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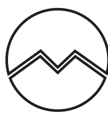

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Production And Characterisation Of Keratinase By Fungi Isolated From Soil Samples At Gwagwalada, FCT – Abuja, Nigeria

Ugoh, Sylvanus Chukwudi and Ijigbade, Bamidele

Department of Biological Sciences, University of Abuja, P.M.B. 117, Abuja-Nigeria.
Email: sylvaugoh@hotmail.com

Abstract: Fifty soil samples were collected from refuse dumps, animal sheds, farm lands, drainage sites and barbers' shops at Gwagwalada, FCT-Abuja and screened for the presence of keratinase- producing fungi by Hair Bait Technique using chicken feather as keratin bait. A total of 51 fungal isolates belonging to two genera and four species of keratinase- producing fungi were observed. Sixteen (31.37 %) of the isolates were from refuse dumps, animal sheds and barbers' shops, both have 11 (21.57 %) isolates each, while farmlands and drainage sites have 10 (19.61 %) and 3 (5.88 %) isolates respectively. *Aspergillus niger* (van Teigh) 17 (33.33 %) was the most abundant species, followed by *Penicillium chrysogenum* (Thom) 13 (25.49 %), *Aspergillus flavus* (Link ex Fr.) 12 (23.53 %) and *Penicillium marneffei* (Hubert) 9 (17.65 %) being the least in abundance. The highest keratinase activities were recorded in *Aspergillus niger* (14.56±1.54 Keratinase unit (Ku)/ml), *Penicillium marneffei* (13.18±2.19 ku/ml), *Penicillium chrysogenum* (12.56±2.75 ku/ml) and *Aspergillus flavus* (11.93±1.80 ku/ml). The rate of prevalence and the quantity of enzymes produced are significantly different (P = 0.05). These non-dermatophytic keratinolytic fungi have potential use in biotechnological processes involving keratin hydrolysis. The results of this work revealed that keratinolytic activity is relatively widespread among common fungi and may have an important role in keratin degradation in the natural environment.
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Keywords: Keratinase, fungi, soil, enzymes, keratinase unit

Introduction

Soil Enzymes are usually offered as "cocktails" of several activities rather than a single enzymatic activity (Agarwal *et al.* 2008; Sakthi *et al*., 2012). However, in many cases the enzyme activities can still act on the same composition, as the composition can have a complex chemical structure having various types of chemical bonds, requiring different enzyme activities for breakdown. Microbial soil Enzymes have become big business, with a wide range of industries using commercial enzymes, in addition to the feed industry. Today enzyme technology mostly depends on microbes like bacteria, fungi and actinomycetes. Fungi in particular have been regarded as treasure of useful enzymes. There is a great variation between various genera as to their ability to produce a specific enzyme the production of particular enzyme varies with the particular medium and pH (Akpan *et al*., 2009). In recent years the potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Pandey *et al*., 2000 ; Abu *et al*., 2005). Many fungi had been found to be good source of keratinase. Mukhopadhay *et al*. (1989) reported keratinase production by Streptomyces sp. He isolated an inducible extracellular homogenous enzyme, which shows 7.5 fold increases in its activity after DEAE cellulose column chromatography. The enzyme-activity was inhibited by reduced glutathione, PMSF and 2-Mercaptaethanol. Keratins are the widely distributed fibrous proteins of our environment found in epithelial cells of vertebrates and characterized by its high content of amino acids, especially cystine, arginine and serine. It is present in hair, feather, hooves, wool, horns, nail, stratum and cornium (Sharma and Prashar, 1997). A vast quantity of chicken are being utilized every day in the society that produces a large amount of feathers waste in poultry industries Keratin-consisting materials have always been plentiful in the nature but restricted in practical usages, mainly because of their insolubility and non-degradability by the ordinary proteolysis, the presence of the disulfide linkages, hydrophobic interactions, and hydrogen bonds, but however, are easily digested by alkali and keratinase enzymes, a group of proteinase enzymes that have high level of activity on insoluble environmental pollution, keratin (Onifade 1998; Fuchs, 1995). The keratineous materials in or on soil are attacked by these keratinophilic microbes, therefore biodegradation takes place.

Materials and Methods

Sterilization of Glassware

The sterilization of glass wares such as conical flasks, beaker and test tubes after washing with detergent was carried out in hot air oven at 160º C for 2 hours according to the procedure given by Harrigan and McCance (1976).

Sampling Site

Gwagwalada is one of the five municpal Councils of the Federal Capital Territory of Nigeria, together with Abaji, Kuje, Bwari, and Kwali; the FCT also includes the City of Abuja. Gwagwalada is also the name of the main town in the Local Government Area, which has an area of 1,043 km² and a population of 157,770 at the 2006 census (Awowole-Browne and Francis 2007). Gwagwalada is where the University of Abuja is located.

Samples collection

A total of fifty (50) soil samples were collected randomly with ten (10) samples each from five (5) different sites in Gwagwalada FCT-Abuja. Samples were collected from farm lands, animal sheds, refuse dumps, drainage sites and Barber's shops (Sharma and Rajak 2003). At each location, 20 g of soil were collected from the superficial layer, at a depth of 10 cm. Soil samples were collected in the sterile polythene bags and brought to the laboratory of Microbiology Department, University of Abuja, for the isolation of keratinase producing fungi and analyzed on the day of collection (Adeniran and Abiose 2009; Amany *et al*., 2009; Ingle *et al*., 2012).

Collection and Sterilization of Chicken Feather

Chicken feather was purchased at the Gwagwalada market and taken to the laboratory of Microbiology Department, University of Abuja, for the isolation of keratinase producing fungi. Sterilization of the feather was done by soaking the feather for 24 hours in diethyl ether and later rinsed 5 times with distilled water and air dry (Sharma and Rajak 2003).

Preparation and sterilization of media

Sabraud's Dextrose Agar was used in this study and prepared according to the manufacturer's instructions thus, 65g of SDA was dissolved in 1000ml of sterile water and then sterilized (autoclaved) at 121ºC and pressure of 15Pa for 15 minutes (Beuchat, 1992). Saboraud's Dexstrose agar was used for the isolation and maintainance of pure cultures of keratinolytic fungi (Sharma and Rajak 2003).

Isolation of keratinolytic fungi

Vanbreuseghem's Hair bait technique as reported by Sharma and Rajak 2003 was used for the isolation of Keratinolytic fungi using chicken feather as keratin bait. Sterile Petri dishes was half filled with the soil samples. Short strand of about 2-3 cm long of sterilized chicken feather was spread over the surface of each soil sample and 5ml of sterile water was added to the soil to facilitate germination of fungal spores on the feather. The preparations were incubated at room temperature (20-25^{oC}) in the dark, for 4 weeks. The Plates were examined periodically for the development of mycelia.

Inoculation of Keratinolytic Fungi on Culture Media

The short strand of feather with fungus growth was remove with the aid of forceps and placed on the prepared Plate of Saboraud's Dextrose agar supplemented with chloramphenicol (0.05mg/l) to inhibiting bacterial growth and incubated for 3-5 days (Kim 2003;Sharma and Rajak 2003; Soomro *et al*., 2007).

Preparation of Pure Cultures of Fungal Isolates

The young colonies of fungi were aseptically picked up and transferred to fresh sterile SDA Plates to obtain pure cultures. The pure cultures on SDA Plates were grown at 30°C for 7 days and stored at 4°C (Sharma and Rajak 2003). The isolate was sub-cultured for further studies.

Identification of keratinolytic fungal isolates

Isolates obtained were characterized and identified on the basis of their morphological assessment that is, macroscopic and microscopic features. Among the characteristics used were colonial characteristics such as size, surface appearance, texture and colour of the colonies (Sharma and Rajak 2003). In addition, microscopy revealed vegetative mycelium including presence or absence of cross-walls, diameter of hyphae, and types of asexual and sexual reproductive structures (Soomro *et al*., 2007). Slide culture method that minimized serious distortion of sporing structures was used. Appropriate references were then made using mycological identification keys and taxonomic description (Harrigan and McCance, 1976; Samson and Reenen-Hoekstra, 1988).

Viable spore count of Keratinolytic Fungal Isolates

The total viable spore number on a Sabdraud's Dextrose Agar (SDA) slant was determined by colony count technique. The spores were suspended in 10ml of distilled water, using a sterile transfer needle and diluted serially up to 10^{10} cells/ml. One ml of spore suspension was poured onto sterile Petri-Plates, containing sterile SDA medium and spread uniformly. The inoculated Petri-Plates were incubated at 30^{0}C for 48 hrs. A Plate that developed between 7 to 200 colonies was selected for counting. The spore density was calculated as the count multiplied by the dilution factor (Sakthi *et al*., 2012).

Determination of Keratinase potentiality by Feather Degradation

Feather degradation was assessed by measuring keratinase activity of isolated fungi according to Awasthi and Kushwaha, (2011). A basal medium which contained the following ingredients per litre of distilled water: Glucose – 2 gm, Peptone – 5 gm, Yeast – 5 gm, K_2HPO_4 – 1 gm, KH_2PO_4 – 3 gm, $CaCl_2$ – 1 gm, $MgSO_4$ – 1 gm and Feather - 200 mg per flask was prepared. The 250 ml Erlenmeyer flasks containing 50 ml of the basal medium and 200 mg of chicken feathers as a keratin substrate were autoclaved at 15 labs pressure for 10 minutes. The pH of the medium was adjusted to 6.0, 7.0, 8.0 and 9.0 respectively before sterilization. Spore suspension of the fungal isolates was prepared by adding 10 ml of sterilized water to 8 days old fungal isolates growing on Plates of potato dextrose agar. The final concentration of the spore suspension was adjusted to about 10^6 mL^{-1}. The flasks were incubated at 30ºC and 40ºC respectively. All the experiments were carried out in triplicates.

Assay of Keratinase Enzyme

At the end of the growth period, the fungal mat and feather were separated from culture medium by filtering through whatman number 1filter paper. The culture filtrate from four test flasks was pooled, centrifuges at 4,000 rpm for 5 min and the supernatant was assayed for keratinase. keratinase was measured as per the method of Rammani and Gupta 2004. Keratnolytic products in the supernatant were determined by reading absorbance at 280 nm against basal medium using UV-Spectrophotometer (JENWAY 6305). An increase of 0.100 in the absorbance was considered as equivalent to 1 unit of KU (keratinase unit).

Statistical Analysis

Production and characterization of keratinase produced by fungi was analyzed, the mean difference was found through statistical procedure applying one way Analysis of Variance (ANOVA) from Ms Excel Statistics. Test applied was F-test statistic at p= 0.05.

RESULTS

Isolation rate of Keratinolytic Fungi

Isolation rate of keratinase-producing fungi from five soil samples at Gwagwalada include *A. niger* was higher in soil samples collected from the refuse arena and farm lands (Table 1).

Table 1: Isolation rate of keratinase producing fungi from soil sample at Gwagwalada FCT-Abuja

Keratinolytic and Amylolytic fungi	Isolation Rate, Number (%)					
	F.L (n=10)	A.S (n=10)	B.S (n=10)	D.S (n=10)	R.D (n=10)	Total (n=50)
Aspergillus niger	4(40)	3(30)	2(20)	2(20)	6(60)	17(33.33)
Aspergillus flavus	3(30)	2(20)	4(40)	0(0)	3(30)	12(23.53)
Penicillium chrysogenum	1(10)	3(30)	3(30)	1(10)	5(50)	13(25.49)
Penicillium marneffei	2(20)	3(30)	2(20)	0(0)	2(20)	9(17.65)
Total	10(19.61)	11(21.57)	11(21.57)	3(5.88)	16(31.37)	51(100)

Keys: F.L=Farm land, A.S=Animal shed, B.S=Barber's shop, D.S=Drainage Site, R.A=Refuse dumps and n=number of soil sample. Isolation Rate, Number (%)

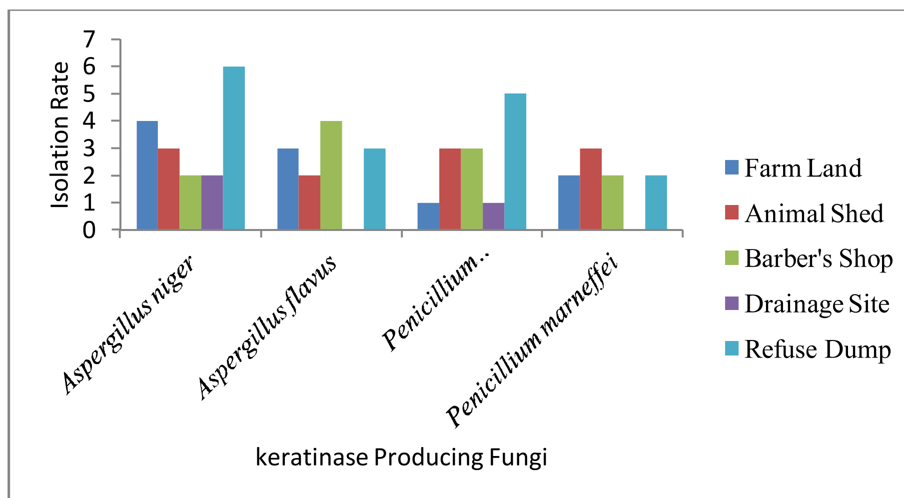


Figure 1: Isolation rate of keratinase producing fungi from soil sample in Gwagwalada FCT-Abuja

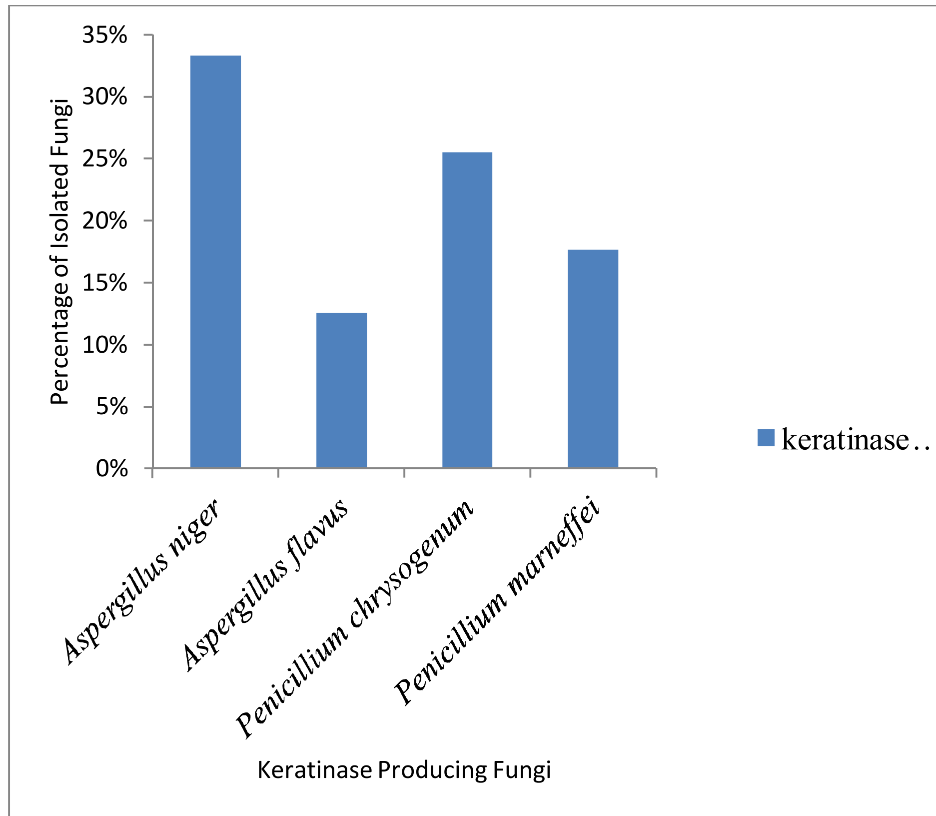
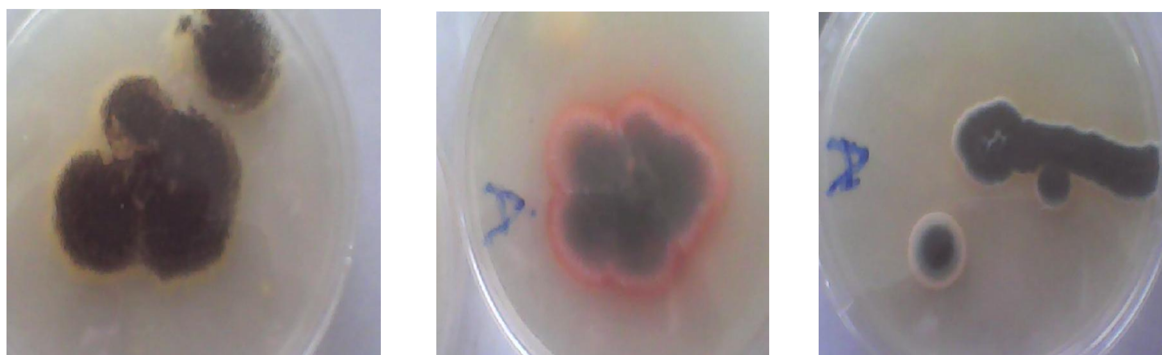


Figure 2: Percentage of Isolation of keratinase producing fungi from soil sample in Gwagwalada FCT-Abuja

Plate 1: Growth of fungi on feather by 'Hair Bait Technique'

IDENTIFICAION OF FUNGAL ISOLATES

The fungal strains were identified on the basis of colony morphology, cultural characters, slide culture, pigmentation and morphology of hyphae and their spores as *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Penicillium marneffei* (Plate 2, Plate 3 and Plate 4) as shown in Table 2.



