0.2
3.5	4.0±0.1	0	6.7±0.3	27.0±0.1
4.5	3.0±0.1	0	6.4±0.1	27.3±0.2

*Mean values followed by the same superscript in each column are not significant different (p>0.05)

Table 2: Mean physico-chemical parameters of the test concentrations *(Ipomoea aquatica*) on *Oreochromis niloticus* using ethanolic extracts

Concentration	Physico – chemical Parameters			
g/L	Do (mg/l)	Salinity($^0/_{00}$)	pH	Temp 0C
0.0	5.9±0.1	0	7.0	26.0±0.6
0.11	5.8±0.1	0	7.0	27.0±0.1
0.21	5.8±0.2	0	7.0	26.9±0.2
0.43	4.1±0.1	0	6.8±0.2	26.8±0.2
0.53	4.0±0.1	0	6.7±0.3	27.0±0.1
1.07	3.0±0.1	0	6.4±0.1	27.3±0.2

*Mean values followed by the same superscript in each column are not significant different (p>0.05)

EFFECT OF AQUEOUS EXTRACT OF *Ipomoea aquatica on Oreochromis niloticus*

The result of the acute toxicity test of aqueous extract of the leaf of *I. aquatica* against *Oreochromis niloticus* juveniles at 24hrs, 48hrs, 72hrs and 96hrs of exposure period is shown in Table 3.

Figure 1 shows the graph of probit response and log – dose drawn from the probit line equation tables, (Microsoft Excel, 2007). The LC_{50} values obtained at 24hrs, 48hrs, 72hrs and 96hrs for aqueous extract were 9.178g/L, 9.157g/L, 4.623g/L and 2.659g/L respectively.

The randomized analysis variance (ANOVA) showed there was significant difference ($P<0.05$) between all the concentrations at 24, 48, 72 and 96 hrs of exposure. Using the Student Newman – Keul's (SNK) test ($P<0.05$) shown. Table 4 shows the mean quantal response of 1.5g/L was significantly different from the control at 48, 72 and 96 hrs exposure. At 72 and 96 hrs exposure period, 0.5g/L and 1.5g/L showed no significant difference so also is the case for 2.5g/L and 3.5g/L at 96 hrs exposure period.

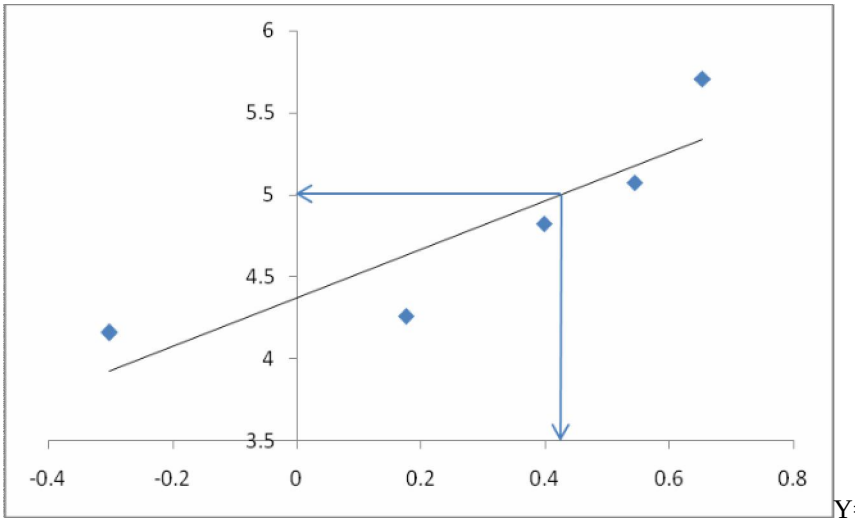
TABLE 3: ACUTE TOXICITY EFFECT OF AQUEOUS EXTRACT OF the leaf of *Ipomoea aquatica* AGAINST *Oreochromis niloticus* juvenile AT 24, 48, 72 AND 96 HOURS EXPOSURE.

Exposure time (hrs)	LC_{50} (95% C.L g/L)	LC_{95} (95% C.L g/L)	LC_5 (95% C.L g/L)	Slope ± S.E	T.F	D.F	Probit Line Equation
24	9.178	41.394	2.035	2.514±0.93	1.00	3	Y=2.579+2.514X
48	9.157	40.822	0.800	1.554±0.49	1.00	3	Y=3.506+1.554X
72	4.623	37.035	0.240	1.280±0.36	1.99	3	Y=4.149+1.280X
96	2.659	31.485	0.225	1.532±0.35	3.35	3	Y=4.349+1.532X

L.C = Lethal concentration; T.F = Toxicity factor; S.E = Standard Error; D.F = Degree of freedom;

C.L = Confidential Limit

$$T.F = \frac{LC_{50} \text{ at 24hrs}}{LC50 \text{ at any other period time}}$$



Y=4.349+1.532X

Fig.1: Linear relationship between probit response and log concentration of Aqueous Extract of *I. aquatica* on juveniles of *O. niloticus*

TABLE 4: MEAN MORTALITY RESPONSE OF *O. niloticus* EXPOSED TO DIFFERENT CONCENTRATION OF AQUEOUS EXTRACT OF THE LEAF OF *I. aquatica* FOR 96 HOURS

Concentration (g/L)	Total no. 0f Organisms	Percentage Mortality Response/Time Hours			
		24	48	72	96
Control	30	0^{a}	0^{a}	0^{a}	0^{a}
0.5	30	0^{a}	3^{ab}	17^{b}	20^{b}
1.5	30	1^{b}	10^{ab}	17^{b}	23^{b}
2.5	30	7^{b}	17^{bc}	30^{b}	43^{c}
3.5	30	13^{b}	27^{cd}	43^{c}	53^{c}
4.5	30	23^{c}	33^{d}	60^{d}	76^{d}

Means followed by the same superscript letter in a column are not significantly different in the SNK test (P>0.05)

TABLE 6: ACUTE TOXICITY EFFECT OF ETHANOLIC EXTRACT OF the leaf of *Ipomoea aquatica* AGAINST *Oreochromis niloticus* juvenile AT 24, 48, 72 AND 96 HOURS EXPOSURE.

Exposure time (hrs)	LC_{50} (95% C.L g/L)	LC_{95} (95% C.L g/L)	LC_{5} (95% C.L g/L)	Slope ± S.E	T.F	D.F	Probit Line Equation
24	0.642	1.692	0.244	3.910±0.64	1.00	3	Y=5.751+3.910X
48	0.391	1.468	0.104	2.863±0.41	1.64	3	Y=6.168+2.863X
72	0.276	1.189	0.064	2.594±0.39	2.33	3	Y=6.450+2.594X
96	0.196	0.842	0.046	2.600±0.41	3.28	3	Y=6.839+2.600X

L.C = Lethal concentration; T.F = Toxicity factor; S.E = Standard Error; D.F = Degree of freedom

C.L = Confidential Limit

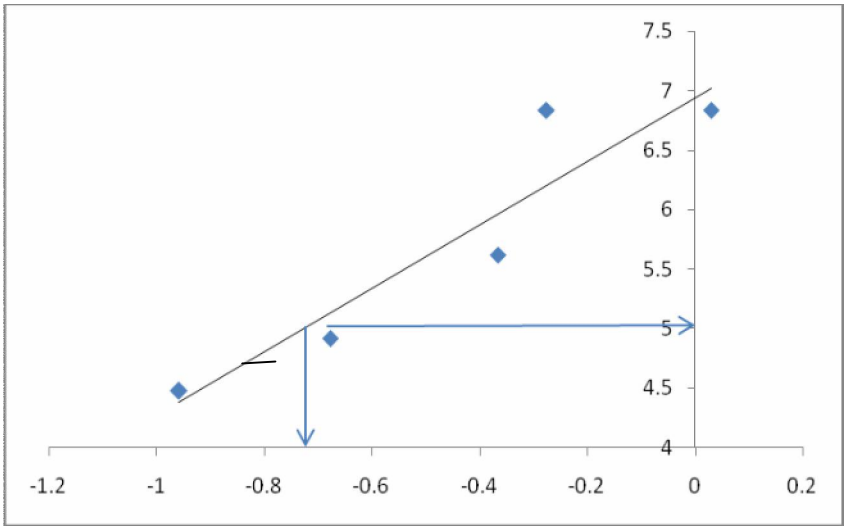
$$T.F = \frac{LC_{50} \text{ at 24hrs}}{LC50 \text{ at any other period time}}$$

EFFECT OF ETHANOLIC EXTRACT OF *Ipomoea aquatica on Oreochromis niloticus*

The result of the acute toxicity test of ethanolic extract of the leaf of *I. aquatica* against *Oreochromis niloticus* juveniles at 24hrs, 48hrs, 72hrs and 96hrs of exposure period is shown in Table 6.

Figure 2 shows the graph of probit response and log – dose drawn from the probit line equation tables (Microsoft Excel, 2007). The LC_{50} values obtained at 24hrs, 48hrs, 72hrs and 96hrs for aqueous extract were 0.642g/L, 0.391g/L, 0.276g/L and 0.196g/L respectively.

The randomized analysis variance (ANOVA) showed there was significant difference (P<0.05) between all the concentrations at 24, 48, 72 and 96 hrs of exposure. Using the Student Newman – Keul's (SNK) test (P<0.05) shown in Tables 7 and 8, the mean quantal response of 0.11g/L was significantly different from the control of 48, 72 and 96 hrs exposure. At 48 and 72 hrs exposure period, 0.11g/L and 0.21g/L showed no significant difference so also is the case for 0.53g/L and 1.07g/L at 96 hrs exposure period.