Pate2: *Aspergillus niger* **Plate 3:** *Penicillium marneffei* **Plate 4:** *Penicillium chrysogenum*

Keratinase fungal strains isolated was identified on the basis of colony morphology, cultural characters, slide culture, pigmentation, morphology of hyphae and their spores as *Aspergillus flavus, Aspergillus niger, Penicillium marneffei* and *Penicillium chrysogenum* respectively.

Characterization of Keratinolytic Fungi From Soil Sample in Gwagwalada FCT-Abuja

The keratinolytic activity of the crude enzyme of the fungal strains cultivated in basal medium as described in materials and methods. Keratinolytic activity of the culture filtrates (Crude enzyme) appeared after three days and reached its maximum after 7 days as shown in Table 2.

Table 2: Keratinase activities of some isolated fungi from soil sample in Gwagwalada FCT-Abuja.

Fungal species	Keratinase Activity (KU/mL)	Optimum PH	Optimum temp(^{o}C)	Optimum Time(days)
Aspergillus niger	14.56±1.54	9	30	7
Aspergillus flavus	11.93±1.80	9	30	7
Penicillium chrysogenum	13.18±2.19	9	30	7
Penicillium marneffei	12.56±2.75	9	30	7

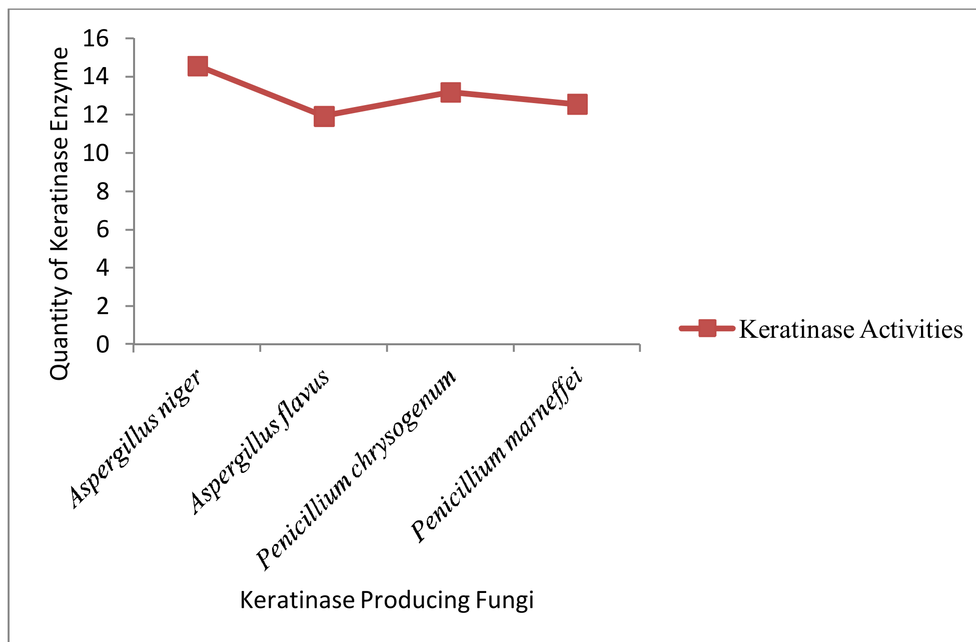


Figure 3: Keratinase activities of some isolated fungi from soil sample at Gwagwalada

DISCUSSIONS

The fungal strains having keratinolytic activity were isolated from soils from different sites at Gwagwalada. They include farmlands, refuse dumps, barber's shops, drainage sites as well as animal sheds. The Hair Bait technique was used, using chicken feather as the keratin bait to screen the fungi for keratinolytic potential as shown in Plate 1. The keratinolytic fungal strains were identified on the basis of colony morphology, cultural characters, slide culture, pigmentation and morphology of hyphae and spores as *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Penicillium marneffei* (Plate 2, Plate 3 and Plate4 respectively) as shown in Table 2. It appears from this study that *Aspergillus niger* is the most prevalent keratinolytic fungus and also dominant species that was isolated from 17 soil samples (cover about 33.33%) of five different regions, followed by *Penicillium chrysogenum* (Thom) 13 (25.49 %), *Aspergillus flavus* (Link ex Fr.) 12 (23.53 %) and *Penicillium marneffei* (Hubert) 9 (17.65 %) being the least prevalent . Isolation rate of keratinolytic fungi including *Aspergillus niger* was higher in soil samples collected from the refuse arena and farm lands (Table 1 and Figure 1).

In order to determine the keratinolytic activity of the crude enzyme, the strains of fungi were cultivated in basal medium as described in materials and methods. Keratinolytic activity of the culture filtrates (Crude enzyme) appeared after three days and reached its maximum after 7 days and *Aspergillus niger* showed the highest keratinase activity (14.56±1.54Ku), followed by *Penicillium chrysogenum* (13.18±2.19), *Penicillium marneffei* (12.56±2.75) and the least was recorded for *Aspergillus flavus* (11.93±1.80) respectively as shown in Table 2 and Figure 2.

The production and characterization of keratinase enzyme by fungi that efficiently degrade feathers is interesting because these fungi play a significant role in the keratin degradation in natural setting. Among 4 filamentous fungi isolated from soil samples at Gwagwalada, 4 species belonging to two genera (*Aspergillus* and *Penicillium*) of ascomycetes were able to grow and produce keratinase in stationary cultures using poultry feather as the only substrate (Marcondes *et al*., 2008). The isolated fungi were able to grow normally, using chicken feathers as their sole source of carbon and nitrogen. The results showed that insoluble non-degradable chicken feathers were gradually decreased with the time, presumably due to keratin hydrolysis by keratinase of these fungi.

CONCLUSION

Research on keratinase has progressed very rapidly over the last five decades and potential industrial applications of the enzyme especially in solid waste management have been identified. Major impediments to exploit the commercial potential of amylase are the yield, stability and cost of amylase production. Although amylase production by microbes have been extensively studied by many researchers. Also, keratinolytic fungi are of great ecological interest not only in pathogenesis but also in keratin degradation. The degradative enzymes produced by *Aspergillus* spp and *Penicillium* spp. are capable of breaking down complex keratinous substrates in nature, and thus are responsible for the biodegradation of keratinized structure in polluted habitats. A thorough review of literature on microbial keratinolytic has shown that some of the fungi which were active in the characterization had been mentioned previously. But there is dearth of information about the ability of *Penicillium marneffei* to produce amylase and keratinase enzymes. However, the present study revealed that *Penicillium marneffei* is a good producer of keratinase enzymes.

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Bioremediation of a Soil Contaminated with Lubricating Oil using Bacteria Consortium

Olusegun Onimisi John-Dewole[1], Ramat Oyenike Sanni-Awal[2]

[1&2]Department of Biochemistry, Lead City University, Ibadan, Nigeria
segunotaru@yahoo.com

Abstract: A pilot study was carried out on soil from toll gate area in Ibadan, Oyo state western Nigeria, contaminated with hydrocarbon (lubricating oil) by artificial simulation to determine the attendant effect associated with the soil physicochemical properties and microbiological composition. Biodegradation of the contaminant using soil microbes and the kinetics of such process was also investigated. Soil parameters such as pH, conductivity, total organic hydrogen, total nitrogen and phosphorus and total petroleum hydrocarbon (TPH) were characterized using standard analytical methods. Trend in growth phase of soil heterotrophic and hydrocarbon utilizing microbes were investigated. Hydrocarbon contamination was seen to affect certain soil properties as a reduction in pH, conductivity, total phosphorus and heterotrophic microbial population was observed. The rate of microbial degradation was found to be dependent on pH and nutrient source. Effective degradation and increased microbial growth occurred between pH 5.3 and 7.2 but recorded reduced microbial growth and rate at much higher pH, thereby defining a suitable pH condition for the process.

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Keywords: biodegradation, lubricating oil, degradation kinetics, soil pollution, total petroleum hydrocarbon.

1. Introduction

Bioremediation is the use of microorganism metabolism to remove pollutants. Bioremediation technologies can be generally classified as *in situ* or *ex situ*. *in situ* bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some examples of current bioremediation technologies include; phytoremediation, bioventing, bioleaching, land-farming, bioreactor, compositing, bioaugmentation, rhizofiltration and biostimulation (Busetti, 2005).

Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators (Akpoveta and Osakwe, 2010).

However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. The assimilation of metals such as mercury into the food chain may worsen matters (Bergey and Breed, 1997). Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use (Mills et al., 1998).

There are recently global concerns over soils contaminated with crude oil or hydrocarbon products in general, after a similar feeling has been around for a while on marine oil-spills, which enjoy more media coverage because of the often spectacular visual effects images conveyed to people (Al-Mailem et al., 2010). There are similarities and differences between inland and offshore crude oil-spills. Similarities include hazards to life in all its forms. Secondly, contamination of valuable fresh water resources from aquifers or desalination plants and long term environmental impact; despite unsubstantiated claims that nature fully recovers in a few years. On the other hand, the differences concern mainly the behavior of spilled oil, its interaction with the surrounding environment and the corresponding approach to remediation (Bamnger et al., 2005).

In the case of soils contaminated by hydrocarbon products, there has been a great deal of work on biologically based treatment processes from several disciplines of the scientific community (Duii et al., 2002). This is not an odd phenomenon since environmental research concerns just as many disciplines and more importantly attracts funding support from government and private sources. However, the diversity of backgrounds of the researchers created a collection of schools of thought as well as, sometimes convenient basis for agreement

or disagreement in interpretation of laboratory or field data on bioremediation (Barrir et al., 2006).

The aim of this study is therefore, to investigate the bioremediation activity of bacteria consortium on a hydrocarbon polluted soil and the kinetics involved in the process.

2. Materials and Methods

Soil samples were obtained from Lead City University premises. The lubricating oil was purchased at mobil filling station Toll gate Ibadan. *S. saprophiticus*, *S. aureus*, *P. aeruginosa*, *E. coli* and Klebs were obtained from the microbiology laboratory of University College Hospital, Ibadan, Nigeria.

2.1 Soil Preparation and Sampling

A representative sample of the soil to be used was collected, dried and sieved using a wire mesh of 2mm. 20g of soil was weighed into five 250ml beakers and the samples were labeled A, B, C, D and E. Samples B,C and D were sterilized by placing it in hot air oven at 180°C and weighed at interval. The sterilization process was completed when the weight remains constant.

2.2 Preparation of Microbial Culture

The bioremediator was made up of an oil-degrading bacteria consortium containing *Staphilococcus saprophiticus, Staphilococcus aureus, Pseudomonas aeruginosa, E. coli* and Klebs. These were previously isolated and sub-cultured using nutrient agar medium. The medium was prepared by first weighing 6.2g of nutrient agar concentrate (with original concentration of 31g/l) and dissolving it in 200ml of distilled water (Chiu et al., 2000 and Dave, 2010). Thereafter, the solution was homogenized by boiling it in a water bath. After homogenizing, the medium was sterilized by autoclaving at a temperature of 121°C for 30minutes. It was allowed to cool for about 30 minutes (during the cooling process, the medium was swirled continuously to avoid solidification). The medium was poured into Mc Artney's bottle and the bottles were left in a slanted position until the medium solidifies (Grassi and Netti, 2000). Using an inoculating needle which has been pre-sterilized by flaming it on the methylated lamp, an inoculum was picked from the original culture and streaked on the surface of the prepared slants. The new isolates were stored in the incubator at 40°C and allowed to grow for 48 hours (Mokolobate and Haynes, 2002a).

2.3 Harvesting

The new culture was obtained from the incubator; about 15ml of peptone water was added into the bottles containing the culture. Using inoculating needle, the microbial cultured was streaked off into the water. Peptone water was used in this case to provide nutrient for the microbial culture (Ramalhosa et al., 2000, Khan et al., 2005 and Olipdri et al., 2009). The solution was then transferred into the contaminated soil sample.

2.4 Experimental Design

20g of sieved soil, which has been thoroughly mixed together was weighed into five 250ml beakers, the beakers were labeled A, B, C, D and E. Four of the samples were contaminated by adding 15ml of lubricating oil. Test carried out on each of the samples is as follows;

Sample A contains unsterilized soil and lubricating oil, this sample was used to monitor the action of the indigenous bacteria on the oil (Bouyouces, 1991). Sample B contains sterilized soil and bacteria consortium; this sample was used to monitor the effect of the introduced bacteria on the uncontaminated soil. Sample C contains sterilized soil and oil; this sample acts as the control (no microbes either foreign or indigenous). Sample D contains sterilized soil, lubricating oil and bacteria consortium; this sample was used to monitor the action of the introduced bacteria on the contaminated soil. Sample E contains unsterilized soil, bacteria consortium and lubricating oil; this sample was used to monitor the effect of combined microbes (both foreign and indigenous) on the contaminated soil (Bray and Kurtz, 1993). The soil samples were incubated for 60 days, after which they were subjected to the following analysis; soil pH, conductivity, Total Petroleum Hydrocarbon (TPH), Polyaromatic Hydrocarbon (PAH) and elemental constituents i.e. hydrogen, nitrogen, sulphur and phosphorus (APHA, 1998) . The values were expressed as Mean ± Standard deviation.

3. Results

The physico-chemical characteristic of the soil influenced by the impact of lubricating oil is shown in Table 1.