Y=6.839+2.600X

Fig.2: Linear relationship between probit response and log concentration of Ethanolic Extract of *I. aquatica* on juveniles of *O. niloticus*

TABLE 7: MEAN MORTALITY RESPONSE OF *O. niloticus* EXPOSED TO DIFFERENT CONCENTRATION OF ETHANOLIC EXTRACT OF THE LEAF OF *I. aquatica* FOR 96 HOURS

Concentration (g/L)	**Total no. 0f Organisms**	**Percentage Mortality Response/Time Hours**			
		24	48	72	96
Control	30	0^{a}	0^{a}	0^{a}	0^{a}
0.11	30	0^{a}	13^{b}	23^{b}	30^{b}
0.21	30	3^{a}	17^{b}	30^{b}	47^{c}
0.43	30	10^{a}	20^{b}	47^{c}	73^{d}
0.53	30	57^{b}	90^{c}	93^{d}	97^{e}
1.07	30	77^{c}	93^{c}	97^{d}	97^{e}

Means followed by the same superscript letter in a column are not significantly different in the SNK test (P>0.05)

DISCUSSION

The result obtained from this study showed that both the aqueous and ethanolic extract of *Ipomoea aquatica* had toxic effect on the juveniles of *Oreochromis niolticus* and the effect of their toxicity increases with time of exposure. The LC_{50} values at 96hrs of exposure of *O. niloticus* to aqueous and ethanolic extracts were 2.659g/L and 0.196g/L respectively.

The difference in the level of toxicity of the extracts could be as a result of the method of extraction used. In the case of aqueous extract, the alkaloids was extracted but the presence of water dilute it, hence reducing it potency, while the alkaloids of ethanolic extract obtained using soxhlet extractor and ethanol as extracting medium remains almost undiluted .

Oreochromis niloticus exhibited erratic movement and aggressiveness (Abalaka and Auta, 2010) when placed in the bioassay tanks. Some attempted to jump out of the tanks. This behavior continued for a few hours after which their movement becomes normal and calm.

Increased physical activity, convulsion, excess secretions of mucus, incessant gulping of air, erratic swimming, respiratory distress, paralysis, sudden quick movement, increase in opercula ventilation and prior to death darkening of fish were associated with *I. aquatica* toxicity in this study. This agreed with the findings of Abalaka and Auta, 2010 on *Oreochromis niloticus* exposed to trichloroform. Omitoyin *et al*., 1999 reported similar observation in *Sarotherodon galilaeus* (Tilapia) fingerlings exposed to piscicidal plant extracts of *Tetrapleura tetraptera.*

The intensity of respiratory distress increased with increasing extracts concentrations but decreased with exposure period for both extracts while nervous abnormality increased with increasing extracts concentrations and exposure period for both extracts (Abalaka and Auta, 2010). Extracts of *I. aquatica* probably poisoned the fish leading to pathological alterations in their tissues and organs (Gabriel *et al*., 2007) which eventually lead to the direct death of the tested organism. Indirect death could also result from changes in the physicochemical conditions of their immediate external environment (Ayoola, (2008), Olufayo, (2009)). The observed respiratory distress may be due to decreased in the dissolved oxygen contents (Dede and Kaglo,2001).

Warren (1997) had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolve oxygen concentration, which will impair respiration leading to asphyxiation. Generally, one could deduce from this research work that the introduction of *Ipomoea aquatica* into water bodies would threaten the life and existence of fish. Therefore, this plant can actually be use as a biological control in eradicating predators and unwanted organisms in the ponds by farmer instead of using agrochemicals.

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Effect of Benzyladenine Foliar Sprays on Offsets Production and Root Growth of *Aloe Barbadensis* Miller.

Saeid hazrati[1], zeinalabedin Tahmasebi Sarvestani*[1], arman beyraghdar[2], faraz mojab[3] and Seyyed jaber hosseini[1]

Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran[1]

Department of Horticulture, Faculty of agriculture, Tarbiat Modares University, Tehran, Iran[2]

Department of Pharmacognosy, Shaheed Beheshti University, Tehran, Iran [3]

saeid.hazrati@yahoo.com; tahmaseb@modares.ac.ir

Abstract: Aloe vera (*Aloe barbadensis*) is one of the most important medicinal plants and used world wide in drug and cosmetic industry. In order to determine the effect of different BA levels on offset production and root growth of Aloe vera, an experiment was conducted; the experimental design was RCBD with four replications placed in greenhouse condition. Treatments were included four different BA levels (0, 500, 1000, 1500 mg.L^{-1}). At the end of growth period, some characteristics such as offset number, offset leaf number, offset height; number of flowering stems, root length, root volume and root fresh and dry weight were measured. Resulted showed that Increasing hormone concentration cause increase offset number and decreased the root growth, so that the highest offset number was at 1500 mg.L^{-1} which was 95.36% more than our control treatment. Thus BA spraying can be used as an appropriate way to increase offset production in Aloe vera, which has low offset production rate.

[Saeid hazrati, zeinalabedin Tahmasebi Sarvestani, arman beyraghdar, faraz mojab and Seyyed jaber hosseini. Effect Of Benzyladenine Foliar Sprays On Offsets Production and Root Growth Of *Aloe Barbadensis* Miller. Nature and Science 2011;9(3):100-104]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: *Aloe barbadensis* Miller, Benzyl Adenine, Offset, Root

1. Introduction

Aloe vera (*Aloe barbadensis*) which belongs to Liliaceae family is a perennial plant with rosette growth pattern compatible with subtropical regions. This species is native to southern and eastern Africa, but is commercially cultivated in different region in America, European and Asia (Reynolds, 2004; Hasanuzzaman et al., 2008). In recent years, aloe vera gel extracts are widely used in cosmetics industry due to physiological and biological properties and also efficiency in healing wounds caused by burns and skin incision, and also because of anti inflammatory, antifungal, antibacterial antiviral and other medicinal properties (Ramachandra and Srinivasa Rao, 2008). Aloe plants are propagated by two methods: sexual and vegetative. In *Aloe barbadensis* species, the most common species in gel production, there is high rate of male sterility which results in cross-pollination; therefore propagation via seed leads to genetic segregation in daughter plants (Natali et al., 1990; Keijzer and Cresti, 1987). For commercial production and increasing leaves yield by increasing area of cultivated land, we have need methods to minimize plantlet production period. Main vegetative propagation method of Aloe plants is by using offsets. Offsets are produced from the end of short stolon and can be used as a propagule in perennial plants propagation (Carey, 2008). Although the offset production rate in Aloe vera plants is high, it is not enough for commercial production, and slow rate of offset production is a serious obstacle in developing its cultivation. Therefore, offset production should somehow be increased. Due to these reasons, using agronomy practices seems to be necessary in order to produce maximum plants number in minimum time. Cytokinin is widely used in ornamental plants production. It is one of the most important plant hormones which regulates plant growth and development and has an important role in promoting cell division with similar functions to kinetin such as differentiation, leaf development and increased nutrients mobility in plants (Duan et al., 2006; Shudo, 1994). Previous study results show that plant growth regulators such as cytokinins could improve shoot growth (Carey, 2008). Spraying cytokinins on *Hemerocallis citrine* shows that this group of plant growth regulators can increase offset production via affecting cell division, offsets size and growth by stimulating lateral buds growth (Amling et al., 2007).

Few studies have been conducted on evaluating the effect of cytokinins on root system of Liliaceae family. Cytokinin is a plant hormone synthesized in root and considering plant type and hormone concentration it has irritating or inhibiting effects on root development. High cytokinins concentration prevents roots growth, but lower concentrations result in improved root development and growth (Zhang and Hasenstein, 1999). Cytokinin is a hormone which can increase flowering stem production in many plants (Carey et al., 2008). The purpose of this study was to determine different BA foliar Sprays application levels on offset production and root growth in Aloe vera.

2. Materials and Methods

Field trials with Aloe vera (*Aloe barbadenisis*) were conducted at the experimental farm of Faculty of Agriculture, Tarbiat Modares University (TMU) in 2009-2010 growth seasons. This experiment was based on Complete Randomized Block Design (RCBD) with four replications. Treatments were included four levels of benzyl adenine (BA_1: 0, BA_2: 500, BA_3: 1000, BA_4: 1500 mg.L^{-1}). Uniform offsets size of 18-20 cm were completely randomly selected then transferred to greenhouse and were planted in pots with capacity of 20 kg soil. Greenhouse temperature during the experiment was 28ºC and 22ºC during day and night, respectively. Plants, based on field water capacity, were uniformly irrigated.

BA application

The cytokinin stock solution was diluted into 10 L aliquots of 0, 500, 1000, and 1500 ml.L^{-1} BA, each containing 10 ml (0.l'%) Tween-20 surfactant. All mixtures were formulated and sprayed in 17th week after planting. Control plants were sprayed with Distilled water plus 10 ml. L^{-1} Tween 20.

Data collection

After 12 months, four plants for each treatment were randomly selected, and were measured stem diameter, number of flowering stem, offset number, offset leaf number, offset length, offset weight, root length, and roots fresh and dry weight characteristics.

Statistical analysis

Data were statistically analyzed using two way analysis of variance (SAS Institute, 9.1.3). The significance of differences among treatment means were compared by Fisher's least-significant difference test (LSD) at $P < 0.05$. The number of replications (n=4) in the table/figures denotes individual plants from each treatment measured for each parameter.