4. Discussions

The physicochemical characteristics of the soil were influenced by the impact of hydrocarbon contamination as observed in table 1 above. A reduction in pH, increase in conductivity and total phosphorus were observed on simulation of the soil with hydrocarbon (lubricating oil) from 7.2 to 5.3, 1891FS/cm to 3990FS/cm and 2.7mg/kg to 4.5mg/kg respectively; while a significant increase in total petroleum hydrocarbon (TPH) from 8.64mg/kg in the control soil to 1894.87mg/kg in the lubricating oil simulated soil was recorded as seen in the table (Dimitrow and Markow, 2000). The weak acidity

observed in the control soil is common with reduced anaerobic soils and sediments in the Niger Delta (Mokolobate and Haynes, 2002a and Maletić et al., 2009). The pH for the unpolluted soil fell within the pH range of between 5-7 which is suitable for most good agricultural soils, since Osuji et al., (2005) reported that most good agricultural soils have a pH between 5 and 7. Increased acidity occasioned by the presence of hydrocarbon (lubricating oil) is a problem for agricultural soil because very low pH values, indicative of acidity, are associated with adverse soil conditions including reduced microbial activity, increased availability and toxicity of heavy metals as well as reduced availability of plant nutrients. Conductivity value recorded in the control soil is due to the presence of soluble polar mobile solutes in the soil. The resulting decrease on contamination is due to the effect of hydrocarbon (lubricating oil) which provides a non polar environment for the soil ions, retarding their movement and immobilizing them, resulting in reduced ionic mobility, velocity and consequently bringing about increased conductivity. Presence of hydrocarbon in soil reduces available forms of phosphorus as has been shown by Okiemen and Okiemen (2005) and Okonokhua *et al*., (2007). The observed reduction in pH and increased conductivity was similar to the findings of Osuji and Nwoye (2007). After the bioremediation process, a decrease in pH (7.2 to 5.3), increased conductivity (1891 to 3990FS/cm) and total phosphorus (2.7to 4.5mg/kg) were observed. Substantial reduction in hydrocarbon concentration thereby providing a polar environment for the soil ions accounted for the increased conductivity. Introduction of exogenous nutrients such as phosphorus, nitrogen and other cat ions from the animal waste used in the bioremediation process possibly explains the observed increase in pH and total phosphorus content. Soil properties such as total nitrogen (0.007 to 0.15 to 0.35mg/kg), and organic phosphate (9.1 to 82.1 to 149.) increased on addition of the hydrocarbon to the soil and subsequently increased after the bioremediation process.

Table 1. Physico-chemical properties of soil at 60th day of study

Samples	pH	Conductivity (µS/cm)	TPH (mg/kg)	Phosphate (mg/kg)	Hydrogen (mg/kg)	% Nitrogen	Sulphate
A	5.43	2000	1153.13	111.87	10.54	0.015	Nd
B	6.91	2720	277.78	149.15	0.004	0.0073	Nd
C	5.31	3990	4333.33	82.17	7.32	0.013	Nd
D	5.90	2590	45833.33	9.14	5.78	0.032	Nd
E	6.27	2170	38555.56	123.69	9.76	0.035	Nd

A – Unsterilized Soil + Oil
B – Sterilized Soil + Bacteria Consortium
C – Sterilized Soil + Oil
D – Sterilized Soil + Oil + Bacteria Consortium
E – Unsterilized Soil + Oil + Bacteria Consortium

Correspondence to:
Olusegun Onimisi John-Dewole
Department of Biochemistry
Faculty of Information Technology and Applied SciencesLead City University, Ibadan, NigeriaE-mail: segunotaru@yahoo.com

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Chemical, Phytochemical and Antimicrobial Screening of Extracts of *B. sapida* for Agricultural and Medicinal Relevances

Olusegun Onimisi John-Dewole[1], Olutomi Oyedunni Popoola [2]

[1]Department of Biochemistry, Lead City University, Ibadan, Nigeria
[2]Department of Biochemistry, College of Natural Sciences, Federal University of Agriculture, Abeokuta, Nigeria
segunotaru@yahoo.com

Abstract: Phytochemical screening of the fruit of *Blighia sapida* confirmed the presence of Saponin, Saponin glycoside, Tannin, Balsam, Cardiac glycoside and Volatile oil. Spectrophotometric analysis for trace metals (such as Mn, Zn, Cu, Ni and Fe), Phosphorus and Sulphur showed that *B. sapida* contained Mn (0.332±0.003 mg/100g), Zn (1.820±0.001 mg/100g), Cu (0.253±0.002 mg/100g), Ni (1.074±0.001 mg/100g), Fe (0.791±0.002 mg/100g), Pb (0.010±0.001), P (49.20±0.200 mg/100g) and S (719.83±0.290 mg/100g). The medicinal and agricultural relevance of the extracts were evaluated in-vitro by antimicrobial and antifungal assays. The aqueous extract (but not methanol and petroleum ether extracts) showed growth inhibitory effects on *Staphylococcus aureus* and *Escherichia coli*, but *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* were resistant to all the plant extracts and the antibiotic controls. The Minimum Inhibitory Concentration (MIC) of the aqueous extract of *B. sapida* on *S. aureus* and *E. coli* were 3.13 mg and 12.50mg respectively. The Minimum Bacterial Concentration (MBC) of the aqueous extract against the test organisms ranged from 12.50mg to 25.00mg.

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Keywords: antimicrobial; *B. sapida;* herbal; pharmaceutical; phytochemical

1. Introduction

The use of plants and plant extracts for medicinal purposes has been going on for thousands of years; it has also form the source of much useful therapy in both herbalism and folk medicine (Sofowora, 1999). The use of medicinal plants in traditional medicine has also generated a lot of interest and concern about their efficacy and safety margin, since 65-70% of the Nigerian population patronizes traditional medicine practitioners in their various forms and methods (Bubayero, 1998 and Sofowora, 2001). Plants produce many chemical compounds that are having various potential values in the treatment of diseases, but a number of them could also be poisonous. Chemical compounds with beneficial effects have been isolated and biologically assayed to establish their medicinal activity. Modern drugs used in orthodox medicine have also been sourced from plants (Sofowora, 2001). It is therefore not surprising that medicinal plants are vastly employed in the treatment of various ailments which include; snake-bite, eye injuries, conjunctivitis, burns, scalds, abdominal colic, peptic ulcer, diarrhea, dysentery, chronic ulcer, measles, hepatitis, arthritis and rheumatism (Esuoso and Odetokun, 2005).

Mere isolation and elucidation of chemical structures of plant extracts may not be too significant, until appropriate bioassays are carried out to establish the biological activity exhibited by the plant extract (Ekong, 2006).

B. sapida, also known as 'Akee apple', belongs to the plant family called *Sapindaceae* and it is noted for its highly distinctive reddish fruits. There are different species of this plant, which include *Blighia sapida*, *Blighia welwitschii* and *Blighia unijugata* (Keay, 1999). *B. sapida* is a familiar tree often planted to provide shade from hot sun. It is known locally as 'isin' in Yoruba, 'gwanja kusa' in Hausa and 'okpu' in Igbo (Keay, 1999). *B. sapida* is about 25m high and 2.5m in girth, with a heavy evergreen crown. The bark is pale brown, while the leave has a stout stalk of about 5–23cm long. The leaflets, 5-15cm long by 3.5-7.5cm broad, are obovate with the lowest part almost circular and close to the base of the leaf-stalk (Keay, 1999). Flowering of the plant begins between October – March. The flowers are small and greenish white in colour. The fruits start appearing between March to September. The fruits are obovoid and about 3.5-6cm long by 3-5cm in diameter, bright red to yellowish in colour and often split open on the tree. The seed is covered with a glossy testa and about 2.5cm long by 2cm broad, while the aril (i.e. the edible part of the fruit) is pale yellow or cream coloured, wrinkled and about 2cm long (Keay, 1999). *B. sapida* is a native of West Africa. It extends from Senegal to Gabon. It is also cultivated in India and tropical America. *B. sapida* is well distributed throughout Nigeria and found in drier forest of the savannah region (Esuoso and Odetokun, 2005).

B. sapida is a medicinal plant commonly used by traditional healers in Nigeria, and highly valued in Africa (Owonubi, 2006) for the treatment of various ailments. Okogun (1996) stated that the bark pulp is used as liniment for oedema and intercostal pains in Cote d'Ivoire, while the bark is powdered and grounded with capsicum and rubbed on the body as a stimulant. The ashes of the dried husks and seeds are used in the preparation of soap, because they are rich in potash. The extracts of the leaves are used as eye drop in ophthalmia and conjunctivitis. Locally, various parts of *B. sapida* plants are used either alone or in combination for the treatment of psychosis, cancer, gonorrhea, stomach ache, hernia, backache, diarrhoea and constipation (Okogun, 1996 and Owonubi, 2006).

Thus, the aim of this study is to investigate the various chemical, phytochemical and anti-microbial components of the husks of *B. sapida* that are available for medicinal, pharmaceutical and agricultural use.

2. Materials and Methods

The fruits of *B. sapida* were harvested from the tree species found in the College of Forestry, Ibadan in Oyo State. The plant species was later identified and authenticated by the Department of Botany, University of Ilorin, Kwara State. The aril and the seeds in the fruits were removed with a sharp knife and the husks were dried at $32^o \pm 2^oC$ for two weeks on a clean pavement prior the analysis. The drying process was further enhanced by the harmattan wind.

Sampling

The dried bulk samples of the husks were pulverized using pestle and mortal, and sieved through a $2mm^2$ wire mesh to obtain a fine powder. The powdered samples were mixed together and quartered to obtain a representative sample weighing 150g.

Aqueous Extract

20g of powdered husks of *B. sapida* was weighed into 250ml beaker and 150ml of distilled water was poured unto the beaker content. The solution was stirred with a glass rod and allowed to soak for 24 h. The aqueous extract was filtered thrice through a plug of absorbent cotton-wool in a glass funnel. The aqueous extract was then filtered through 11cm Rundfilter paper MN713. The solution was concentrated by gentle evaporation on a heating mantle and poured into a 100ml beaker.

Methanolic Extract

200ml of methanol was measured into the round-bottom flask of the soxhlet. 20g of the powdered husks of *B. sapida* was placed in the thimble of the soxhlet extractor. The apparatus was coupled and the system was switched on at thermostat temperature of 65^oC. The sample was continuously extracted under reflux for 3 h, and the extract was poured into 100ml flask. Methanolic extract of the sample was concentrated by gentle evaporation on a heating mantle.

Petroleum Ether Extract

200ml of petroleum ether was measured into the round-bottom flask of the soxhlet. 20g of the powdered husks of *B. sapida* was placed in the thimble of the soxhlet. The apparatus was coupled and the system was switched on at thermostat temperature of 60^oC. The sample was continuously extracted under reflux for 3 h and the extract was poured into 100ml beaker after some of the petroleum ether had been recovered. The 100ml extract of the sample was concentrated by gentle evaporation on a heating mantle.

Phytochemical Screening of Crude Extracts

Phytochemical screening of the crude extracts for saponin, saponin glycoside, tannin, anthracene, alkaloid, volatile oil, balsam and cardiac glycoside were carried out by the methods described by Evans (2002) and Sofowora (2001).

Spectroscopic Analysis of Crude Extracts

Methods of Howtz (1999), Skoog et al., (2006) and Pavial et al., (2007) were used for spectroscopic analysis of the samples, using Atomic Absorption Spectrophotometer (A200).

Colorimetric determination of Phosphorus was done using Vanadomolybdate (Yellow) method (AOAC, 2000). Spectrophotometric determination of Sulphate was done using Turbidometric method (AOAC, 2000). Antimicrobial assay of crude extracts of *B. sapida* was done using the methods described by Egwari (1999), Ntiejumokwu and Kolawole (1999), and WHO (1999) to test the effects of crude extracts on the following pathogenic microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Saccharomyces cerevisiae*. Determination of antibiotic activity and antibiotic control was done by using the Disc Diffusion and Agar Diffusion techniques as described by WHO (1999). Determination of Minimum Inhibitory Concentration (MIC) of the crude extracts was done by using Tube Dilution method as described by Rotimi et al., (1999).

3. Results

Table 1 gives the phytochemical compounds present in crude extract of the husks of *B. sapida*. The extracts were positive for some of the following compounds; alkaloids, anthracene, balsam, cardiac glycoside, saponin, saponin glycoside, tannin and volatile oil indicating their presence in the extract.

Table 1. Phytochemical Compounds in the husks of *B. sapida*

Phytochemical Compounds	Remarks
Alkaloids	+ve
Anthracene	-ve
Balsam	+ve
Cardiac glycoside	-ve
Saponin	++ve
Saponin glycoside	+ve
Tannin	++ve
Volatile oil	-ve

Key: ++ve = strongly positive, +ve = positive, -ve = negative

Table 2 shows the trace metal contents of the plant extract in mg/100g. The extracts contained Manganese, Zinc, Copper, Nickel, Lead and Iron, while Table 3 shows the concentration of Phosphorus and Sulphur content of the extract in mg/100g.

Table 2. Trace Metals content in mg/100g

Elements	Conc. (mg/100g)
Manganese	0.332±0.003
Zinc	1.820±0.001
Copper	0.253±0.002
Cobalt	ND
Cadmium	ND
Nickel	1.074±0.001
Iron	0.791±0.002
Lead	0.010±0.001

The value represents mean ± SD (N=3), ND = Not Detectable

Table 3. Phosphorus and Sulphur concentration of the extract

Elements	Conc. (mg/100g)
Phosphorous	49.20±0.200
Sulphur	719.83±0.290

The value represents mean + SD (N=3)

Table 4 gives the inhibitory effects of extract of *B. sapida* husks at 15mg. *Staphylococcus aureus* and *Escherichia coli* were sensitive to aqueous extract of *B. sapida* with zone diameters of inhibitions of 14mm and 20mm respectively. *S. aureus, P. aeruginosa* and *E. coli* were all resistant (i.e. shows no growth inhibition) to both methanolic and petroleum ether extracts of *B. sapida*. The active ingredients in the plant extracts seemed more soluble in aqueous medium. The plant extracts exhibited no antifungal effects on *Saccharomyces cerevisiae*. *S. aureus* and *E. coli* were sensitive to the antibacterial effects of Ampicillin trihydrate (15mg) and Tetracycline hydrochloride (15mg) which were used as positive controls, with zone diameter of inhibition of 22mm and 26mm respectively (for ampicillin) and 26mm and 27mm respectively (for tetracycline). However, *P. aeruginosa* showed no growth inhibition on the antibiotic controls. Table 5 and 6 show the Minimum Inhibitory Concentrations (MIC) of the extracts on pathogens; the MIC of extracts of *B. sapida* on *S. aureus* and *E. coli* were 3.13mg and 12.50mg respectively.