3. Results and Discussion

Results showed that BA application has a significant effect on offsets number, weight, height and leaves number (Table 1). Means comparison of treatments showed that higher BA levels result in significance increase on all offsets characteristics. Highest offset number (6.32) offsets leaves number (5.8), offsets weight (38.42) and offsets height (23) was in treatment with 1500 ml.L^{-1} which was 95.36%, 75.51%, 71.89%, 82.74% higher than control treatment, respectively (Figure 1,Table 3). In this experiment, BA increased offsets number that can be attributed to decreased apical dominance by main stem (Duck et al., 2004). Our result was in accordance with those achieved by Carey et al., (2008). Considering the cytokinins effects, it was entirely predictable that spraying BA on plants stimulates cell division and increased cell number (Schmulling, 2002); therefore, application of BA results in higher offsets number, higher offsets leaves number, higher offsets height and weight. These results were in concordance with Carey et al., (2008) on other Liliaceae family plants (Echeveria and Sempervivum). Analysis of variance showed that application of BA had significance effects on roots length, root fresh and dry weight (Table. 2). As indicated in mean comparison Table 3, these traits were simultaneously decreased by increasing BA levels. Foliar application of BA with concentration of 1500 ml.L^{-1} had the shortest root, while the longest root was related to control treatment. Lowest fresh and dry weights was achieved in treatment with 1500 ml.L^{-1} BA; while highest fresh and dry weight was in control treatment (Figure 2). Analysis of variance showed that different BA levels had a significance effect on Aloe vera stem diameter (Table 2). As showed in mean comparison Table 3, foliar application of BA resulted in higher stem diameter which was the highest in treatment with 1000 ml.L^{-1} BA. Response of Aloe vera was the same as polyanthes which showed higher flowering stem diameter by foliar application of BA (Shoor et al., 2005). Stem diameter increasing is a result of BA role in cell division and assimilated transport (Schmulling, 2002; Halmann, 1990). According to the biological effects of cytokinin compounds, the results were entirely predictable that foliar application of BA stimulates cell division and increased cell number and therefore can result in increased offset number, offsets length, offsets weight and stem diameter. These increases were in accordance with those results achieved by (Khalighi et al., 2006). In a study by Garner et al., (1998), results showed that

BA application on hosta plants can increase bud development and propagule production ratio. Baque et al., (2010) showed that application of BA, Tidiazuron, and a combination of these two substances decreased roots fresh and dry weight in *Morinda citriofolia*. Earlier studies on Sedum, a succulent plant, showed that application of BA increased vegetative growth, flowering percentage and prevented root development (Boe et al., 1972). Aloe vera plants grow slowly and offset formation rate is slow in them. Application of BA-type cytokinin hormone increases cell division and lateral bud formation. Foliar application of BA had significantly increased flowering stems number (Table 1), but there was no statistical difference between levels of 500 and 1500 $ml.L^{-1}$. Our results were in agreement with those achieved by Boyle (1992), on Easter cactus in which BA had increased flowering stems number. In another study by Carey (2008), application of BA results in highest flowering stems number in *Echeveria setosa* and *Salvia nemorosa* which was in accordance with our results.

4. Conculation

Based on our results it can be concluded that foliar application of BA with concentration of 1500 $ml.L^{-1}$ can increase offsets number and give rise to higher levels of BA prevents root growth.

Table 1: The analysis of variance for Influence of application BA foliar spray on characterize offset in aloe vera plants

S.O.V	df	Offset				Number of Flower Stalks
		number	weight	Number leaf	height	
BA	3	* *	**	**	**	**
Error	9	0.83	43.77	0.29	4.90	0.09
C.V	--	23.03	25.29	12.41	15.75	17.19

* and** Significant at the 5% and 1% levels of probability, ns: no significant

Table 2: The analysis of variance for Influence of application BA foliar spray on characterize root in aloe vera plants

S.O.V	df	stem Diameter	Root			
			Length	fresh Weight	Volume	dry Weight
BA	3	**	*	**	ns	**
Error	9	9.27	9.15	13.46	22.48	0.21
C.V	---	6.69	13.6	13.55	21.70	12.51

* and** Significant at the 5% and 1% levels of probability, ns: no significant

Table 3: Results of mean comparison the influence of application foliar BA on some characterize in aloe vera plants

Treatment	Offset weight (g)	Number of offset leaf	offset height (cm)	Number of Flower Stalks	Diameter of stem (mm)	Length root (cm)
control	9.41 ± 4.21^{d}	1.68 ± 0.75^{c}	3.97 ± 1.78^{d}	0.55 ± 0.13^{b}	43.82 ± 1.02^{b}	24.8 ± 1.55^{a}
BA 500 $mg.L^{-1}$	24.79 ± 4.45^{c}	4.396 ± 0.70^{b}	11.93 ± 2.15^{c}	2.30 ± 0.21^{a}	43.73 ± 0.85^{b}	24.32 ± 1.41^{a}
BA 1000 $mg.L^{-1}$	32.10 ± 2.87^{b}	5.59 ± 0.24^{a}	17.32 ± 1.11^{b}	2.25 ± 1.9^{a}	48.16 ± 0.75^{a}	21.37 ± 1.28^{c}
BA 1500 $mg.L^{-1}$	38.42 ± 2.88^{a}	5.80 ± 0.19^{a}	23.00 ± 1.93^{a}	1.97 ± 0.06^{a}	46.34 ± 0.79^{a}	18.25 ± 2.17^{c}
LSD at (5%)	4.71	0.38	1.57	0.48	2.16	5.05

Means with different superscripts are significantly different at $P< 0.05$ level of significance using the Fisher's least significant different (LSD).

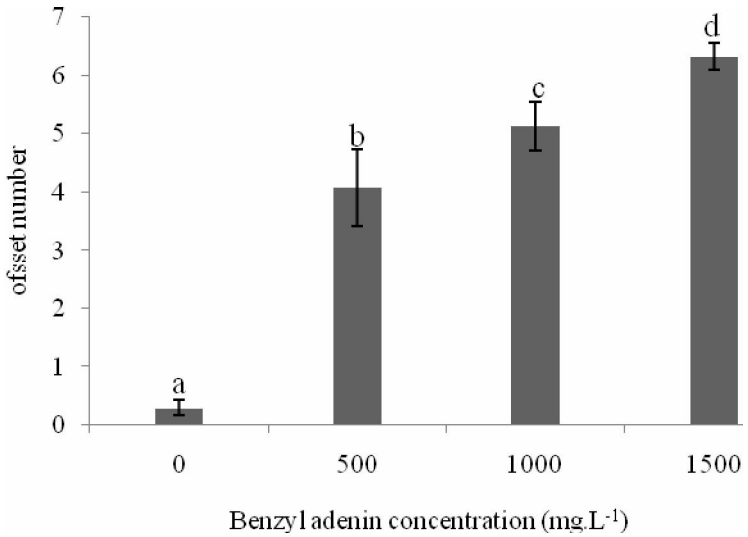

Figure 1: Effect of BA spray foliar on offset number in aloe vera plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at $P < 0.05$.

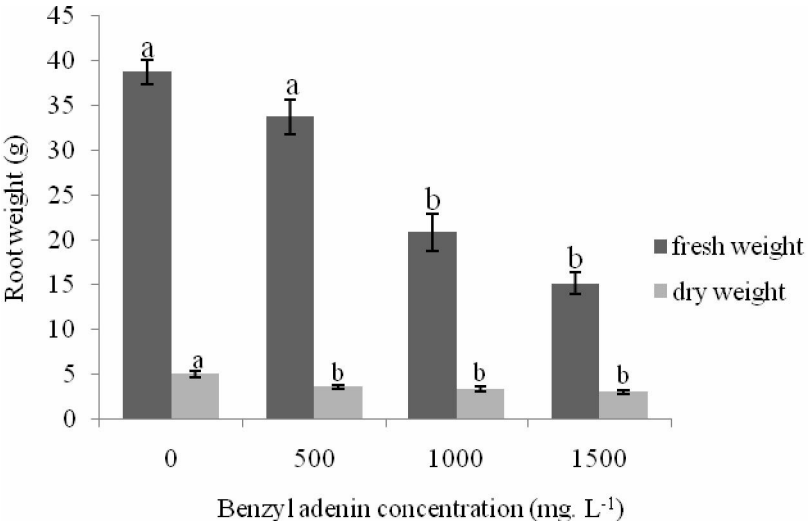

Figure 2: Effect of BA spray foliar on root fresh and dry weight in aloe vera plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at $P < 0.05$.

Correspondence to:

zeinalabedin Tahmasebi Sarvestani
Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
Cell phone: +98-09122185864
E-mail: tahmaseb@modares.ac.ir

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Antimicrobial proteins and oil seeds from pumpkin (*Cucurbita moschata)*.

A. B. Abd EI-Aziz and H.H. Abd EI-Kalek.

Microbiology Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. abdelazizamany@gmail.com

Abstract: The nutritive value and biological activity of pumpkin (*Cucurbita moschata*) seeds cultivated in Egypt were evaluated. Chemical analysis of fiber, protein, ash, carbohydrates, and fatty acids present in the non irradiated and irradiated seeds was conducted. The results show that the values for the indices are within recommended levels for edible oils. Seeds were found to be rich in oil (44.45±2.83 %). The oil contains an appreciable amount of unsaturated fatty acids (71.10±4.32 %) and found to be a rich source of linoleic acid (52.64±0.90 %).Gamma irradiation of pumpkin increased significantly ($P<0.05$) the yield of free fatty acid, acid value and peroxide value of extracts. Results showed decreases in the iodine value after irradiation at doses up to 10kGy. The antimicrobial effect of irradiated and unirradiated pumpkin oil seeds was studied. Gamma radiation up to 10kGys don't affect on the antimicrobial activity of pumpkin oil. Three different proteins were extracted from the pumpkin rinds, seeds, and pulp. All the extracted proteins were screened for their antimicrobial activity against the tested microbial isolates. The total protein and antimicrobial effect of all extractions were decreased at gamma irradiation doses used.
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Key words: pumpkin seed, Pumpkin seed oil, Oil Quality, fatty acid, Antimicrobial, Antibacterial protein.