Table 4: Inhibitory Effects of Extracts of Leaves of *B. sapida* (15mg)
Zone diameter (mm) of growth inhibition

Pathogens	Aqueous	Methanol	Pet. Ether	Ampicillin Control	Tetracycline Control
Staphylococcus aureus	14	0	0	22	26
Pseudomonas aeruginosa	0	0	0	0	0
Escherichia coli	20	0	0	26	27
Saccharomyces cerevisiae	0	0	0	0	0

Table 5: The MIC of *B. sapida* on *S. aureus*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g)
A1	25.00	Nil	
A2	12.50	Nil	
A3	6.25	Nil	
A4	3.13	+	3.13
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

Table 6: The MIC of *B. sapida* on *E. coli*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g
A1	25.00	Nil	
A2	12.50	Nil	12.50
A3	6.25	+	
A4	3.13	+	
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

4. Discussions

The crude extracts of the husks of *B. sapida* was chemically and microbiologically assayed for the presence of phytochemical compounds which could be responsible for their medicinal use in traditional medicine, as anti-amoebic, anti-diarrhea, anti-helminthic and treatment of Broncho-pneumonia (Tona 2008; Sofowora, 1999 and Owonubi, 2006). The study showed (Table 1) that the leaves of *T. diversifolia* contained Saponin, Alkaloids, Saponin glycoside, Tannin and Balsam. This result agrees with similar research done by Kela et al. (1999); Menut *et al.* (2002) and Okogun (1996). These phytochemical compounds have pharmacological effects and have been the basis of chemical synthesis of drugs used in modern medicine responsible for their medicinal use in traditional medicine (Sofowora, 2001) and (Okogun, 1996). Saponins are found in most plants as nitrogen-free glycosides, each consisting of a sapogenin and a sugar molecule. Glycosides are large and varied groups of naturally occurring plant products, characterized, on hydrolysis, by the formation of sugar and non-sugar moiety. Schuster et al. (1999) and Egwari (1999) have isolated steroidal glycosides such as Hecogenin, Progesterone, Testerone and Diosgenin from plants and are now being used therapeutically as hormones and contraceptives in medicine. Evans (2002) reported that the fruit *B. sapida* contains saponins which are haemolytic and probably toxic, but Baruah et al., (2000) reported that the toxic agent is neither saponin nor alkaloids. They concluded that the toxic compound is a water soluble substance that is stable at 100°C and is not precipitated by ethanol. The toxic compounds were named hypoglycine A and B. Ekong (2006) also reported that the ingestion of unripe fruit walls, seeds and white aril of *B. sapida* causes `vomiting sickness' that is characterized by marked hypoglycemia and a mortality rate ranging from 40-80%. Bello (1999) reports that there is a high content of hypoglycine A in immature fruit of *B. sapida* while Tongma *et al*., (2008) used Reversed Phase Liquid Chromatography for the determination of hypoglycine A in a canned ackee fruit samples. The high mortality rate associated with the ingestion of unripe ackee fruits was further confirmed by Kela et al., (1999). They reported the high epidemic of fatal encephalopathy in preschool children in Burkina Faso associated with the consumption of unripe ackee fruit. The poisoned children were observed to have common symptoms of hypothermia, vomiting, convulsion and coma.

Apart from those symptoms observed in school children, Bello (1999) reported that extract of *B. sapida* produces leucopenia and thrombocytopenia in mice. The toxic property in *B. sapida* has been advantageous in formulations of various insecticides and pesticides. Kela *et al.*, (1999) reported the molluscicidal activity of *B. sapida*, while Ntiejumokwu and Kolawole (1999) reported the pesticidal activity of *B. sapida* and other selected plants. Cardiac glycosides, digitoxin and digoxigenin have varying effects in the cardiovascular systems of human. They are used in the treatment of heart disorders and high blood pressure (Groth, 1994 and Stenlake, 1997). Tannins are polyphenolic compounds also used for medicinal purposes e.g. catechol, hydroquinine and resorcinol are phenolic salicylates used as analgesics, antipyretics and as internal antiseptics in medicine and surgery (Bello, 1999 and Stenlake, 1997). This research however showed that *B. sapida* has growth inhibitory effect on *S. aureus* and *E. coli*. The toxic property in Hypoglycine A isolated from *B. sapida* may be responsible for the antibacterial activity of this plant. Thus, the presence of these phytochemical compounds in *B. sapida* could be responsible for the observed pharmacological effects and their medicinal use in traditional medicine.

The seed and seed-oil of matured *B. sapida* have some beneficial uses. Esuoso and Odetokun (2005) reported that the seeds and seed-oils of *B. sapida* are rich sources of protein, carbohydrate, fatty acid and amino acid, which could be used in animal feed formulations (Akobundu and Agyakwa, 1997).

Trace Elements are essential components of the body enzymes, haemoglobon, vitamin B_{12} and thyroxin which are important for life processes and metabolism; and are sourced mainly from plants. Analysis of trace metals, sulphur and phosphorus content revealed Mn(0.332$\pm$0.003 mg/100g), Zn (1.820$\pm$0.001 mg/100g), Cu (0.253$\pm$0.002 mg/100g), Ni (1.074$\pm$0.001 mg/100g), Fe (0.791$\pm$0.002 mg/100g), Pb (0.010 $\pm$ 0.001), P (49.20$\pm$0.200 mg/100g) and S (719.83$\pm$0.29 mg/100g) (See Table 2 & 3). The concentration (mean$\pm$SD) of the elements analyzed showed Sulphur > Phosphorus > Zinc > Nickel > Iron > Manganese > Copper > Lead. The high concentration of sulphur could be responsible for the plant's antimicrobial properties. The clinical effectiveness of sulphanilamides in the control of bacterial infection has led to their effective use against pneumonia and *streptococci* infection (Groth, 1994 and Stenlake, 1997). Sulphanilamides interfere with the synthesis of folic acid, and important bacterial growth factor, by utilization of *para aminobenzoic acid* (PABA) necessary for the synthesis of trihydrofolic acid. The study showed that aqueous extracts of the husks of *B. sapida* possess antimicrobial effect against the growth of pure isolates of *S. aureus* and *E. coli*. This is similar to what is reported by Egwari (1999). The result of zone

diameters of inhibition of the plant extract on the growth of *Staphylococcus aureus* and *Escherichia coli* (Table 4) compared favourably with that of standard antibiotic controls consisting of *Tetracycline hydrochloride* (15mg) and *Ampicillin trihydrate* (15mg) (WHO, 1999 and Cheesebrough, 2000). *P. aeruginosa* was resistant to all the plant extract and antibiotic controls. This observation agrees with that of Timothy and Nelson (1992) and Cheesebrough (2000). The plant extract had no antifungal activity against *Saccharomyces cerevisiae*.

5. Conclusion

The phytochemical screening of the husk extracts of *B. csapida* tested positive for the presence of alkaloids, saponin, saponin glycoside, tannin and balsam. The concentration (mean±SD) of elements analysed in mg/100g showed S > P > Zn > Ni > Fe > Mn > Cu > Pb. The high concentration of sulphur and phosphorus is an index for the plant's medicinal properties. The medicinal properties of the plant as evaluated *in-vitro* by antimicrobial assay revealed that aqueous extract showed growth inhibitory effects on *S. aureus* and *E. coli*. However, *Pseudomonas aeruginosa* was resistant to the plant extract and antibiotic controls. The plant extracts have no antifungal effects on *Saccharomyces cerevisiae.*

Recommendations

Further work is recommended on isolation and characterization of active chemical compounds responsible for the antimicrobial/antibacterial properties of the plant. The antibacterial effect of the methanol extract and the antifungal effects of the plant extract should be re-evaluated. Medicinal plants are also known to exhibit seasonal variation in chemical properties and bioactivity, which could also affect their medicinal properties at any given period of time. Therefore, there should be an investigation to mitigate the seasonal chemical properties variation of this plant.

Correspondence to:
Olusegun Onimisi John-Dewole
Department of Biochemistry
Faculty of Information Technology and Applied Sciences
Lead City University, Ibadan, Nigeria
E-mail: segunotaru@yahoo.com

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Examining relation between qualities of work life based on Walton model and staff efficiency of Islamic Azad University, Shoushtar

Masoud Ahmadinejad[1], Ommehkolsoum Gholamhosseinzadeh[2] Mahmoud yaghobi doust[3]

[1]Department of Management, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran
[2]Assistant Professor, Sari branch, Islamic Azad University, Sari, Iran
[3]Department of Sociology, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

Abstract: This research has been done with the main goal of examining relation between quality of work life based on Walton theory and staff efficiency amount of Islamic Azad University, Shoushtar branch. The use and method of the present research is descriptive-correlation. The statistical society is consisting of whole staff of this university that according to Karaji and Morgan 100 persons were chosen randomly as sample among 320 non-academic member staff occupying in university. The statistical descriptive and presumptive methods are consisting of percentage, frequency, average, variance and criteria deviation in order to analyze. The present research founds indicate the presence of the positive and meaningful correlation coefficient between quality of work life pattern based on Walton and staff efficiency amount occupying in Islamic Azad university, Shoushtar branch. Also research founds indicate the presence of the positive and meaningful correlation coefficient between different dimensions of work life as the independent variants and efficiency amount of staff as the dependent variant.
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Keywords: quality of work life, Walton theory, efficiency, staff of Islamic Azad University, Shoushtar branch

1. Introduction

One of the most basic matters related to manage today societies of manpower between them is designing and collecting the quality of work life of staff occupying in the governmental and private sections that has been always background of appearing a lot problems for societies. Many existing disorders and dissatisfactions in relations of staff and institutes owners or their managers are result of losses and unsuitable work life of their staff.

In these cases, if a multilateral and universal quality of work life program is designed regarding to society culture that staff have a desired mental imagination about the physical and mental environment of own work place, so staff accept it and also they consider benefits and goals of organizations owners, a lot of these disorders such as the less work, absence and changing job, work accidents, wasting sources, job dissatisfaction and decreasing use quality and so on are eliminated and job satisfaction and so use of all staff occupying in organization are promoted (Walton, R. E., 1973).

The work life quality programs emphasize strongly on creating an environment that leads to satisfy people needs. The work life quality emphasizes on methods that change organization that all members it be able to interference in decisions that affect their job especially their work environment. So it causes partnership and their job satisfaction in the more work following increasing efficiency and decreasing stresses (Oshagbemi, T., 1997).

Regarding to the economical problems and two-digit price of inflation, authorities Islamic Azad university, Shoushtar branch tries to eliminate their unsuitable work life quality level with setting the suitable policies and diplomacies about choosing a strategy and creating life quality program of staff with university. So it needs to evaluate the present and future needs of staff in this case. Examining and measuring the power amount of quality of work life pattern indexes because of its effect on staff efficiency amount can improve the individual, group and organizational performance. Researches of Eric Trist and colleagues in Tavistoc institute of London indicated that promoting quality of work life of staff causes increasing the higher levels of career credits, variety and progress in work and their partnership. They tried to provide a feedback from job information with independence (or freedom of act) for staff.

A research as change in organization and quality of work life has been done in three different institutes in New Zealand IN 1996. Results of this research indicate that staff suggestions about improving job condition cause the organizational ownership feeling and increasing products use. Of course staff tendency to perform these suggestions, group work and using equipment and partnership in making decision were the most important suggestions of staff (Heinonen, S., Saarimaa, R., 2009). They examined effect of quality of work life (GWL) on staff efficiency of electric force distribution office of Khouzestan province in MA thesis.

Founds of this research indicate existence of the direct and meaningful relation between the independence variants of quality of work life and the dependence variant of efficiency in the mentioned company. Considering above topics, this research has been designed that by performing it with the scientific method, it is specified that how much existence of relation between work life based on Walton model and efficiency amount of staff occupying in Islamic Azad University, Shoushtar branch is valid? In this research, eight components that form quality of work life pattern of Walton are axis as the independence variant.

So the basic question of research topic is that do we know there is a meaningful relation between Walton work life quality pattern and efficiency amount of staff occupying in this university? How much is effect of each work life quality components based on Walton model on efficiency amount of occupying staff if there is a relation? Which will be more effective on efficiency amount?

1.1. The theoretical bases of research:

Walton work life quality pattern:

Richard Walton as one of the famous researchers has proposed some indexes after many researches that possibility access to quality of work life (QWL) is provided in each organization with examining them. These criteria are respectively:

1.1.1. Fair payment:

Payment must be such that person can live in a rational level and fit to own skill. The equal payment for the equal work and being proportional payments with the social and staff criteria and its genesis with other jobs and in the other word, what individuals in own view have right of its receiving in comparison to others. They may conclude that they have a fair receipt or not. If they conclude this comparison is fair or based on equality, it is very possible that they become satisfied, if not, they become dissatisfied and will try less.

1.1.2. Law tendency:

Purpose of law tendency in organization is existing freedom of expression of staff without fear from revenge of the higher authority and or being effective law power on the human power. If the legal norms are ruling in organization, individual tastes and nobody can decide based on the personal view. Also persons express own words without fear from the next revenge.

1.1.3. Opportunity of theconstantgrowth: Providing background of the individual abilities improving, progress opportunity and learning opportunity of the obtained skills consisting of duties referring with training and also rebuilding individual knowledge and introducing them with the new methods and techniques and finally creating the sufficient opportunity for their progress in the official hierarchy.

4- The social dependence:

The social dependence points to perception method (the mental perception) of staff about the social responsibility of organization.

5- Providing the constant security:

Purpose of providing the constant security is an employment that supply constancy in work, as employee is secure rationally in her/his future.

6- Developing the individual abilities:

Developing the individual and human abilities is applied to provide opportunities such as using independence and self-control in work, enjoying different skills and access to the suitable information about work.

7- The secure and healthy work environment:

It consists of the rational and standard work time during week and payment to person in lieu of the additional work hours and the suitable physical conditions in work environment, so illness risk or events from work decrease.

Organization must choose the security-healthy plans which individuals are involved in the less material or social-mental damages. If organization be able to provide a secure, health and comfortable environment to work, increasing success for attracting and maintaining the qualified labors and producer is possible. Organization which is famous as an insecure place will make difficult access to the qualified labors.

8- The social integration and correlation:

Staff must feel belonging to work environment and it needs to create the suitable work environment and atmosphere. If staff feel that organization doesn't support them and their work, they aren't able to use own potential force to achieve organization goals. The social integration and correlation point to create work environment, so staff feel belonging (Walton: 1973, pages 11-21).

1.2. Research goals:

1. Examining relation between quality of work life pattern of Walton and efficiency amount of staff occupying in Islamic Azad University, Shoushtar branch.

2. Examining and describing amount of each index that form quality of work life pattern of Walton.Examining and describing amount of staff efficiency.

2. Material and Methods

The present research type is unity. The statistical society of this research are all staff or non-academic members occupying in Islamic Azad university, Shoushtar branch that they have been considered without attention to gender, age, job, kind and degree of academic paper consisting of all staff

(education-official sections) that their whole number is 319 persons. 100 persons of staff have been considered as the sample volume.

In this research, sampling method has been performed randomly and simply that according to measure table, sample is calculated on the basis of society measure that has been presented by three scientists called Kohn, Morgan and Karjsai.

2.1. Analyze method of data:

In the present research, different statistical methods have been used:

A) The descriptive statistical methods: They are consisting of average calculation, standard deviation, maximum and minimum of grade, frequency, frequency percentage and so on.

B) The presumption statistical methods: They are consisting of Pierson correlation coefficient, analyzing the multivariate regression, Scheffe post hoc test, Cronbach Alpha method in order to calculate the permanency coefficients. Also computer software (SPSS) has been used to analyze the collected data exactly.

2.2.Measurement tools:

A) Quality of work life questionnaire: This questionnaire based on Walton model has been used to measure amount of quality of work life that this questionnaire has been translated and designed by Mr. Allameh in 2000 within 35 questions according to Likert five degree spectrum. Its Cronbach Alpha is %85 (Allameh: 1997).

B) Efficiency questionnaire: This questionnaire is according to Likert five degree spectrum that has been designed and collected by Nanchian and his colleagues in "management strategies" book, designing directory of questions of research questionnaires consisting of 15 questions has been used to measure efficiency amount of staff. Its Cronbach Alpha is %83.

3. Results

3.1.) Results related to the secondary hypothesize of research:

3.1.1. The first hypothesis: There is a meaningful relation between the fair and sufficient payments and efficiency of staff occupying in Islamic Azad University, Shoushtar branch

Table (1): Correlation coefficient between the fair payments and staff efficiency

Variants	Efficiency of staff	
The fair payments	Correlation coefficient	The meaningful level
	0/61	0/0001

As founds of table (1) Show, there is a positive and meaningful relation between the fair payments and efficiency of staff occupying in Islamic Azad university, Shoushtar branch: (r=0/61 and p<0/0001). In the other word, whatever payments in university is fair, just and sufficient, efficiency of staff occupying in university increases. So regarding to this result, the first secondary hypothesis is confirmed.

3.1.2. The second hypothesis: There is a meaningful relation between the safe and healthy work conditions and efficiency of staff occupying in Azad Islamic university of Shoushtar.

Table (2): Correlation coefficient between the safe work conditions and staff efficiency:

Variants	Efficiency of staff	
The safe and healthy work conditions	Correlation coefficient	The meaningful level
	0/51	0/0001

As we observe in table (2), there is a positive and meaningful relation between the safe and healthy work conditions of Islamic Azad university, Shoushtar branch and efficiency of staff occupying in it: (r=0/51 and p<0/0001) that means whatever environment and work conditions of university are safe and healthy, occupying staff efficiency increases. So regarding to this result, the second secondary hypothesis is confirmed.

3.1.3. The third hypothesis: There is a meaningful relation between opportunity for using and developing the individual abilities and efficiency of staff occupying in Islamic Azad university, Shoushtar branch.

Table3: Correlation coefficient between developing the human abilities and staff efficiency:

Variants	Efficiency of staff	
Developing the human abilities	Correlation coefficient	The meaningful level
	0/75	0/0001

As founds of table (3) show, there is a positive and meaningful relation between opportunity for using and developing the individual abilities and efficiency of staff occupying in Islamic Azad university, Shoushtar branch: (r=0/75and p<0/0001) that means whatever the opportunity is provided to use and develop the individual abilities in university and staff are able to fulfill and develop own abilities and potential talents, their efficiency increases. So regarding to this result, the third secondary hypothesis is confirmed.

3.1.4. The fourth hypothesis: There is a meaningful relation between opportunity for supplying security and the constant growth in the future and efficiency of staff occupying in Islamic Azad University, Shoushtar branch.

Table (4): Correlation coefficient between opportunity for supplying security and the constant growth in future and staff efficiency:

Variants	Efficiency of staff	
Opportunity for supplying security and the constant growth in future	Correlation coefficient	The meaningful level
	0/54	0/0001

As we observe in founds of table (4), there is a positive and meaningful relation between opportunity for supplying security and the constant growth in university in the future and staff efficiency: (r=0/54 and p<0/0001). In the other hand, whatever staff are secure in own job future and its progress way, their efficiency increases. So the fourth secondary hypothesis of research is confirmed.

3.1.5. The fifth hypothesis: There is a meaningful relation between the social integration and cohesion and efficiency of staff occupying in Islamic Azad University, Shoushtar branch.

Table (5): Correlation coefficient between the social integration and cohesion and staff efficiency:

Variants		Efficiency of staff
The social integration and cohesion	Correlation coefficient	The meaningful level
	0/65	0/0001

As we observe in table (5), there is a positive and meaningful relation between the social integration and cohesion efficiency of staff occupying in Islamic Azad university, Shoushtar branch: (r=0/65 and p<0/0001). In the other word, whatever university creates a favorable work environment that staff knows itself part of university and strengthen feeling of belonging of staff to university to them, staff efficiency increases. Regarding to this result, the fifth secondary hypothesis is confirmed.

3.1.6. The sixth secondary hypothesis:

There is a meaningful relation between law tendency in organization of work environment and efficiency of staff occupying in Islamic Azad University, Shoushtar branch.

Table (6): Correlation coefficient between law tendency and staff efficiency:

Variants	Efficiency of staff	
Law tendency in work place organization	Correlation coefficient	The meaningful level
	0/54	0/0001

As founds of table (6) show, there is a positive and meaningful relation between law tendency in organization of work environment and efficiency of staff occupying in Islamic Azad university, Shoushtar branch: (r=0/54 and p<0/0001) that means if staff feel that work in an open, warm, protector and legal environment and express own view without fear from the higher authorities and they will been behave according to law and regulations if they make problem and also law ruling in organization is more effective than authorities ruling, this law tendency atmosphere have a great effect on increasing efficiency of staff. So regarding to obtained results, the sixth secondary hypothesis is confirmed.

3.1.7. Seventh hypothesis:

There is a meaningful relation between work and total life environment and efficiency of staff occupying in Islamic Azad University, Shoushtar branch

Table (7): Correlation coefficient between work and total life environment and efficiency of staff:

Variants	Efficiency of staff	
total and whole life environment	Correlation coefficient	The meaningful level
	0/50	0/0001

As founds of table (7) show, there is a positive and meaningful relation between work and total life environment and efficiency of staff: (r=0/50 and p<0/0001) that means whatever job of staff is more interesting, challenging and rich and also total life environment of staff is healthy and favorable that an appropriate balance is established between work life and other life parts of staff, efficiency amount of staff increase. So regarding to obtained results, the seventh secondary hypothesis of research is confirmed.

3.1.8. The eighth hypothesis: There is a meaningful relation between the social dependency and connection of work life and efficiency of staff occupying in Islamic Azad University, Shoushtar branch.

Table (8): Correlation coefficient between the dependence and connection of work life and staff efficiency:

Variants	Efficiency of staff	
The dependence and connection of work life	Correlation coefficient	The meaningful level
	0/67	0/0001

As founds of table (8) show, there is a positive and meaningful relation between the dependence and connection of staff work life and their efficiency: (r=0/67 and p<0/0001).

In the other word, if staff understand that university is responsible to own staff and society and they have a suitable conclusion about own work and responsibility, their efficiency increases. So the eighth secondary hypothesis of research is confirmed.

2) Results related to the essential hypothesis of research:

2.1. The essential hypothesis: There is a meaningful relation between quality of work life and efficiency of staff occupying in Islamic Azad University, Shoushtar branch

Table (9): Correlation coefficient between quality of work life and staff efficiency:

Variants	Efficiency of staff	
Quality of work life	Correlation coefficient	The meaningful level
	0/79	0/0001

As founds of table (4-3-9) show, there is a meaningful relation between quality of work life and efficiency of staff occupying in Islamic Azad university, Shoushtar branch: (r=0/79 and p=0/0001). In the other word, efficiency amount of staff increase with increasing quality level of work life of them. So regarding to these results, the essential hypothesis of research is confirmed.

4. Discussion:

One of the most basic matters related to management and managing today societies of manpower between them is designing and collecting quality of work life programs of staff occupying in the governmental and private sections that has been always background of a lot problems for societies and its different sections. Topic of this research is examining relation between quality of work life based on Walton model and efficiency of staff occupying in Islamic Azad University, Shoushtar branch. Examining and studying eight dimensions of quality of work life based on Walton model is purpose of it. The obtained results of analyzing the basic hypothesis of this research are compatible with founds related to the done researches by researchers in the past.. In this chapter regarding to data analyses and the obtained results, all hypothesizes and their analyzed results are explained. Then they will be discussed

4.1.The basic hypothesis of research:

There is a meaningful relation between quality work life and efficiency amount of staff and efficiency amount of staff occupying in Islamic Azad University, Shoushtar branch**.** As founds show, there is a strong, positive and meaningful relation between two variants of quality work life and efficiency amount of staff. According to that, we can certainly say that attention to quality work life factors is equal with staff efficiency level. In other word, whatever quality of staff life increases, staff efficiency will improve and promote. So the basic hypothesis of research is confirmed. The obtained results from analyze of the basic hypothesis of this research is compatible with founds of -Bastami, H., 1995.

4.2. The first hypothesis:

There is a meaningful relation between the fair and sufficient payments and efficiency of staff occupying in Islamic Azad University, Shoushtar branch. As founds show, there is a strong and positive relation between two variants of the fair payment and staff efficiency. According to that, we can certainly say that attention to the fair payment in organization and pay that person is able to live in a rational level suitable with skill will cause to improve and promote staff efficiency level. So whatever payments are fairer and more just, staff efficiency improve and promote. The obtained results of data analyze related to the first secondary hypothesis are compatible with research results of Zamani Fard and Sandi Ghale Abdol Shah (2006).

4.3. The second hypothesis:

There is a meaningful relation between the safe and healthy work environment and conditions and efficiency amount of staff occupying in Islamic Azad University, Shoushtar branch r. As founds show, there is a positive and meaningful relation between two variants of the safe and healthy work conditions and efficiency amount of staff. So we can certainly say that attention to the fair work hours and security and healthy design of work environment will cause to promote staff efficiency level. In other word, the safe and healthy work conditions in university cause to increase staff and organization efficiency level. Results of this research are compatible with research founds of Zamani Fard (2006) equal with (r=22 and p=0/04) and research of Sandi Ghale Abdol Shah (r= 0/52 and p=0/01).

4.4. The third hypothesis:

There is a positive and meaningful relation between opportunity for using and developing the individual abilities and efficiency amount of staff occupying in Islamic Azad University**,** Shoushtar branch. Considering results related to founds, it is clear that there is a meaningful relation between two variants of opportunity for using and developing the individual abilities and efficiency amount of staff. We can certainly say that creating opportunities such as independence and autonomous work, enjoying different activities and access to information compatible with work will cause to promote and improve efficiency level of staff. Results of this research are compatible with research results of Ansari, M., Esmaeili. A., Bagheri, K., Salehi A. 2010).

4.5. The fourth hypothesis:

There is a meaningful relation between opportunity for supplying security and the constant growth in the future and efficiency amount of staff. According to this information, there is a positive relation between opportunity for supplying security and the constant growth in the future and efficiency amount of staff. So we can certainly say that regarding to result of the mentioned hypothesis, whatever staff is secure in own job future and have opportunities for job promotion in the future, their work performance improve and so their efficiency increases. Results of this research are compatible with research founds of Zamani Fard (2006) and Sandi Ghale Abdol Shah.

4.6. The fifth hypothesis:

There is a meaningful relation between the social integration and cohesion and efficiency amount of staff. As the obtained results of founds show, there is a meaningful relation between two variants of the social integration and cohesion and efficiency amount of staff occupying in Azad Islamic university of Shoushtar. So we can certainly say that attention to create trust environment between colleagues, their belonging feeling to organization, doing works based on following the official hierarchy and avoiding discrimination between staff will cause to promote efficiency level of staff. Results of the done researches are compatible with the present research and researches of Lau RSM (2000).

4.7. The sixth hypothesis:

There is a meaningful relation between law tendency in work environment organization and efficiency amount of staff occupying in Islamic Azad University, Shoushtar branch. As founds show, there is a positive and meaningful relation between two variants of law tendency in work environment organization and efficiency amount of occupying staff. So we can certainly say that attention to the fair behavior with staff, freedom of expression of staff without fear from the higher authorities and also being effective and ruler the legal norms in organization cause to improve and promote efficiency level of staff. Results of this research are compatible with research founds of Lau RSM (2000).

4.8. The seventh hypothesis:

There is a meaningful relation between work and total life environment and efficiency amount of staff. As founds show, there is a positive and meaningful relation between two variants of work and total life environment and staff efficiency. We can explain that different factors such as work and total life environment of staff affect efficiency of staff occupying in university.

4.9. The eighth hypothesis:

There is a meaningful relation between the social dependency and connection of work life and efficiency of staff occupying in Islamic Azad University, Shoushtar branch. As founds show, there is a meaningful relation between two variants of the social dependency and connection of work life and efficiency amount of staff. The obtained results of analyzing this hypothesis are compatible with found of the done researches by Hassanzadeh, D., 2002.

4.10. Suggestions:

1. Selecting a payment system and the fair and sufficient advantages.
2. Creating and developing the safe and healthy work environments.
3. Developing law tendency in organization.
4. Developing the individual abilities in organization**.**
5. Creating balance between work and total life environment.
6. Empowering staff and their interfering in making decisions and university programming.
7. Developing the human relations in work environment and strengthening staff self-confidence.
8. Enriching and redesigning of jobs.

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Corresponding Author:
Masoud Ahmadinejad
Department of Management, Shoushtar Branch,
Islamic Azad University, Shoushtar, Iran

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Asymptomatic *Plasmodium* Parasitaemia in Ilorin, North Central Nigeria

[1]Udeze AO, [1]Nwokocha EJ, [2]Okerentugba PO, [1]Anibijuwon II, [2]Okonko IO

[1]Department of Microbiology, University of Ilorin, P.M.B. 1515 Ilorin-Nigeria
[2]Medical Microbiology Unit, Department of Microbiology, University of Port Harcourt, P.M.B, 5323 Choba, East-West Road, Port Harcourt, Rivers State, Nigeria
*Corresponding author: austok90@yahoo.com, udeze.ao@unilorin.edu.ng, Phone: +234(81)35586003

ABSTRACT: This study was carried out to determine the prevalence of malaria parasites infection among apparently healthy students of the University of Ilorin, Ilorin-Nigeria. A total of 200 students {98 (49.0%) males and 102 (51.0%) females; age range: 16-30 years} were recruited for the study after informed consent. Both thick and thin films were made and stained using parasitological standard procedures after collection of blood samples from the students. Analysis of the result showed that 100 (50.0%) of the subjects had malaria parasitaemia. Highest prevalence (56.9%) was recorded among the females than the males (42.9%). Age distribution showed 48.8% among subjects of age range 15-20 years, 48.3% among subjects of age range 21-25 years and 40.0% among subjects of age range 26-30 years. The result also showed that 84.0% of the infection occurred among subjects with haemoglobin genotype AA while 16.0% of the infection occurred among subjects with haemoglobin genotype AS. Frequency distribution of the Plasmodium species showed that *Plasmodium falciparum* has the highest occurrence (75.0%). This is followed by *Plasmodium malariae* with a frequency of 13.0% and *Plasmodium ovale* with a frequency of 8.0% while *Plasmodium vivax* has the least occurrence of 4.0%. This finding has further confirmed the endemicity of this infection among students population. Control measures to stop the spread are therefore advocated.
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Keywords: Plasmodium, Parasitaemia, Haemoglobin genotype, Students

1. INTRODUCTION

Malaria remains a global health problem despite all efforts aimed at eradicating it. The disease remains a major public health problem in Nigeria where it is endemic especially in rural populations, as is the case elsewhere in Africa (Klinkenberg *et al.,* 2005). In Nigeria, malaria results in 25% infant and 30% childhood mortality (FMH, 2005a). More than 90% of the total population is at risk of malaria and at least 50% of population suffers from at least one episode of malaria each year (RBM, 2005; FMH, 2005b). The parasites are known to show resistance to common first line anti malarial drugs, in various part of the country (Falade *et al*., 1997). The disease affects about 500 million people and kills about 2 million, mostly children, each year (WHO, 2000). The burden of this disease falls heaviest among children below the age of five in sub- Saharan Africa.

Human malaria is commonly caused by four species of plasmodium, a unicellular protozoan. It includes species of *P. falciparum, P. vivax, P. malariae, and P. ovale.* All of these have different manifestations with *P. falciparum* having been described as the most dangerous (Snow *et al*., 2003). *Plasmodium knowlesi* is one of the five malaria species known to cause human malaria (Cox-Singh and Singh, 2008).

Transmission is principally by bite of an infected female Anopheles mosquito and rarely by direct inoculation of injected red blood cells through blood transfusion, congenital transfer or sharing of needles. Among the predisposing factors of transmission are stagnant water, overcrowding and improper sanitation (Atif *et al.,* 2009). Host immunity and types of haemoglobin (Hb) genotype have been found to be crucial to the rate of parasite invasion, multiplication, and destruction as well as outcome of the disease (Pasvol and Weatherall, 1980). However, the relationship between Hb genotype and level of protection conferred against severe forms of malaria remains unclear. Children with heterozygous sickle cell traits have been reported to have lower parasite rates and less fatal infections as compared to children with HbAA (Okam, 2002). On the other hand, some other researchers have reported that children with sickle cell disease are not immuned to cerebral malaria (Konotey-Ahulu, 2001; Jones, 2008). The seasonal incidence of malaria infection depends on the feeding and resting habits of female Anopheles mosquitoes, its infectivity, climatic factors and the presence of susceptible human population and transmission.