1. Introduction

The pumpkin is an angiosperm belonging to the cucurbitaceae family. *Cucurbita moschata* is more tolerant to harsh environmental conditions than other cucurbitaceae species [1]. Pumpkin fruit has many nutritional components including pumpkin polysaccharides, active proteins, essential amino acids, carotenoids, and minerals. It has been received considerable attentions in recent years because of the nutritional and health protective value of these components [2].

Pumpkin seeds have a high nutritional value, provides good quality oil, and excellent source of protein [3]. In addition to good health benefits, pumpkin seeds are less expensive and are widely distributed. In the traditional medicine in North America and Mexico, pumpkin seeds have been used as an anthelmintic agent and for supportive treatment in functional disorders of the bladder [3].

The healing powers of plants have been used for hundreds of years; about 80% of the available therapeutic substances are originated from medicinal plants [4, 5]. Scientists showed that the plants had medicinal properties for their biological activities ranging from antimicrobial to antitumor. The antimicrobial activity of plants has many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [6, 7].

While some of the oils used on the basis of their reputed antimicrobial properties have well documented *in vitro* activity [8]. The seed of pumpkin has pharmacological activities such as anti-diabetic [1], antifungal, antibacterial and antiinflammation activities, and antioxidant effects [9]. The most critical health benefit attributed to pumpkin seed oil is preventing the growth and reducing the size of the prostate [10].

In this study, the pumpkin (*Cucurbita moschata*) was screened for antimicrobial compounds from seeds oil extracts and all of its under-utilized products (rinds, seeds and pulp).

Recently increased attention has been focused on the utilization of under-utilized agricultural products, as well as by-products and wastes from food processing to produce food and feed. Such utilization would help maximize available resources and minimize waste disposal problems.

Pumpkin seeds, rinds and pulp, that remain in large quantities as waste product after the removal of the flesh could be utilized. The seeds of pumpkin are rich in oil and protein and the crop could potentially become another source of vegetable oil and protein.

The aim of the present study was to investigate the detailed proximate chemical composition, the physicochemical properties of oil and the antimicrobial effect of the extracted oil and proteins. The effect of gamma irradiation on the physicochemical properties and antimicrobial effect of pumpkin oil and extracted proteins were studied.

Sylvia, 2004). These microbes benefit for the plant through different mechanisms action, including the production of secondary metabolities, antibiotics and hormone like substances (Ozbay and Newman 2004; Harman *et al.* 1996). The production of siderophores, antagonistic to soil borne root

pathogens (Dubeikovsky *et al.* 1993; Siddiqui *et al.* 2008) has been also reported.

The bio-efficiency of compost therefore, could also be further enhanced by fortifying it with plant nutrients or biocontrol inoculants such as *Trichoderma* spp. *Trichoderma harzianum* alone or in combination with compost has been documented as the most common and effective biocontrol agent for disease control in various host-pathogen systems (Elad 2000; Ibrahim 2005; Siddiqui *et al.* 2008).

Therefore, this study was carried out to determine the efficiency of compost fortified with *T. harzianum* as an alternative to chemical fungicide and Top.Znformulation on morpho-physical growth and occurrence of root rot disease of orange and mandarin citrus seedlings. The effect of different treatments on rhizosphere soil microflora was also studied.

2. Material and Methods:

Experimental procedure

Sample collection and irradiation

Pumpkin *(Cucurbita moschata)* fruits were purchased from Agricultural Research Center (ARC) in Giza (Egypt). The ripe pumpkins were cut and the seeds were extracted from them, washed, dried in an oven at 60 – 70°C to constant weight. Samples of pumpkin seeds were sealed in polyethylene bags and irradiated to 1, 3, 6, and 10 kGy using a Cobat-60 gamma chamber 4000A. INDIA, the average dose rate of this gamma radiation source was 2.5 kGy/h. The irradiation facilities were located at the National Center for Radiation Research and Technology, NCRRT (Nasr City, Cairo, Egypt).

Oil extraction

Control (Non-irradiated seeds) and γ-irradiated pumpkin seeds were ground in an electric grinder to pass through 0.4 mm screen and fed into a soxhlet extractor with methanol as a solvent. The solvent was then distilled off under vacuum at 45 °C in a rotary evaporator [11].

Proximate composition

Moisture content was determined according to AOAC, 1995 [12]. Crude oil was determined by a Soxhlet extractor using methanol as a solvent. Crude proteins were calculated from the nitrogen content by Kjeldahl method (AOAC, 1995) [12]. Crude fiber was determined according to the gravimetric procedure of AOAC, 1995 [12]. Ash was determined by incinerating at 550°C in a muffle furnace for 6 hrs [12]. The total carbohydrate content (on dry weight basis) was determined by subtracting the sum of the percentages of moisture, crude fat, fiber, protein and ash from 100 [100-(crude protein + crude lipids + ash + crude fiber)]. All the analyses were done in triplicate.

Oil quality attributes

Physicochemical properties of oil extracted from irradiated and non-irradiated pumpkin seeds were determined. Peroxide value (mg/kg), iodine value (g/g), free fatty acid (%) and acid value (mgKOH/g) were estimated according to AOAC, 1995 [12].

Fatty acids composition of pumpkin seed oil.

Individual fatty acids were determined by gas liquid chromatography (GLC) [13].

Microorganisms, inoculum and sample preparation

Microorganisms

The bacterial and fungal strains used in this study were obtained from the Microbiology Laboratory, Department of Microbiology, and National Center for Radiation Research and Technology, NCRRT.

Seeds oil (extracted from non-irradiated seeds and γ-irradiated seeds) were screened for their antimicrobial activity using broth micro dilution method. The yeasts species tested were *Candida albicans* and *Rhodotorula rubra*, the mold species tested were *Trichoderma viride*, *Penicillium chrysogenum*, *Rhizopus oligosporus*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Aspergillus niger*

The bacterial species tested were two gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and three gram-negative bacteria (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Escherichia coli*)

All the microorganisms used were checked for purity and maintained at 4°C in slants of nutrient agar and sabouraud dextrose agar (SDA) for bacteria and fungi, respectively.

Preparation of inocula

A loopful of cells were transferred from new slant into 25 ml broth medium in 250-ml Erlenmeyer flasks and cultivated on a rotary shaker at 200 rpm for 18 hrs at 37 °C for bacteria, and at 48 hrs for yeasts. Inocula were prepared by transferring morphologically similar colonies of each organism into (5 ml) 0.9 % sterile saline solution to obtain the required working suspensions, 10^8 cfu/ml for bacteria and 10^7cfu/ml for yeasts [14]. For the fungi, fungal spores were harvested after 7 days old SDA slant culture was washed with 10ml saline in 2% Tween 80 with the aid of glass beads to help in dispersing the spores. The spores' suspensions were standardized to 10^5cfu/ml.

Preparation of plates

Stock solutions of the seeds oil and the positive control drugs ampicillin for bacteria and ciprofloxacin for fungi (Sigma-Adrich, UK) were prepared in dimethyl sulphoxide (DMSO) at the concentrations of 100 mg/mL and 1.6 mg/mL,

respectively. Microdilution susceptibility testing was performed in flat-bottom 96-well clear plates containing broth medium (0.1 ml) in each well. Sample solutions (0.1 ml) were subsequently serially diluted two-fold in the plates with the broth, starting with the final concentration of 1000 mg/L for plant extracts and 8 mg/L for standard antibiotics. The working inoculum suspension (0.1 ml) was added. Sterility and growth controls in the presence of organic solvents employed in sample preparation were also included. No inhibitory effects were observed in the presence of DMSO at the highest concentration used (0.5% v/v). The plates were incubated at 37 °C for 24 - 48 hrs for bacteria, and at 30 °C for 5-7 days for yeasts and fungi, respectively. The amount of growth in the wells containing the agent was compared visually with the growth in the growth control wells. The concentration with a prominent decrease in turbidity was determined as the MIC. Each isolate was tested in three separate replicates. Sample with the final concentration of 1000 mg/L for plant seeds oil extracts were not effective with some tested microorganisms so concentrations of 2000 mg/L and 3000 mg/L of plant extracts were also tested for them.

Extraction and irradiation of crude protein from different parts of pumpkin

One hundred grams of dried pumpkin rinds, seeds and pulp were homogenized in extraction buffer (50 mM Tris/HCl, pH 6.8 glycerol 10%w/v, ascorbic acid 0.1%, cyctiene hydrochloride 0.1w/v) using laboratory blender (Stomacher 400). The homogenate was centrifuged and soluble extracts were separately extracted in cold acetone for 30 min. After centrifugation all the extracts were concentrated under vacuum and stored at 4 °C. The filtrates were used as crude extracts. All the procedures were performed at about 4 °C.

Extracted pumpkin protein from seeds, pulp and rinds samples were irradiated at 1, 3, 6 and 10 kGy using a Co^{60} gamma-irradiator at the National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. The dose rate was 2.5 kGy/h. The unirradiated and irradiated collected protein fractions were subjected to an antifungal and antibacterial assay against the tested isolates.

Polyacrylamide Gel Electrophoresis

SDS-PAGE was run in a gel containing 15% (w/v) polyacrylamide and 0.1% (w/v) SDS [15]. The silver staining method for protein was used, as a sensitive and detective stain of little protein (2µg) in a single band [16]. The total protein content for each extracted protein was determined [17]. The proteins gels were scanned for band R_f using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6 C, Fullerton CA 92631). The different M.W. of bands were determined against PCR marker amresco 100 bp K180 by unweighted pair-group method based on arithmetic mean (UPGMA)

Antimicrobial Growth Inhibition Assay

Antibacterial activity assay of extracted proteins.