In Nigeria, works have been carried out to determine the prevalence and distribution of the parasite in different subpopulations (Anosike *et al*.,

2004; Eke *et al*., 2006; Adefioye *et al*., 2007; Epidi *et al*., 2008; Ibekwe *et al*., 2009; Ilozumba and Uzozie, 2009; Okonko *et al*., 2009). However, continuous study is required in order to keep track of the ever dynamic nature of the infection. This study was carried out to determine the distribution of the different species of Plasmodium parasites among the students of the University of Ilorin, Ilorin Nigeria.

2. MATERIALS AND METHOD

2.1. Study population

A total of 200 {98 (49.0%) males and 102 (51.0%) females} asymptomatic students of the University of Ilorin who gave their consent after thorough explanation of the purpose of the study were recruited for the study. The age range of the students was from 16 to 30 years.

2.2. Collection of Samples

The method of sample collection employed was venepuncture technique (Carmel *et al*., 1993; Ibhanesebhor *et al*. 1996; Okocha *et al*. 2005). Soft tubing tourniquet was fastened to the upper arm of the patient to enable the index finger feel a suitable vein. The puncture site was then cleansed with methylated spirit (methanol) and venepuncture made with the aid of a 21g needle attached to a 5 ml syringe. When sufficient blood had been collected, the tourniquet was released and the needle removed immediately while the blood was transferred into an EDTA bottle. Biodata of the students were collected from students' clinical records which include; gender, age and genotype.

2.3. Examination of Blood for Malaria Parasites

Microscopy is the main tool for laboratory diagnosis of malaria (WHO, 1992). Thick and thin smears of blood samples were made from the subjects under study. The smears were stained using 2% Giemsa solution for the thick and 100% Leishman solution for the thin film for the identification and speciation of the parasite respectively. The ring forms of the merozoites were identified and counted in 100 filed using x 100 objective lens. The count was done per 200 leucocytes, assuming a leucocyte number of $600mm^{-3}$.

3. RESULTS

This analysis showed that 200 subjects were recruited out of which 98 were males and 102 were females. Prevalence of *Plasmodium* parasitaemia among these subjects was 50%. Presence of ring forms of *Plasmodium* and Trophozoites of *Plasmodium* indicated positive results. Gender distribution showed a higher prevalence (56.9%) of *Plasmodium* parasitaemia among the female subjects and 42.9% *Plasmodium* parasitaemia among the male subjects as shown in Table 1.

Table 1: Gender distribution of *Plasmodium* infections

Gender	No. tested (%)	No. positive (%)
Males	98 (49.0)	42 (42.9)
Females	102(51.0)	58 (56.9)
Total	**200(100.0)**	**100 (50.0)**

Figure 1 shows frequency and distribution of *Plasmodium* parasitaemia in relation to age. The results showed that age group 15-20 years has the highest prevalence (48.8%) followed by age group 21-25 years with prevalence of 48.3% while the least prevalence (40.0%) was observed in age group 26-30 years.

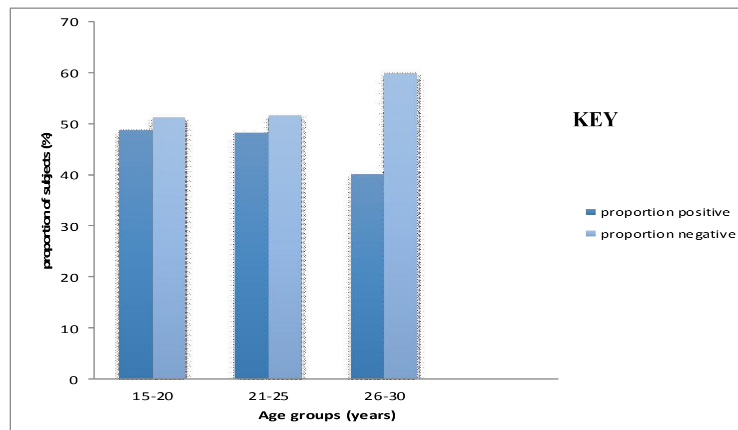

Figure 1: Frequency and distribution of *Plasmodium* parasitaemia in relation to age.

The results also showed highest prevalence (84.0%) of *Plasmodium* parasitaemia among subjects of HbAA genotype followed by subjects of HbAS genotype with prevalence of 16.0%. None of the subjects had HbSS or HbSC genotype (Table 2).

Table 2: Frequency distribution of *Plasmodium* species among subjects of different Haemoglobin genotypes

Genotype	% Distribution				Total (%)
	P. falciparum	*P. vivax*	*P. malariae*	*P. ovale*	
HbAA	68	3	8	5	84
HbAS	7	1	5	3	16
HbSS	0	0	0	0	0
HbSC	0	0	0	0	0
Total	**75**	**4**	**13**	**8**	**100**

Analysis of the results also showed highest frequency of *Plasmodium falciparum* (75.0%) among the subjects indicating that the bulk of the infection is as a result of this parasite. This is followed by *Plasmodium malariae* with frequency of 13.0%, while the least frequency (4.0%) was observed for *Plasmodium vivax* as shown in Figure 2.

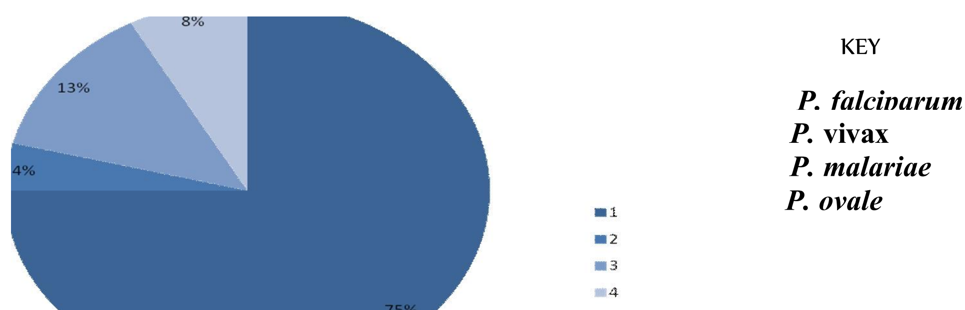

Figure 2: Frequency distribution of different *Plasmodium species* in the study population

4. DISCUSSION

Despite advances in the understanding of the pathogenic and clinical aspects of malaria, it is not well known why some people tolerate malaria infection with few or no symptoms whereas others are severely affected (Azeez and Raji, 2007). In this study, 50.0% of the 200 students of the University of Ilorin who were tested had *Plasmodium* parasitaemia yet they are asymptomatic. The result also shows a higher prevalence (56.9%) among the female students than the male students with prevalence of 42.9%. This finding is similar to Epidi *et al.* (2008) who reported a total prevalence of 51.5% among blood donors in Abakiliki, Southeastern Nigeria. Earlier, Eke *et al*. (2006) reported a slightly lower prevalence of 45.1% in a suburb of Aba Town, also in Nigeria. In a similar study conducted among first year students of Nnamdi Azikiwe University, Awka, Southeastern Nigeria, a prevalence of 80.0% was reported (Ibekwe *et al*., 2009). These findings further confirm the endemicity of this infection even among the students' population.

The higher prevalence (56.9%) observed for the females in this study is in agreement with findings of researchers who have observed a similar trend (Ibekwe *et al*., 2009; Okonko *et al*., 2009). Other investigators however, have found higher prevalence among the males than the females (Eke *et al*., 2006; Epidi *et al*., 2008; Ilozumba and Uzozie, 2009). However, there appears to be no scientific evidence linking malaria prevalence to gender.

Generally, our finding showed that the highest prevalence of malaria was in age group 15-20 years, followed by age group 21-25years while the least prevalence was among age group 26-30 years. This result agrees with the finding of Munyekenye *et al.* (2005) that parasite density falls as age increases suggesting age-dependent immunity to Plasmodium among adults.

The result also shows that 84.0% of the *Plasmodium* parasitaemia occurred among students

with haemoglobin genotype AA while 16.0% of the parasitaemia occurred among students with haemoglobin genotype AS. This result is in consonance with earlier finding by Akhigbe *et al.* (2011).

Analysis of the result also showed that *Plasmodium falciparum* has a prevalence of 75.0% thereby accounting for the bulk of the infection. The finding is consistent with report by other investigators in other locations and populations in Nigeria (Ahmed *et al*., 2001; Ibekwe *et al*., 2009; Ilozumba and Uzozie, 2009; Nebe *et al*., 2002). *Plasmodium malariae* is the second most prevalent species in our study population with a prevalence of 13.0%. This species was reported as the second most prevalent by Ibekwe *et al*. (2009) and third most prevalent by Ilozumba and Uzozie (2011). *Plasmodium ovale* is the third most prevalent in this study with a prevalence of 8.0% while the least prevalence (4.0%) was observed for *Plasmodium vivax*.

Immunity/tolerance to malaria parasitaemia does not occur naturally, but only in response to repeated infections with multiple strains of malaria, especially among adults in areas of moderate or intense transmission conditions (Farnert *et al.,* 2009; WHO, 2010). Detection of the parasite in asymptomatic subjects could be as a result of acquired immunity in the subjects due to repeated exposure to mosquito bites. The subjects included in this study are students who are undergoing medical screening at the University health center most of who must have come from rural areas of the country where the infection is highly endemic. Although levels of transmission in urban area may be lower than in contiguous rural areas, high population densities and possible lower immunity may result in more disease impact in urban setting (Klinkenberg *et al*., 2005). Therefore mixture of these subjects with the relatively unprotected people of Ilorin metropolis (urban area) could lead to more disease impact if the chain of transmission is not broken.

Vector control (reducing mosquito breeding grounds by spraying or destruction of habitat), use of insecticide-treated nets (ITN), indoor residual spraying, and targeted chemoprophylaxis for those most at high risk, e.g. pregnant women and travelers are therefore advocated.

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Effect of Harvest Period on Senescence and Grain Yield in Some Varieties of Cowpea (*Vigna Unguiculata* (L.) Walp)

[1]Aliko, A.A., [1]Mukhtar, F.B., [2]Aminu S.U., [3,4]Gashua, I.B.

[1]Department of Plant Biology, Bayero University, P.M.B. 3011, Kano - Nigeria.
[2]Jigawa State College of Education, P. M. B. 1002, Gumel – Nigeria
[3]School of Applied Sciences University of Wolverhampton. Wulfruna, WV1 1SB, UK.
[4]Department of Science Laboratory Technology, Federal Polytechnic Damaturu-Nigeria.
i.b.gashua@wlv.ac.uk

Abstract: Studies were conducted in the rainy and dry seasons of 2009 at the International Institute of Tropical Agriculture (IITA), Kano research station - Nigeria on four cowpea varieties namely: Kanannado, IT89KD-288, IT99K-82-2 and IT99K-1060 to assess the effect of different harvesting period on senescence and grain yield. Three harvesting periods - depodding at physiological maturity, deseeding at physiological maturity and depodding at agronomical maturity were adopted for this study. Data obtained on the progression of senescence showed significant difference among the different treatments. Senescence occur very late in all varieties given physiological maturity harvest treatment and greater yield was observed by delaying these varieties of cowpea to senesce. Average number of days to total death of plants in "Kanannado" harvested at physiological maturity was observed to be 138.83 days during the rainy season, while it was 124.67days in the same variety harvested at agronomical maturity. The mean number of pods was observed to be 13.20 in "Kanannado" harvested at physiological maturity which is greater than 6.83 observed in the same variety harvested at agronomical maturity during same season.
[Aliko, A.A., Mukhtar, F.B., Aminu S.U. and Gashua, I.B. **Effect of Time of Harvest on Senescence and Grain Yield in Some Varieties of Cowpea (*Vigna Unguiculata* (L.) Walp).** *Nat Sci* 2013;11(10):29-33]. ISSN: 1545-0740. http://www.sciencepub.net/nature. 6

Key word: Cowpea, Kanannado, Depodding, Agronomical maturity, Senescence.

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an important grain legume to millions of people living in the savannah regions of the tropical and subtropical Africa. It has the ability to fix atmospheric nitrogen which allows it to grow on soils that are deficient in plant nutrient. Cowpea is one of the principal food crops cultivated in the region and its importance is mainly attributed to the high protein content in its edible parts. According to Singh, (1997), Cowpea grain contains about 23 - 25% protein. This makes valuable in situation where people cannot afford other protein foods such as meat and fish. One understated physiological processe in which the reproductive activity of cowpea [*Vigna unguiculata* (L.) Walp] can be characterized is senescence, usually viewed as an internally programmed process that occurs in many different tissues and serves different purposes. Senescence is one of the causes of low yield in cowpea due to the photoperiodic effect, this is because the local varieties are short day plants and flowering is early in the dry season (Mukhtar, 2007) which leads to early death of the plants. Most cowpea plants die after producing the first flush of pods and this is most drastic in local cultivars which are photoperiod-sensitive (Ismail and Hall, 1998).

Senescence has been well authenticated and studied experimentally in cowpea by several workers including (Abdelbagi, et al., 2000). Workers on plant senescence had been determining the extent of senescence by visually examining the degree of chlorophyll loss (leaf yellowing) which gives an idea on the extent of actual cell death. In grain legumes, the extent of leaf senescence during podding varies among genotypes and may also be modified by sink reduction (Owen et al., 2007). Reproductive activity in cowpea [*Vigna unguiculata* (L.) Walp] is characterized by two separate flushes of pod production but many plants die after producing the first flush of pods. It was also observed by Ismail and Hall, (1998), that the second flush yields in cowpea is relative to the number of plants that survive to produce the second flush.

It is therefore necessary to adequately understand the phenomenon of senescence in cowpea so as to enable crop scientists develop strategies required to overcome its effect thereby increasing yield which will be of tremendous benefit especially in the cultivation of photoperiod-sensitive cowpeas during the dry season. This study was therefore, carried out to determine the effect of time of harvest on reproductive life span of the cowpea varieties with respect to onset and duration of senescence and yield.

2.0. Materials and Methods

2.1. Study material and experimental design

Seeds of four cowpea varieties namely "Kanannado" (local cultivar), IT89KD-288, IT90K-82-2 and IT99K-1060 (improved varieties) were obtained from the International Institute of Tropical Agriculture, Kano (located in North western Nigeria within Latitude $11^0$30' and longitude $8^0$30' E) and sown at a 2–3cm depth on prepared pots for both Rainy and Dry season trials. Pot preparation was done by mixing Sandy soil and manure of ratio 5:1 and stacked into planting pots of 250mm diameter for both experiments. The mixture was then watered and allowed for two days to saturate as recommended by nursery tending operations and establishment standard (Kano State Ministry of Environment, 2005) the experiment was laid in a Randomized Complete Block Design (RCBD) with six replications for each season to minimise error.

2.2. Agronomic practice

After Germination was observed (3 to 4 and 5 to 7 days for dry and rainy seasons trial respectively), plants were thinned to two plants per pot at two weeks after germination and were managed by daily watering (during dry season experiment only). Hand weeding was done using hand hoe on the emergence of weed so as to minimise nutrient competition. However, spraying with sherpa plus was done three times during the trial against attacking pest. This was done at bud initiation stage (at 35 -45 days after planting), beginning of pod initiation stage (50 – 60 days after planting) and at rapid pod filling stage (70 days after planting).

2.3. Data collection

Three (3) harvest treatments during the period of life cycle were adopted. (Harvest at physiological maturity, deseeding at physiological maturity and harvest at agronomic maturity). Pods sampled for physiological maturity harvest treatment were harvested from each of the cowpea varieties when they have attained physiological maturity but before they senesced and dried up. Samples for deseeding at physiological maturity harvest were extracted from the pods by dissecting one side of the pods using a scalpel. The pods were left on the plant and the seeds removed, then dried in the glass house which was estimated at eleven (11) days after pod initiation from trial experiment. Pods sampled for dry maturity harvest treatment were removed at agronomical maturity when the pods have senesced and dried which is the usual harvesting time of cowpea. Observations were also done on days to onset of senescence and days to total senescence when the leaf colour changed completely to yellow due to chlorophyll loss on the leaves. Chlorophyll content was quantified for the study using a Minolta spad 502plus meter made by Spectrum Technologies, Inc. at 3, 5, 7, 8, 9 and 11 weeks after planting.