In vitro antibacterial activity of extracted proteins was determined by the agar disc diffusion method. 18 ml of sterilized Mueller Hinton agar medium was taken in each Petri dish and then spread with a suspension of the tested micro-organism (average concentration is 10^8 cells/ml). 150 µg aliquots of each extract was applied on sterile paper discs and placed on the seeded agar plates and then incubated at 37°C for 24 hrs. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions in millimeter [18].

Antifungal activity assay

Fungal fragments were placed in the center of potato/ dextrose/agar (PDA) plates, and the plates were then incubated at 28°C for 60 hrs. After incubation, sterilized blank paper disks were placed around the seeded fungi and 150 µg aliquots of each protein extract was then delivered to the disks. The plates were incubated at 28°C for 72 hrs [19].

Analysis of uptake with acridine orange.

To determine whether extracted proteins induce permeabilization of the microbial cell membrane, we assayed the uptake of the vital dye, acridine orange, by conidia and vegetative cells of yeasts and bacteria. Microbial cells non treated with extracted proteins were used as a control [20].

Statistical Analyses

The statistical package used was SAS system version 9.1.3 (Cary, NC). Each determination was carried out on three samples. Averages and least significant differences were calculated. A P value of <0.05 was considered significant.

3. Results and Discussion

Proximate chemical composition.

The proximate chemical composition of dried pumpkin seeds is shown in Table 1. The contents of moisture, protein, lipid, ash, fiber and Carbohydrate were: 7.73 ± 1.22, 39.25 ± 0.51, 44.45 ± 2.83, 4.41±0.32, 3.60 ± 0.45 and 8.52 ± 0.98%, respectively. However, most of those components were in accordance with those reported for *Cucurbita moschata* seeds by many investigators (21-23).

The whole seed moisture contents was quite low (7.73 ± 1.22%) and was within the range of moisture The pumpkin seeds are a good source of protein (39.25 ± 0.51 %), oil (44.45%) and carbohydrates (8.52 ± 0.98%). Similar values for

protein and oil contents of the pumpkin seeds were reported [22].The crude protein value compared favorably with high protein seeds and legumes like soybeans (35%) and cowpea (22.7%); however, it is higher than others such as lima beans (19.8%) and chickpeas (19%) [23].

The total ash content of the seeds in the present study (4.41±0.1) is similar to those obtained by another study [21]. Which ranged from 3.5-5.3%. These values are similar to those of soybean (5.0%), cotton seed (4%), sesame (3.8% and sunflower seed (4.1%) [23].

The carbohydrate content (8.52 ± 0.98%) was lower than that reported, meanwhile, the crude fiber content of kernel was similar to that reported [23].

The high oil and protein content makes the seed a potential source of commercial vegetable oil and protein.

Effect of Gamma Irradiation on Chemical Constituents:

The effect of gamma irradiation (1, 3, 6, and 10 kGy) on the proximate chemical composition for both non-irradiated and irradiated pumpkin seeds are presented in table 1. There are no appreciable differences in proximate composition of samples irradiated to 1, 3 and 6 kGy (Table 1). The moisture content was not substantially affected by gamma irradiation. Similar findings showed that gamma irradiation has no real effect on moisture content of oil seeds [24]. Protein, fiber, and ash content were not significantly ($P<0.05$) changed. This result is in agreement with other study, which reported that gamma irradiation did not induce any change in protein, fiber, and ash content of groundnut [25]. Similarly another study reported that the protein and crude fiber contents of almonds did not change after irradiation to 3, 7 and 10 kGy [26]. No significantly ($P<0.05$) differences were observed in carbohydrates content and in the total lipids was observed with 10 kGy radiation dose. No significant differences between irradiated and unirradiated walnuts in moisture, ash and protein contents were found [27]. Data obtained by other authors also showed that gamma irradiation, using doses up to 10 kGy, did not induce significant loss in water soluble components such as sugars and proteins [28]. The oil content of non-irradiated and irradiated seeds with 10 kGy was 44.45% and 42.80%, respectively. It was also found that there is an inverse relationship between oil content and irradiation dose, namely, if irradiation dose increases, oil content decreases [29].

Table (1): Effects of different doses of gamma irradiation on proximate chemical composition of pumpkin seeds.

*Nutritive Content (g/100g d.w.)	Control	Radiation Dose			
		1 kGy	3 kGy	6 kGy	10 kGy
**Moisture	7.73^{a} ± 1.22	7.37^{a} ±1.20	7.68^{a} ±0.08	7.63^{a} ±0.24	7.20^{a} ±0.37
Protein	39.25^{a} ± 0.51	39.09^{a} ±0.74	39.09^{a} ±0.50	39.25^{a} ±0.50	39.00^{a} ±1.10
Lipid	44.45^{a} ± 2.83	44.45^{a} ±2.83	44.40^{a} ± .20	43.63^{a} ±3.10	42.80^{a} ±2 .05
Ash	4.41^{a} ± 0.32	4.41^{a} ± 0.65	4.41^{a} ± 0.60	4.38^{a} ± 0.60	4.34^{a} ± 0.62
Crude fiber	3.60^{a} ± 0.45	3.60^{a} ± 0.50	3.60^{a} ± 0.40	3.55^{a} ± 0.85	3.50^{a} ± 0.35
Carbohydrate	8.52^{a} ± 0.98	8.41^{a} ±1.13	8.46^{a} ±1.07	9.20^{a} ±0.33	10.22^{a} ±0.47

*Each value in the table is the mean of three replicates (n=3), ±SEM. SEM: standard error of the mean
$^{A, b, c}$ Means with different superscripts in the same row are statistically different ($P<0.05$) according to Least Significant Test (LSD). **Moisture content (g/100g f.w.)

Physicochemical properties

The physicochemical properties of the pumpkin seed oil (control) were determined. It was found that, acid value (mg KOH/g of oil), peroxide value (equiv.g O_2/Kg of oil) iodine value (g /100g of oil) and free fatty (%) of the oil were 4.54±1.42, 0.85±0.58, 105.53±12.29, and 2.27±0.42, respectively.

The oil extracted from the dried pumpkin seeds was liquid at room temperature. Acid value is an indicator for edibility of oil and suitability for industrial use. Pumpkin seeds oil has 4.54±1.42 mgKOH/g while, this falls within the recommended codex of 10 mgKOH/g for edible oils [30]. The results suggest that the oils are edible and can also be used in the manufactured of paints and vanishes as comparable to the values of [31].

The iodine value which is useful in predicting the drying properties of oils is 105.53 (g /100g of oil).The

high iodine value of this oil indicates that they have a high content of unsaturated fatty acids suggests that the oil may be used for cooking purpose [30]. The iodine value is also an index for assessing the ability of oil to go rancid [32]. The high iodine values indicate that the oils have longer time to undergo oxidative deterioration [33].

The peroxide value was low (0.85 ± 0.46 meq peroxide /kg), as the Codex Alimentarius Commission stipulated permitted maximum peroxide levels of 10 meq peroxide/kg oil for soybean, cottonseed, rapeseed, and coconut oils. [30] These values were comparable to those reported for pumpkin seed oil [23, 34, and 35).The peroxide index is an indication of the amount of hydroperoxides present in oil. These compounds arise from lipid oxidation. High peroxide values are associated with higher rate of rancidity. The low peroxide values of the oils indicate that they are less liable to oxidative rancidity at room temperature [28, 29]. This shows the commercial potential of the oil, which is enhanced by the low peroxide value, free fatty acids, and acid values.

Effect of Gamma Irradiation on Oil Quality Attributes:

Two cases were studied, firstly pumpkin dry seeds were exposed to gamma radiation (0, 1, 3, 6, and 10 kGy) and oil extracted from these irradiated seeds. The second case was the irradiation of oil which extracted from non irradiated seeds with the same gamma radiation doses as the first case.

The effect of gamma irradiation (0.0, 3, 6 and 10 kGy) on iodine value, acid value, peroxide value, and free fatty acids for both of seeds and oils are illustrated in table 2. The results indicate that, tested doses of gamma radiation have no significant ($P<0.05$) effect on the seeds. The highest iodine values were acquired from the oil of the sample which is not exposed to irradiation; and relating to the irradiation dosages this values decreased significantly ($P<0.05$), and finally the lowest values recorded in the sample treated with 10 kGy irradiation. However, the changes were higher for acid and peroxide values, the changes in irradiated samples were higher than that of non-irradiated, and the changes in irradiated oil were higher than irradiated seeds. Irradiated oil was more sensitive (1kGy) to gamma irradiation than irradiated seeds. The effects of gamma irradiation on irradiated oil appear with the radiation dose 3 kGy and above. Similar findings were reported [36] for the iodine value of sunflower and soybean oil which decreased significantly with high gamma radiation (1, 5 and 20 KGy) while the acid values were increased. The decrease in iodine value may be attributed to the saturation of the double of unsaturated fatty acids bonds [27].

Our results are consistent with other study, which also reported a decrease in the iodine value upon irradiation [37]. Radiation probably broke some double bonds and induced oxidation processes in the fatty acids resulted in saturation [29]. These results also agree with other studies, which found that, the unirradiated samples have highest iodine values, suggesting saturation of oils as a result of irradiation [27, 38].

It was found that, prior to irradiation, pumpkin seeds and seeds oil exhibited very low peroxide values, indicative of good product quality in terms of degree of lipid oxidation. PV increased with an increase in irradiation dose. Maximum peroxide value was observed in pumpkin oil irradiated at 10kGy. Present results are in general agreement with those obtained for soybean oil [28], walnuts oil [39] and almonds oil [40].