The data recorded during the trial were statistically analysed according to Snedecor and Cochran, (1980). Least significant difference (LSD) was used to separated the means and declared significant at $p<0.05$.

3. Results and Discussion

Senescence with regards to its onset and progression was significantly affected by harvesting period in both the rainy and dry season trials, but was found to be pronounced during the rainy season. Senescence progressed at faster rate in all the varieties harvested at agronomical (dry) maturity. Thus, duration to 50% and 90% senescence was extended in varieties harvested at physiological (green) maturity and those de-seeded (Table1 and 2). It can therefore be explained that plants harvested at agronomical (dry) maturity had continued to utilize the plant nutrients from the source (leaves) thereby exhausting the essential nutrients for the second flush of pods. Whereas assimilates left in the leaves of the plants harvested at physiological (green) maturity and those de-seeded were utilized by the plants for second flush of pods. Hence, there were two harvests in those two treatments which tremendously raised the number of pods and grain yield.

There was significant difference with regards to the number of pods per plant in both rainy and dry season trials. Pod length measurements during the rainy season planting varied. Pods were longer in "Kanannado" and IT99K-82-2 given agronomical maturity harvesting treatment but in IT89KD-288, it was longer in plants de-seeded at green mature while in IT99K-1060 were longer in plants harvested at physiological maturity (Table 3). For dry season, pods harvested at physiological maturity were observed to be longer except in IT99K-1060 but shorter in varieties given agronomical maturity harvest treatment (Table 4). However, number of pods per plant was observed to be greater in all varieties harvested at physiological maturity while plants harvested at agronomical maturity were observed to produce least number of pods across all trials. Khanna-Chopra and Reddy (1988) found that regulation of leaf senescence by reducing the reproductive sink intensity suggests the involvement of senescence signal from the developing seeds to the leaf. Removing the pod at physiological maturity may allow the plants a better survival strategy, since the plant can put most of its accumulated energy and resources into seed production rather than saving some for the plant to overwinter, which would limit

seed production (Lawton, et al., 1990; Nooden et al., 1997). Increased number and weight (g) of seeds observed in plants harvested at physiological maturity could be associated with the number of pods which were greater in this treatment resulting from the removal of the matured pods. Senescence was delayed in varieties given physiological maturity harvest treatment but earlier in varieties harvested at agronomic maturity. Therefore, grain yield was improved upon physiological maturity harvest and deseeding treatments in relation to the delay in progression of senescence. This improvement can be seen in both photoperiod sensitive and photoperiod insensitive varieties with greater yield in the rainy season trial.

Conclusion

Findings of this study revealed that, plants harvested at agronomical maturity had lower chlorophyll content at maturity and senesced earlier. Senescence was greatly delayed by removing green mature pods as well as seeds in cowpea, and the delay significantly improved grains production by allowing second flush of pods. This would be very valuable to farmers as one of the means to control senescence and thus, maximise cowpea grains' yield. This idea can be exploited for all varieties of cowpea during rainy or dry season of a year based on findings of this trial.

Table 1. Effect of Harvesting Treatments on the Onset and Progression of Senescence in Four Cowpea Varieties Grown in 2009 Rainy Season

Variety	Treatment	Days to 50% Senescence	Days to 90% Senescence	Days to Total Death of Plant
A. Kanannado				
	P.M.H	114.50	138.83	138.83
	A.M.H	109.17	121.00	124.67
	D.S.H	113.17	131.50	135.67
	Mean	112.28	129.11	133.06
	LSD	1.88	3.03	3.07
B. T89KD-288				
	P.M.H	114.00	134.00	138.83
	A.M.H	108.17	118.33	121.83
	D.S.H	115.00	134.33	139.33
	Mean	112.39	128.89	133.33
	LSD	2.16	3.41	3.56
C. IT99K-82-2				
	P.M.H	74.50	96.83	100.17
	A.M.H	65.83	75.83	78.00
	D.S.H	69.17	92.83	95.67
	Mean	69.83	88.50	91.28
	LSD			
D. IT99K-1060				
	P.M.H	70.17	95.67	98.33
	A.M.H	64.33	72.50	75.50
	D.S.H	70.33	91.17	94.33
	Mean	68.28	86.45	89.39
	LSD	2.08	3.95	3.93

P.M.H= Physiological Maturity Harvest, A.M.H= Agronomical Maturity Harvest, D.S.H=De-Seeding Harvest, LSD=Least Significant Differences $p<0.05$.

Table 2. Effect of Harvesting Treatments on the Onset and Progression of Senescence in Four Cowpea Varieties Grown in 2009 Dry Season

Variety	Treatment	Days to 50% Senescence	Days to 90% Senescence	Days to Total Death of Plant
A. Kanannado				
	P.M.H	73.17	83.00	87.50
	A.M.H	69.33	75.33	79.83
	D.S.H	72.67	83.67	87.50
	Mean	71.72	80.67	84.94
	LSD	1.63	2.43	2.37
B. T89KD-288				
	P.M.H	74.17	83.67	88.00
	A.M.H	70.67	76.17	81.67
	D.S.H	74.00	82.83	87.17
	Mean	72.95	80.89	85.61
	LSD	1.58	2.28	2.09
C. IT99K-82-2				
	P.M.H	79.00	87.00	91.17
	A.M.H	76.67	81.33	85.50
	D.S.H	80.00	88.17	91.33
	Mean	78.56	85.50	89.33
	LSD	1.47	2.16	2.05
D. IT99K-1060				
	P.M.H	71.17	80.67	85.17
	A.M.H	68.33	73.17	78.17
	D.S.H	72.50	81.00	86.83
	Mean	70.67	78.28	83.39
	LSD	1.64	2.37	2.42

P.M.H= Physiological Maturity Harvest, A.M.H= Agronomical Maturity Harvest, D.S.H=De-Seeding Harvest, LSD=Least Significant Differences.

Table 3. Effect of Harvesting Treatments on Yield Attributes of Four Cowpea Varieties Grown in 2009 Rainy Season

Variety	Treatment	No. of Pods/Plant	Pod Length (cm)	Seed Weight/Pod (g)	100 Seed Weight (g)	Dry Matter Content (g)
A. Kanannado						
	P.M.H	13.67	11.38	9.83	15.10	44.22
	A.M.H	6.83	12.47	6.35	16.20	38.67
	D.S.H	13.17	11.73	9.36	14.10	39.88
	Mean	11.22	11.86	8.51	15.13	40.92
	LSD	2.20	0.84	1.55	1.15	1.93
B. T89KD-288						
	P.M.H	13.83	12.02	9.83	14.10	36.10
	A.M.H	7.67	11.97	6.18	15.20	42.37
	D.S.H	13.50	12.18	10.00	15.10	43.28
	Mean	11.67	12.06	8.67	14.80	40.58
	LSD	2.20	1.97	1.66	0.88	2.23
C. IT99K-82-2						
	P.M.H	8.83	10.83	5.78	12.02	15.18
	A.M.H	3.17	12.13	2.57	14.05	17.10
	D.S.H	5.67	11.53	4.76	12.02	18.63
	Mean	5.89	11.50	4.37	12.70	16.97
	LSD	1.90	0.91	1.44	1.22	1.48
D. IT99K-1060						
	P.M.H	12.17	10.87	7.06	11.02	14.28
	A.M.H	4.50	10.75	3.18	12.06	12.40
	D.S.H	8.50	10.83	6.24	13.03	14.00
	Mean	8.39	10.82	5.49	12.04	13.56
	LSD	2.21	0.42	1.61	1.13	1.13

P.M.H= Physiological Maturity Harvest, A.M.H= Agronomical Maturity Harvest, D.S.H=De-Seeding Harvest, LSD=Least Significant Differences.

Table 4. Effect of Harvesting Treatments on Yield Attributes of Four Cowpea Varieties Grown in 2009 Dry Season

Variety	Treatment	No. of Pods/Plant	Pod Length (cm)	Seed Weight/Pod (g)	100 Seed Weight (g)	Dry Matter Content (g)
A. Kanannado						
	P.M.H	5.33	9.80	3.26	13.80	14.90
	A.M.H	3.50	8.93	3.48	213.60	11.30
	D.S.H	4.33	8.98	3.77	14.30	15.00
	Mean	4.39	9.24	3.50	13.90	13.73
	LSD	1.07	0.79	0.01	1.81	1.64
B. T89KD-288						
	P.M.H	3.83	11.83	3.55	15.00	20.10
	A.M.H	2.83	10.58	2.85	14.00	22.10
	D.S.H	4.00	10.73	3.07	13.50	20.80
	Mean	3.55	11.05	3.16	14.17	21.00
	LSD	0.89	0.93	0.67	0.98	1.14
C. IT99K-82-2						
	P.M.H	4.33	10.98	3.25	14.90	22.60
	A.M.H	3.00	10.17	2.82	14.40	13.00
	D.S.H	4.33	10.47	3.03	14.60	11.80
	Mean	3.89	10.54	3.03	14.63	15.80
	LSD	0.99	0.72	0.09	0.11	2.74
D. IT99K-1060						
	P.M.H	4.50	9.75	3.24	13.80	14.90
	A.M.H	3.50	9.53	2.71	13.60	11.30
	D.S.H	3.50	10.02	3.50	13.90	15.00
	Mean	3.83	9.77	3.15	13.77	13.73
	LSD	0.83	0.33	0.72	0.40	1.64

P.M.H= Physiological Maturity Harvest, A.M.H= Agronomical Maturity Harvest, D.S.H=De-Seeding Harvest, LSD= Least Significance Differences.

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Epidemiological studies of abomasal nematodes of sheep of Kashmir Valley with particular reference to *Haemonchus contortus.*

Irfan-ur-Rauf Tak[1], M. Z. Chishti[1] and Fayaz Ahmad[2]

[1] Centre of Research for Development, University of Kashmir, Srinagar – 190 006
[2] Department of Zoology, University of Kashmir, Srinagar-190 006, Kashmir
irfanrauftak@yahoo.in

Abstract: The valley of Kashmir owes its great excellence not only to the charming scenic beauty but is bestowed with rich flora and fauna present in wide variety and diversity. Sheep are among those animals which were first tamed by man. Sheep being a close grazer is regarded as museum of parasites especially for helminths. *Haemonchus contortus* is a blood sucking intestinal helminth that lives in the abomasum of small ruminants worldwide. This parasite can be devastating to producers as it causes decreased production levels due to clinical signs such as anaemia, edema and death. The abomasae of sheep in which this parasite resides were collected from abattoirs of various districts during the study of one year from November, 2011 to December, 2012 and were then carried to laboratory for screening. In case of collection sites falling in far areas, the organs were screened on spot. The parasites were placed in petridish containing 0.05M PBS (pH 7.4) for initial washing to remove host material and allow regurgitation of gut contents. The length and width of each parasite was measured and segregated into *Haemonchus contortus* based on standard body lengths: *Haemonchus contortus*: female (18 to 30 mm), male (10 to 20 mm). The regular record of the entire process was properly maintained. During the study period, a total of 310 sheep abomasum were examined, out of which 198 (**63.87%**) were found to be infected. Of these, 191 **(61.61%)** were found to be infected with *H. contortus* and 112 **(36.12%)** were found to possess mixed infection. The infection was found highest in summer **(80.80%)** and lowest in winter **(37.5%) (P<0.05).** The prevalence of the parasite was highest in lower age groups (**78.35%**) and lowest in higher age groups (**39.65%**) (**P<0.05**). The males (**63.03%**) showed significantly higher prevalence as compared to females (**60.00%**) (**P>0.05**). The study indicates the prevalence of *Haemonchus contortus* varies in different seasons and in different age groups.

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Keywords: *Haemonchus contortus*; Helminths; Abomasum; PBS.

1. Introduction

The state of Jammu and Kashmir is strategically located on the northern most part of India. It is geographically located between 32° 17′ and 36° 58′ northern latitude and 37° 26′ and 80° 30′ eastern longitudes. In Jammu and Kashmir, livestock activity has a contribution of about 11% in the Gross Domestic Product of the state as per Integrated Sample Survey (ISS) report 2007-08. The total livestock population as per census 2003 was 98.993 lakhs out of which sheep were 34.107 lakhs and goats were 20.549 lakhs together constituting almost 50% of the total livestock (17th Indian Livestock Census, Jammu and Kashmir, 2003). The sheep plays a significant role in national economy and rural socioeconomic conditions in the country. The overall development of the rural hilly areas could not be achieved by neglecting the development of the agricultural commodities like sheep and goats. Helminths play an important role in decreasing the sheep production in the world. Sheep have numerous gastrointestinal helminth parasites. The prevalence of gastrointestinal nematode infection is very high in Kashmir valley.

Haemonchus contortus (Rudolphi, 1803) Cobb, 1898 is a blood sucking intestinal helminth that lives in the abomasum of small ruminants worldwide. This parasite can be devastating to producers as it causes decreased production levels due to clinical signs such as anaemia, edema and death. Economic losses are especially increased in tropical and subtropical regions where *H. contortus* thrives and consumption of goat meat is higher than other food animals. Control programmes in the past included pasture management strategies combined with intensive anthelmintic treatment and prophylaxis which were effective in reducing losses of meat and wool in sheep and goats. There are anthelmentics still available but multiple drug resistant *H. contortus* strains have quickly developed and producers and veterinarians are now faced with seeking alternative methods of treatment and prevention (Sangster, 1999; Miller *et al*., 1987 and 1994; Jackson *et al*., 2001; Terrill *et al*., 2001). The principal aim of the present study was to investigate the prevalence of

Haemonchus contortus and to identify its diversity in sheep of Kashmir Valley.

2. Material and Methods

Naturally infected guts were obtained from slaughtered sheep on the day of slaughter from local slaughterhouses in particularly three districts namely Anantnag, Pulwama and Srinagar of Jammu and Kashmir. Guts were examined thoroughly especially the abomasum part and nematode particularly *Haemonchus contortus* was collected and placed in petridish containing 0.05M PBS (pH 7.4) for initial washing to remove host material and allow regurgitation of gut contents. The length and width of each nematode was measured and segregated into *Haemonchus contortus* based on standard body lengths of adult nematode: *Haemonchus contortus*: female (18 to 30 mm), male (10 to 20 mm) and general morphology (Soulsby).

2.1. Determination of prevalence

During the collection period, the season of the collection, age of the host as well as the gender of the host was noted down. After the collection was complete, the prevalence of was calculated as given here under:

2.1.1. Prevalence

The prevalence of infection of any parasite indicates the percentage of the hosts infected by the parasite among the ones observed for the infection. The prevalence can be recorded in different ways depending upon the season, age and gender of the host.

Prevalence = Number of infected specimens ×100
Number of observed specimens

Prevalence is the percentile representation of infected hosts divided by hosts examined multiplied by 100.

2.1.2. Seasonal prevalence

The season has a marked influence on the prevalence of infection caused by any parasite. The components of the season like temperature, humidity *etc*. determine the abundance of the parasites in the host. The seasonal prevalence was calculated by the formula as:

Number of infected hosts in a particular season ×100 Number of hosts observed in that season

2.1.3. Age-wise prevalence

The age has also been reported to influence the prevalence of the parasites in the host because of the resistance/immunity present in some age group or the preference of the parasite to a particular age group over the other. The age wise prevalence was calculated by the formula as:

Number of infected hosts of a particular age group served number of hosts of that age group×100

2.1.4. Gender-wise prevalence

The gender of the host may also sometimes affect the abundance of the parasites present in the host. This probably may be due to the different types of hormones secreted by the male and female individuals. The gender wise prevalence was calculated as per the given formula:

Number of infected hosts of a particular genderObserved number of hosts of that gender×100

3. Results and Discussion

3.1. Epidemiology of *H. contortus* in sheep

During the present study of one year from November, 2011 to December, 2012, abattoirs of different districts were surveyed and regular record of the entire process was properly maintained.