Peroxide value characterizes quantity of peroxides formed in the oils as intermediates of oxidative reactions under irradiation and at high temperatures [41]. Previously, an increase in the peroxide value was attributed to interaction of gamma radiation with fat molecules, which triggered oxidation, dehydration and polymerization reactions [42]. A previous study of cashews irradiated to 7 kGy revealed approximately a five-fold increase in the peroxide value [43].

The increase in free fatty acids of the oil might be due to slight and random hydrolysis of triglycerol molecules to free fatty acids and diacylglycerols [29, 27].

Fatty acid composition

The fatty acids composition of pumpkin seeds oil is presented in Table3. The main monounsaturated fatty acid (MUFA) present in non-irradiated seeds was oleic acid (17.2±0.63), with minute levels of Erucic (0.71±0.13%) and palmitoleic acid (0.15±0.06) present. The major polyunsaturated fatty acid (PUFA) present was linoleic acid (52.64±0.90) with small amounts of linolenic acid (0.40±0.32). The major saturated fatty acids present were palmitic acid (19.01±0.86) and stearic acid (9.5±1.23) with small amounts of myristic acid (0.39±0.05).

Our results were in agreement with other studies which, observed that, linoleic acid was the principal fatty acid followed by oleic acid in pumpkin seed oil [21, 44]. This result indicates that the fatty acid composition of pumpkin seed oil is quite close to that of melon seed oil [45]. The presence of high amounts of the essential linoleic acid suggests that the pumpkin seed oil is highly nutritious.

Table (2): Effect of different doses of gamma irradiation on physico-chemical parameters (properties) of oil quality of irradiated pumpkin oil.

parameters	Irradiation case	Radiation Dose (kGy)				
		0	1	3	6	10
Acid value (mg KOH/g of oil	Seeds	4.54^{a} ±1.42	5.54^{a} ±1.02	6.06^{a} ±0.80	6.15^{a} ±0.72	6.67^{a} ±0.69
	Oil	4.54^{c}±1.42	7.24^{bc}±0.42	8.16^{abc}±0.72	9.86^{ab}±0.52	12.7^{a} ±0.50
Peroxide value (equiv.g O_2/Kg of oil)	Seeds	0.85^{a} ±0.58	0.88^{a} ±0.50	1.10^{a} ±0.34	1.30^{a} ±0.28	2.25^{a} ±0.63
	Oil	0.85^{b}±0.58	1.53^{b}±0. 26	2.63^{b} ±0.36	4.80^{b}±0.40	42.5^{a} ±3.22
Iodine value (g /100g of oil)	Seeds	105.53^{a} ±12.2	101.96^{a} ±10.2	97.76^{a} ±11.69	94.39^{a} ±9.85	87.25^{a} ±9.3
	Oil	105.53^{a} ±12.2	84.43^{ab}±8.13	76.56^{ab} ±7.40	72.80^{ab} ±8.20	63.46^{b}±7.37
Free Fatty acid (Oleic acid %)	Seeds	2.27^{a} ±0.42	2.77^{a} ±0.57	3.03^{a} ±0.38	3.07^{a} ±0.25	3.33^{a} ±0.56
	Oil	2.27^{c}±0.42	3.62^{bc}±0.64	4.08^{bc} ±0.30	4.93^{ab} ±0.72	6.35^{a} ±0.62

*Each value in the table is the mean of three replicates (n=3), ±SEM.
SEM: standard error of the mean
A, b, c Means with different superscripts in the same row are statistically different (P<0.05) according to Least Significant Test (LSD).

Table 3. Fatty acid composition % of *Cucurbita moschata* seeds

Fatty acid (FA)	Mean value
Myristic (C14:0)	0.39±0.05
Stearic (C18:0)	9.5±1.23
Palmitic (C16:0)	19.01±0.86
Erucic (C22:1)	0.71±0.13
Palmitoleic (C16:1)	0.15±0.06
Oleic (C18:1)	17.2±0.63
Linoleic (C18:2)	52.64±0.90
Linolenic (C18:3)	0.40±0.32
Total saturated FA	28.90±2.14
Total Monounsaturated FA	18.06±0.82
Total Polyunsaturated FA	53.04±1.22
Total unsaturated FA	71.1±2.04
R1=% TSFA/% TUSFA	0.406

The changes of fatty acid content of irradiated pumpkin oil and oil extracted from irradiated seeds are given in Table 4. At low irradiation doses, small changes were observed in saturated and unsaturated fatty acids components, and the changes in fatty acids composition of oil extracted from irradiated seeds were not significant (P<0.05).

It was observed that, for irradiated oil, parallel to the increases in irradiation doses, there is an significant (P<0.05) increase in total saturated fatty acid (SFA) from 28.90 % to 54.29 %, i.e., there is a decrease in total unsaturated fatty acid (TUSFA) from71.10% to 45.71% for irradiated oil, respectively (Table 4). Another study suggested that, the decrease in unsaturated fatty acids during the irradiation exposure of oil is mainly due to a molecular structure change in fatty acids [38].

It is apparent that higher the unsaturation of fatty acids the higher is their oxidation potential. Thus, the increase in SFA concentration may be explained by the oxidation of PUFA and MUFA, respectively. The ratio of total unsaturated over total saturated acids (PMUFA+PPUFA/PSFA) was used [46] to predict the shelf life of hazel nuts; indicating that the lower the ratio, the longer was product shelf life. In the present study, this ratio was 0.406 prior to irradiation increasing to 0.474 and1. 188 after irradiation of seeds and oil, respectively.

The high level of unSFA in these oils was due to their high levels of linoleic acid. This showed that these oils are good sources of unSFA, mostly PUFA, with linoleic acid (an essential fatty acid) being the most abundant (52.64%). Linoleic acid is the most important essential fatty acid, for it must be got from food [47].

Some reported no significant changes in poly-unsaturated fatty acids of almond kernels irradiated up to a dose of 7 kGy, while monounsaturated fatty acids decreased with a respective increase in saturated fatty acids [40]. They also added that, the highly unsaturated fatty acids were very sensitive and readily destroyed by irradiation. Generally, most saturated components are increased as a function of irradiation dose whereas unsaturated components decreased with increasing total dose [40].

Antimicrobial activity of pumpkin seeds oil.

Methanolic extracts of pumpkin seeds (crude oil) were exposed to gamma-rays radiation at dose levels of 1, 3, 6, and 10 kGy. The effects of different doses of gamma irradiation on the antimicrobial activity were studied. The non-irradiated methanol extract of the oil used as control.

Table 4. Fatty acid composition % of non-irradiated seeds and irradiated pumpkin oil

Seeds	Fatty acids	Radiation doses (kGy)

		0	1	3	6	10
	Monounsaturated	18.06[a] ±1.42	17.61[a] ±2.58	17.51[a] ±1.25	17.46[a] ±2.29	17.06[a] ±2.78
	Polyunsaturated	53.04[a] ±2.36	52.81[a] ±0.95	52.76[a] ±2.78	52.77[a] ±3.42	50.74[a] ±2.69
	Total unsaturated	71.1[a] ±3.25	70.42[a] ±1.87	70.27[a] ±4.13	70.23[a] ±3.25	67.80[a] ±2.63
	Total saturated	28.90[a] ±1.75	29.58[a] ±0.40	29.73[a] ±1.29	29.77[a] ±4.02	32.20[a] ±2.41
	R1=% SFA/%USFA	0.406	0.420	0.423	0.424	0.474
Oil	**Monounsaturated**	18.06[a] ±1.42	17.55[a] ±2.58	16.78[a] ±1.25	15.37[a] ±2.29	14.69[a] ±2.78
	Polyunsaturated	53.04[a] ±2.36	50.45[ab]±2.10	39.47[bc] ±1.34	35.20[bc] ±3.55	31.02[c]±1.22
	Total unsaturated	71.1[a] ±3.25	68.0[ab] ±3.10	56.25[bc] ±2.06	50.57[c] ±1.42	45.71[c]±1.55
	Total saturated	28.90[c] ±1.75	32.0[bc] ±1.47	43.75[ab] ±2.33	49.43[a] ±3.54	54.29[a] ±2.65
	R1=% SFA/%USFA	0.406	0.470	0.778	0.978	1.188

*Each value in the table is the mean of three replicates (n=3), ±SEM. SEM: standard error of the mean
[A, b, c] Means with different superscripts in the same row are statistically different (P<0.05) according to Least Significant Test (LSD).

Antibacterial activity

In the present study, two-fold serial dilution technique was used to determine the minimal inhibitory concentration (MIC) of crude methanolic extracts against the selected bacterial strains. The results revealed (Table5) that the methanolic seeds extracts of un-irradiated (control) samples have antibacterial effects against *Bacillus subtilis*, *Staphylococcus aureus* , *Escherichia coli* and *Klebsiella pneumonia* local isolates at concentration levels of 1.0, 2.0, 2.0 and 3.0 mg/ml, respectively. The plant extracts were found ineffective against *Pseudomonas aeruginosa* at concentration levels up to 3000 µg/mL. The results regarding the effect of gamma irradiation on antibacterial activities showed that there was no difference in the activity of the irradiated extracts up to a dose level of 10 kGy. No information in the literature is available on the effect of gamma irradiation on the antibacterial activity of pumpkin oil seeds. Little is also known for other plant materials.

Previous studied [48] showed that gamma-radiation treatment did not have any detrimental affect on the antimicrobial activity of the *Nigella sativa* seed up to 10 kGy radiation doses. Similarly, the microbial decontamination of tea by gamma irradiation was studied [49] and the results showed that, the antimicrobial and sensory properties of the samples were unaffected by radiation treatment within a dose of 10 kGy. Another study [50] on ciprofloxacin showed that the antimicrobial activity was not affected by gamma-irradiation treatment up to 100 kGy treatment.

Antifungal activity

The methanolic extracts of irradiated and unirradiated (control) pumpkin oil seeds samples were checked for antifungal activity using two-fold series dilution method. The minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2µl, from the micro plate wells without visible growth, into micro plates containing 100µl of broth per well and further incubation for 72hrs at 28°C. The lowest concentration with no visible growth was defined as the MFC, indicating=99.5% killing of the original inoculum.

The results indicated that the unirradiated oil have antifungal activity against *Rhodotorula rubra* and *Candida albicans* at 0.5 and 1.0 mg/ml concentrations, respectively and *Rhodotorula rubra* was the most susceptible isolate to the seeds oil. The effective concentration of pumpkin oil seeds methanolic extracts were 1.0 mg/ml against *Penicillium chrysogenum* and *Aspergillus parasiticus* and 2.0 mg/ml against *Aspergillus flavus (A)* , respectively.

No antifungal activity was detected against other selected fungi. Following exposures to gamma rays, the irradiated samples of the plant did not show any change in antifungal activity. This revealed that the antifungal activities of plant extracts against selected fungi were not affected by gamma irradiation up to 10 kGy doses.

This is in agreement with a previous study [48] on *Nigella sativa* seeds, which showed that gamma irradiation did not affect the antifungal activity.

Table 5. Effect of different doses of gamma irradiation on the antimicrobial activity of pumpkin seeds oil.

Microorganism (strain)	Irradiation dose (kGy)

	0	1.0	3.0	6.0	10
Pseudomonas aeruginosa	>3.0	>3.0	>3.0	>3.0	>3.0
Klebsiella pneumoniae	3.0	3.0	3.0	3.0	3.0
Escherichia coli	2.0	2.0	2.0	2.0	2.0
Staphylococcus aureus	2.0	2.0	2.0	2.0	2.0
Bacillus subtilis	1.0	1.0	1.0	1.0	1.0
Candida albicans	1.0	1.0	1.0	1.0	1.0
Rhodotorula rubra	0.5	0.5	0.5	0.5	0.5
Aspergillus niger	>3.0	>3.0	>3.0	>3.0	>3.0
Aspergillus flavus(A)	2.0	2.0	2.0	2.0	2.0
Trichoderma viride	>3.0	>3.0	>3.0	>3.0	>3.0
Aspergillus flavus(H)	>3.0	>3.0	>3.0	>3.0	>3.0
Penicillium chrysogenum	1.0	1.0	1.0	1.0	1.0
Rhizopus sp	>3.0	>3.0	>3.0	>3.0	>3.0
Aspergillus fumigates	>3.0	>3.0	>3.0	>3.0	>3.0
Aspergillus parasiticus	1.0	1.0	1.0	1.0	1.0

*MIC values expressed in (mg/ml)
*Maximum concentration tested was 3 mg/ml
*>3.0=No inhibitory effect up to the concentration level of 3000 µg/ml Experiments run in triplicate.

Antimicrobial activity of different crude extracted proteins from pumpkin.

This study determined the inhibitory activity of different extracted crude proteins, (extracted from seeds, rinds and pulp) for growth of different microorganisms, including bacteria (Gram +ve and Gram -ve), yeasts and fungi. The average of the diameters of the growth inhibition zones obtained on the experiments is shown in Table 6.

Extracted pumpkin seeds crude protein had a higher antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*, the inhibition growth zone diameter were 10.0 and 8.0 mm, respectively.

The study revealed that the extracted rinds crude protein had a higher antimicrobial activity of (27.5, 25.0, and 23.0 mm) against *Penicillium chrysogenum*, *Aspergillus flavus(A)*, and *Aspergillus flavus(A)*, respectively.

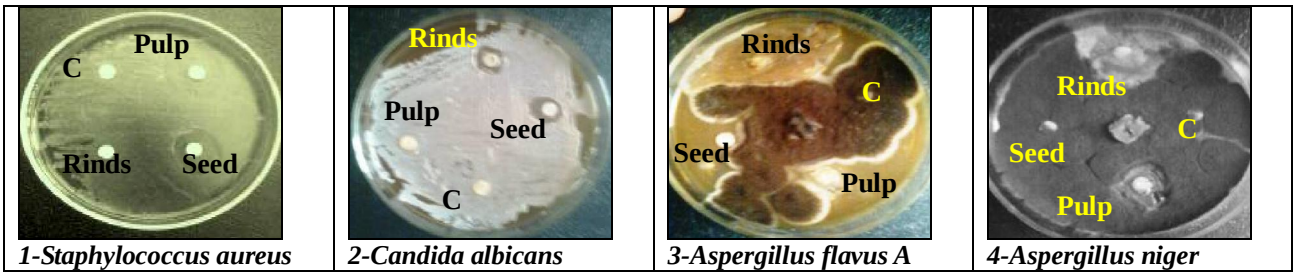
It is clear from the results (Table 6) that the seeds protein was effective against tested Gram +ve bacteria and had no effect against Gram –ve bacteria.

Pulp protein was effective on all tested bacteria, on the other hand, the rinds protein had no effect against all tested bacteria and *Aspergillus parasiticus* by the tested concentrations used in this study.

In another study, Gram-negative bacteria have been reported to be more resistant than Gram-positive to proteins and oils antimicrobial effect because of their cell wall lipopolysaccharides which may prevent these active compounds reach the cytoplasmic membrane of gram-negative bacteria [51].

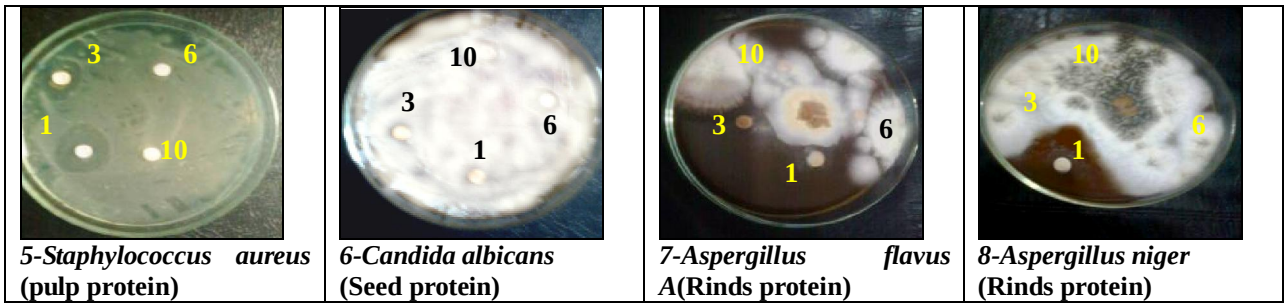
The study evaluated the *in vitro* effect of all extracted proteins on the growth of the tested fungi and bacteria after gamma irradiation (1, 3, 6 and 10 kGy). Antimicrobial effect of the extracted proteins was found to be decreased with the increased of the irradiation doses used. Another study attributed this decrease in the antimicrobial effect of the extracted proteins to the changes in protein fractions which may be related to some cross linking or aggregation of proteins as a result of gamma irradiation which could affect in protein nitrogen [52].

The antimicrobial activities of the extracted proteins and the effects of gamma radiation on it were visible on the microbial growth inhibition zones as shown in (Figs 1, 2).



1-Staphylococcus aureus *2-Candida albicans* *3-Aspergillus flavus A* *4-Aspergillus niger*

Fig 1. Inhibitory activity of extracted proteins for microbial growth.



5-Staphylococcus aureus **(pulp protein)** *6-Candida albicans* **(Seed protein)** *7-Aspergillus flavus A***(Rinds protein)** *8-Aspergillus niger* **(Rinds protein)**

Fig 2. Effect of different doses of gamma radiation (1, 3, 6, 10kGy) on the inhibitory activity of extracted proteins for microbial growth.

Table 6. The antimicrobial activity of extracted crude proteins from different parts of pumpkin

Microorganisms	Extracted crude proteins		
	Seeds	Rinds	Pulp
Pseudomonas aeruginosa	-	-	2.0
Klebsiella pneumoniae	-	-	1.0
Escherichia coli	-	-	1.5
Staphylococcus aureus	10.0	-	12.0
Bacillus subtilis	8.0	-	9.0
Candida albicans	5.0	4.0	-
Rhodotorula rubra	9.0	6.0	8.0
Aspergillus niger	-	5.0	4.5
Aspergillus flavus(A)	10.0	25.0	4.0
Trichoderma viride	-	4.0	-
Aspergillus flavus(H)	-	4.0	-
Penicillium chrysogenum	10.0	27.5	-
Rhizopus sp	1.0	2.0	1.0
Aspergillus fumigates	-	23.0	-
Aspergillus parasiticus	-	-	-

*(150 µg /disc) *Diameter of inhibition in millimeters (mm) *- No inhibition with the tested concentration

The final preparations gave single bands on a 15% SDS-PAGE gel. The molecular mass of the purified proteins were determined to be 56, 59, and 30 kDa for rinds, pulp, and seeds, respectively by 15% SDS-PAGE gel Fig (3). The computer analyses of protein spots on SDS-PAGE gels were carried out. The results in Table (7) showed that 21 specific different proteins were detected in rinds and pulp, respectively and 23 specific different proteins were detected in seeds. Their molecular weights (M_w) were ranged from 183 to 28 kDa. The computer analysis of the proteins showed 91.19 % similarity between the rinds proteins and pulp proteins and there were 84.40% similarity between them and seeds proteins.

Antimicrobial proteins from pumpkin have been previously identified. Three basic proteins

(MAP2, MAP4 and MAP11) from the pumpkin seed inhibited the growth of yeasts [53]. Cucurmoschin, an antifungal peptide isolated from black pumpkin seeds inhibited growth of *Botrytis cinerea, Fusarium oxysporum* and *Mycosphaerella oxysporum* [9]. A ribosome-inactivating protein derived from *Cucurbita moschata* had antibacterial activity against phytopathogenic bacteria *Phytophora infestans, Erwinia amylovora* and *Pseudomonas solanacearum* [54]. In addition, PR-5, with a molecular mass of 28 kDa and high homology to thaumatin, was isolated from pumpkin leaves that exhibited a synergistic effect with nikkomycin, a chitin synthase inhibitor, against *Candida albicans* [55].

PR-5 proteins have been isolated from *A. thaliana* [56], corn [57], beans [58], and many other plants [59, 60, and 61]. The majority of PR-5 proteins have molecular masses of; 22 kDa and are stabilized by eight disulfide bonds. This highly stabilized structure allows PR-5 proteins to be very resistant to protease degradation [62].

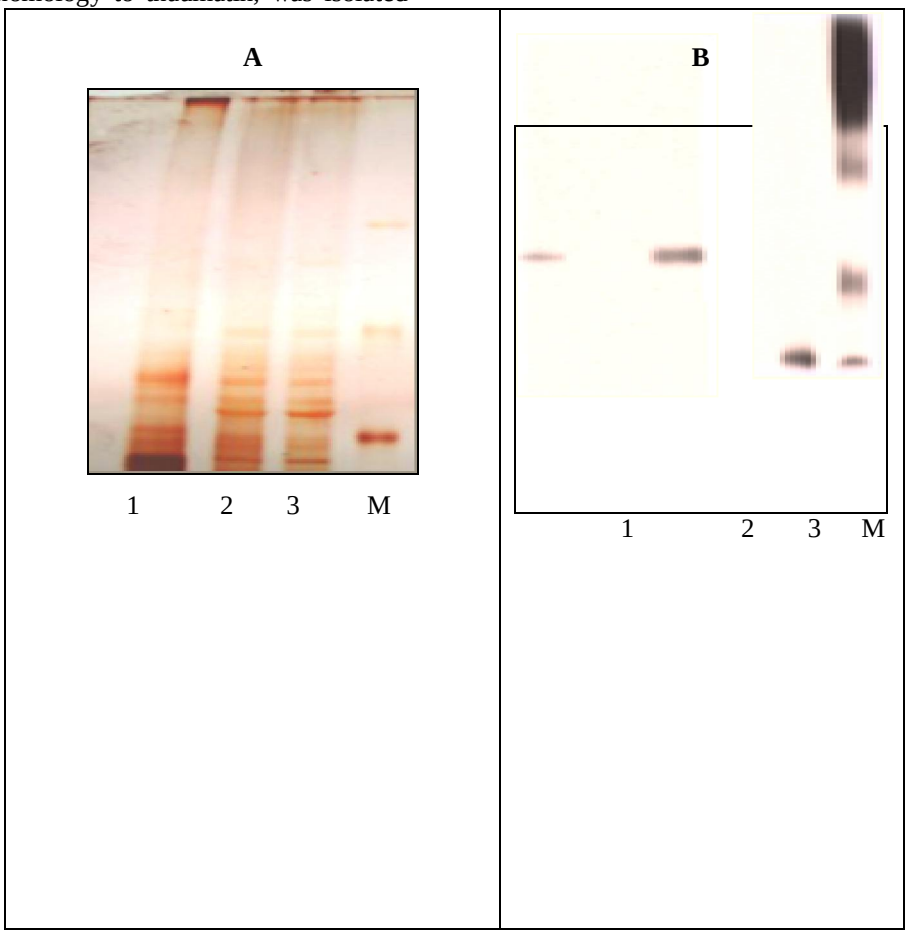


Fig 3. SDS–PAGE of crude (A) and purified (B) proteins analyzed by SDS-PAGE on a 15% separating gel with silver nitrate. A-crude proteins extract from pumpkin rinds (lane 1), crude proteins extract from pumpkin pulp (lane 2), crude proteins extract from pumpkin seeds (lane 3), molecular size marker (lane M). B- Purified protein extract from pumpkin rinds with M_w 56 (kDa) (lane 1), purified protein extract from pumpkin pulp with M_{w59} (kDa) (lane 2), and purified protein extract from pumpkin seeds with $M_{w\ 30}$ (kDa) (lane 3). Positions of the marker (M) proteins (116, 66 and 45 kDa) were represented on the right hand side of gel.

Fig 4. Similarity between the extracted proteins.
*1-rinds proteins 2- pulp proteins and 3- seeds proteins.

Table 7. Molecular weight and percentage (%) of extracted proteins from different parts of pumpkin.

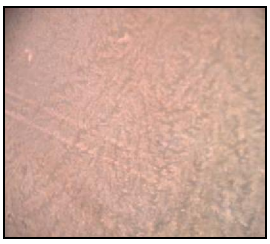
Peak number	Rinds proteins		Pulp proteins		Seeds proteins	
	M_w (kDa)	%	M_w (kDa)	%	M_w (kDa)	%
1	183	4.95	183	2.43	183	3.29
2	170	11.60	177	6.62	177	8.36
3	115	3.42	119	0.86	119	2.46
4	103	2.32	111	3.52	98	5.72
5	97	1.26	99	5.02	80	7.47
6	89	5.24	77	4.59	76	3.60
7	75	5.98	66	4.10	66	4.95
8	72	1.38	63	1.02	65	2.03
9	69	1.24	61	4.13	62	1.72
10	63	2.00	59	22.83	60	2.40
11	61	4.62	56	4.31	58	2.12
12	56	15.07	52	3.27	56	1.94
13	54	0.49	50	5.33	55	4.75
14	52	4.09	45	2.45	52	3.05
15	46	5.04	43	6.99	49	6.05
16	44	3.15	40	3.23	45	1.57
17	40	6.89	37	3.66	43	5.60
18	39	1.66	35	5.77	40	3.35
19	36	8.66	32	1.34	37	3.24
20	34	3.07	30	5.37	35	6.10
21	28	7.87	27	3.15	31	1.08
22	-	-	-	-	30	15.61
23	-	-	-	-	27	3.54

- M_w= Molecular weight

Although the precise mechanism of action of PR-5 proteins is not completely understood, there are a number of interesting observations that may eventually lead to a unified hypothesis for how these proteins function to kill fungi [60, 61]. Several antifungal proteins cause cell permeability changes in fungal cells with a cell wall but have no or little effect on protoplasts [62]. Uptake of the vital stain, SYTOX Green, was enhanced when fungal conidia were treated with Pr-1 suggesting that the protein has membrane permeabilization activity. The author suggested that, Pr-1 induces the damage of the plasma membrane of fungal cell directly, with resultant leakage of cytoplasmic components to the exterior of cell. [63].

The effect of the extracted protein on the cell wall proteins of the tested microorganisms were studied by staining the fungal spores and vegetative cells of bacteria and yeasts by acridine orange. It is clear (Fig 5) that the tested proteins may be having lyses or destruction effect on the sporangia cell wall and the cells of yeasts and bacteria.

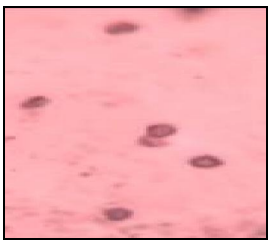
Acridine orange, the first stained dye in yeast, turns into green fluorescence in viable cells and orange fluorescence in dead cells. Acridine orange used for the detection of contamination in beer and food as it has the ability to differentiate viable and dead cell distinction [64].



1-Candida albicans **(Control)**

2-Candida albicans **(Seed protein)**

3-Aspergillus flavus A **(Control)**



4-Aspergillus flavus A **(Rinds protein)**

Fig 5. Effect of extracted proteins on the viability of microbial cells. live cells (1, 3). Uptake (2, 4) of acridine orange by dead cells (orange stained)

4. Conclusion and Recommendations:

This study showed that the Egyptian pumpkin seeds had high content of protein and oil indicating high nutritive value. The high content of pumpkin seeds oil indicates that this oil can be extracted and refined for uses.

These oils are very rich in essential fatty acids (linoleic acid). The acceptable acid and peroxide values, high linoleic and low linolenic acid levels of these oils suggest that they could be sources of good edible oils. The abundance of linoleic followed by oleic acid in these oils makes them good oils for reducing serum cholesterol and LDL and increasing HDL levels, hence could be good oils for the fight against cardiovascular illnesses.

The extracted crude pumpkin oil and proteins (seeds, rinds, and pulp) were examined for antimicrobial activities, before and after gamma irradiation. Gamma radiation at dose level above 3 kGy affect on the physico-chemical properties of the pumpkin oil. No changes in the antibacterial activity against tested pathogens after radiation treatment up to10 kGy. In conclusion, the present study indicates that radiation at higher doses may be not good for certain biological activities and may cause degradation or changes in chemical structures of some biologically active important ingredients. This investigation suggests that radiation treatment up to 3 kGy is safe and beneficial for pumpkin seeds.

These findings also indicate that the extracted oil and proteins from pumpkin may be of importance to clinical microbiology and have therapeutic applications.

Corresponding author
Amany. B. Abd El-Aziz
Department of Microbiology. National Center for Radiation Research and Technology. Atomic Energy Authority. P.O. box 29, Nasr City, Cairo, Egypt
abdelazizamany@gmail.com

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