3.1.1. Overall prevalence

A total of 310 sheep abomasum were examined of which 198 (**63.87%**) were found to be infected. Out of these, 191 were found to be infected with *Haemonchus contortus* and 112 **(36.12%)** were found to have the mixed infection. The number of individuals of the parasite found varied from individual to individual. **Thus the overall prevalence of *Haemonchus contortus* in sheep was found out to be 61.61% (Figure 1).**

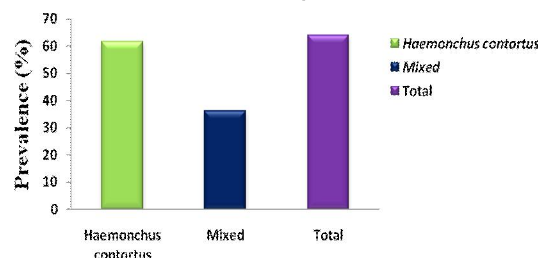

Figure 1. Showing overall prevalence of *H. contortus* in sheep of Kashmir Valley

Raza *et al.* (2009) recorded 37.18% prevalence of *H. contortus* in sheep and 31.10% prevelence in goats; Durrani and Hayat (1964) recorded prevalence percentage of haemonchosis as 32.40% in sheep and goats; Vercruysse (1985) reported 78% prevalence of *H. contortus* in sheep; Tariq *et al.* (2008) recorded 38.0% prevalence of *O. circumcincta* and 59.6% prevalence of *H. contortus;* Pal and Qayuum (1992) found *H. contortus* to be the most prevalent among the helminthes recovered and recorded 90.43% prevalence; Maqsood *et al.* (1996) reported 65.2 % and 47.1% prevalence of haemonchosis in sheep and goats, respectively; Jabeen *et al.* (2000) recorded overall infection of *H. contortus* as 54.77% in sheep; Tariq *et al.* (2003) reported 38% prevalence for haemonchosis; Lone *et al.* (2012) reported nematodes of which prevalent were *Haemonchus* (82%), *Trichuris* (74%), *Nematodirus* (60%), *Trichostrongylus* (58%),

Chabertia (52%), *Strongyloides* (42%), *Oesophagostomum* (46%).

The present results are in accordance with **Vercruysse (1985), Tariq *et al.* (2008), Maqsood *et al.* (1996), Lone *et al.* (2012), Jabeen *et al.* (2000)**, who observed almost similar prevalence percentages of the two parasites as recorded in the present study. The differences in the prevalence percentages between the present study and many of the above mentioned workers may probably be due to the environmental conditions present at the collection places, overall climate of the valley, different hosts or different breeds of same host and also to the low frequency of intermediate hosts.

3.1.2. Seasonal prevalence of *H. contortus*

The study revealed seasonality of infection showing highest prevalence of infection in summer and lowest in winter. The prevalence in spring and autumn was found to be falling in between summer and winter seasons. During winter months, 27 sheep were found infected out of 72 examined giving the prevalence of **37.5%**. During the spring season, a total of 87 sheep were examined and out of these 62 were infected showing prevalence of **71.26%.** During the summer season, a total of 99 sheep were examined and 80 were infected showing prevalence of **80.80%**. In autumn season, 29 sheep were found to be infected out of 52 examined, giving prevalence of **55.76%. Thus the prevalence was recorded highest (80.80%) in summer followed by spring (71.26%) then by autumn (55.76%) and lowest (37.5%) in winter. (Figure 2 and Table 1).** By using Chi Square test, **(P=0.03),** which means data is statistically significant (P<0.05). The highest incidence of infection during summer and spring may be correlated with the seasonal/climatic pattern and conditions. These seasons provide optimum conditions for the herbage growth and the necessary moisture for the optimum development of the parasites. The rainy season that starts in spring and early summer in valley makes the environmental conditions more favourable for the development and survival of pre-parasitic stages and causes increased availability of infective larvae in the rainy and post rainy season. The hot and humid weather provides favourable condition for the development and survival of exogenous stages of *H. contortus* (Kates, 1950). Lower prevalence percentages in the winter may be because in winter, the temperature is low and atmosphere is dry which might have inhibited the development of eggs and larvae. Besides weather conditions, self cure phenomenon may also be the reason for the decrease in infection during colder months.

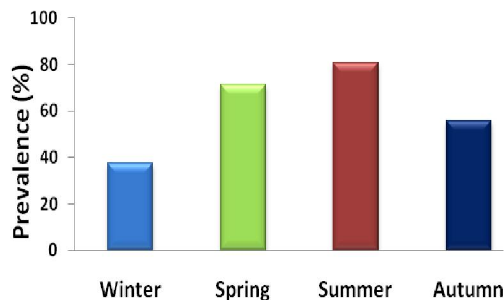

Figure 2. Showing prevalence of *H. contortus* in sheep in different seasons of Kashmir Valley

Makhdoomi *et al*. (1995) observed highest infection of *H. contortus* during summer (82.27%) and lowest (44.23%) during winter season; Jabeen *et al*. (2000) recorded highest prevalence during summer (89.55%) and lowest during winter (20.02%); Nasreen *et al.* (2005) also observed the highest infection (33.18%) in summer and lowest (15.25%) in winter in case of *H. contortus;* Lone *et al.* (2012) reported 40% of Helminth infections in spring followed by 74% in summer, 51% in autumn and 18% in winter in sheep; Tariq *et al*. (2008) recorded highest infection in summer and lowest in winter; Similar results were obtained by Mbuh *et al*. (2008) and Rahman *et al*. (2012), who observed highest prevalence in summer. The present results are in accordance with **Makhdoomi *et al*. (1995), Jabeen *et al.* (2000), Tariq *et al.* (2008), Lone *et al.* (2012),** who reported almost similar prevalence percentages as recorded in the present study.

3.1.3. Age-wise prevalence of *H. contortus*

Table-1. Showing prevalence of *H. contortus* in sheep in different seasons of Kashmir Valley

Seasons	**Number Examined**	**Positive**	**Prevalence (%)**
Winter	72	27	37.5%
Spring	87	62	71.26%
Summer	99	80	80.80%
Autumn	52	29	55.76%
Total	**310**	**198**	**63.87%**

P<0.05

The 310 abomasae were collected for parasite screenings from animals of different age groups. The age groups selected were - <1 year, 1-2 years, 2-3 and >4 years. 97 lambs having age less than 1 year were examined out of which 76 were found to be infected with *H. contortus* showing prevalence of **78.35%** and 61 were found to have mixed infection showing prevalence of **62.88%.** In age group of 1-2 years, 83 were examined out of which 55 were found to be infected with *H. contortus* showing the prevalence of **66.26%** and 42 were having mixed infection showing prevalence of **50.60%.** Similarly in age group of 2-3 years, 72

abomasae were examined out of which 37 were found to be infected with *H. contortus* and 18 were found to have mixed infection showing prevalence of **51.38%** and **25.00%** respectively. At last in age group of >4 years, 58 abomasae were examined, out of which 23 were found to be infected with *H. contortus* showing prevalence of **39.65%** and 7 were found to have mixed infection showing prevalence of **12.06%. Thus, the prevalence was found highest in age group <1 year followed by age group 1-2 years and then by age group of 2-3 years and least prevalence was found in age group >4 years (Figure 3 and Table 2).** By using Chi Square test, **(P=0.001),** which means data is statistically significant (P<0.05).

Table-2. Showing prevalence of *H. contortus* in sheep of different age groups of Kashmir Valley

Age Group	Number Examined	Infected	
		***H. contortus* (%)**	**Mixed (%)**
<1	97	76(78.35%)	61(62.88%)
1-2	83	55(66.26%)	42(50.60%)
2-3	72	37(51.38%)	18(25.00%)
>4	58	23(39.65%)	7(12.06%)
Total	**310**	**191 (61.61%)**	**128 (41.29%)**

P<0.05

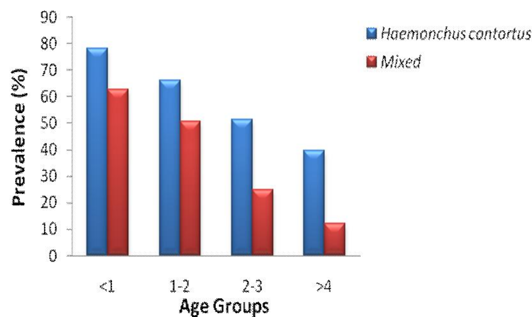

Figure 3. Showing prevalence of *H. contortus* in sheep of different age groups of Kashmir Valley

The lower age groups of animals found to be more infected is because of the high susceptibility and low resistance found in them. The lower levels of infection reported in adult sheep is because of the development of significant immune capability, which increases with the duration of exposure to infection (Ahmad *et al*., 2007).

Biu *et al.* (2009) reported 35% GI parasite infection in younger ones (Age 1-2.5) and 19% prevalence in older ones (3-4); The high rate of infection with *H. contortus* in young lambs has also been observed by Maqsood *et al*. (1996) who reported it as 67.1% in lambs of less than two years age and 40.4% in sheep of more than two years age; Qamar *et al.* (2009) also reported in case of haemonchosis that the infection in sheep was higher below 9 months (46.43%) than above 9 months (34.48%) and similarly in goats the rate of prevalence was higher below 9 months (44.44%) than above 9 months (31.84%); Lone *et al.* (2011) also reported 49% prevalence of nematodes in 2-4 month goats followed by 58% in 5-12 months and 34% in >1 year of age; Lone *et al.* (2012) also reported 94.73% and 97.77% prevalence of helminth parasites in 0-1 year age group in sheep and goats respectively and 29.41% and 51.28% in older age group in sheep and goats respectively; **Thus the present results are in conformity with the studies carried out by earlier workers.**

3.1.4. Gender-wise prevalence of *H. contortus*

The total 310 examined organs were taken from both the genders. 165 organs were taken from male specimens of which 104 were found to be infected with *H. contortus* showing a prevalence of **63.03%** and 69 were found to have mixed infection showing a prevalence of **41.81%.** In case of females, 145 organs were taken out of which 87 were found to be infected with *H. contortus* and 59 were having mixed infection showing prevalence of **60.00%** and **40.68%** respectively. **Thus the infection was found little higher in males as compared to the females (Figure 4 and Table 3).** By using Chi Square test, **(P=0.9),** which means data is statistically insignificant.

Table-3. Showing prevalence of *H. contortus* in male and female sheep of Kashmir Valley

Gender	Number Examined	Infected	
		***H. contortus* (%)**	**Mixed (%)**
Male	165	104(63.03%)	69(41.81%)
Female	145	87(60.00%)	59 (40.68%)
Total	**310**	**191(61.61%**	**128(41.29%)**

P>0.05

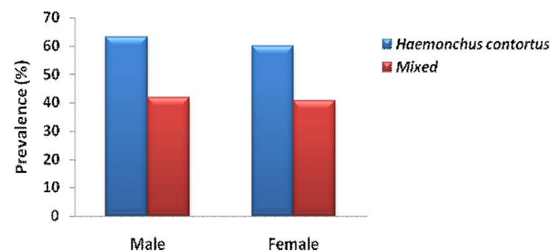

Figure 4. Showing prevalence of *H. contortus* in male and female sheep of Kashmir Valley

The influence of gender on the susceptibility of animals to parasitic infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control. Management and climatic conditions also have a greater role to play in the onset of infections.

Gorski *et al*. (2004) reported that males were more infected with nematode species than females; Tariq *et al.* (2010) reported 57.8% prevalence of nematodes in males as compared to 52.7% in

females; Gulland and Fox (1992); Gauly *et al.* (2006); Ahmad *et al*. (2007) and Tariq *et al*. (2008) also observed a little higher percentage prevalence of *H. contortus* in males than female sheep. Tariq *et al*. (2003); Qamar *et al*. (2009) recorded no significant difference in infection percentage between males and females. Lone *et al.* (2011) also reported 42.5% prevalence of nematodes in males and 57.2% in females in case of goats; Raza *et al.* (2009) also recorded 34.11% prevalence of *H. contortus* in males and 39.22% in case of females in sheep while 29.91% in males and 31.90% in females in case of goats; Javed *et al.* (1992); Maqsood *et al*. (1996) and Khan *et al*. (2010) observed more infection in females than males. Therefore, it seems that both sexes are equally susceptible to nematode infection and the differences reported could be the effect of management conditions of the host animals and also may be due to differences in sample size.

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Corresponding author: Irfan-ur-Rauf Tak
Research Scholar
Parasitology Research Lab
Centre of Research for Development,
University of Kashmir-190006
Email**:** irfanrauftak@yahoo.in

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7/25/2013

IN-VITRO STUDY OF BIODEGRADATION OF SPENT LUBRICATING OIL BY *ASPERGILLUS NIGER*

[1*]Stephen, E., Emmanuel, O.E., Okpanachi, O.S., Emmanuel, S., Temola, O.T., Musa, K. and Ebiloma, I.P[2]

1. Department of Microbiology, Kogi State University, Anyigba, Nigeria
2. Department of Science, Kogi State Polytechnic, Lokoja, Nigeria

psychsea07@gmail.com

Abstract: Biodegradation of spent lubricating oil by *Aspergillus niger* was studied *in vitro* for 16 days. The pH, turbidity, nitrate and gas chromatographic analysis (GC-MS) of the medium was carried out. The result showed increase in pH and turbidity in the course of the study while nitrate concentration declined over the same period. There were significant differences ($p<0.05$) in pH, turbidity and nitrate concentrations between the controls and the inoculated samples. The GC-MS revealed that alkanes were degraded into carboxylic acids while benzene and azulene were not degraded by *Aspergillus niger*. This study suggests that *Aspergillus niger* can grow and metabolize some compounds in spent lubricating oil.

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Key words: Biodegradation, In-Vitro, Aspergillus niger, Turbidity, GC-MS

1. Introduction

Biodegradation refers to biological activities resulting in the breakdown of a compound (Ismail, 2008). Biodegradation of complex molecules usually involves the interactive effort of mixed populations of microorganisms and relies on the metabolic versatility of bacteria and fungi and the rate of degradation depends on the composition of the molecules (George and Metting, 1993). According to Hawrot and Nowak (2006), biological degradation of hydrocarbons in the environment is linked to a number of physical and chemical factors including the concentration and chemical structure of contaminant, moisture, oxygen, temperature and pH.

The rate and efficiency of biodegradation depends on the occurrence of adequately numerous and active microflora in the contaminated environment (Hawrot and Nowak, 2006, Anene and Chika, 2011). Organisms that have been reported to be capable of degrading hydrocarbons include *Pseudomonas aeruginosa, Bacillus subtilis, Alcaligenes, Acinetobacter iwoffi, Flavobacterium* spp, *Micrococcus roseus, Cornybacterium, Trichoderma* spp, *Candida* spp, *Aspergillus* spp, *Rhizopus* spp (Anene and Chika, 2011, Khaled et al., 2012). *Aspergillus* spp especially *A. niger* and *A. flavus* have been reported as fungi that degrades hydrocarbons. Kuiper et al. (2004), Okereke et al. (2007), Chikere et al. (2009) reported the presence of *A. niger, A. flavus* and *A. fumigatus* in oil spilled site and their crude oil degradation abilities. Watkinson and Morgan (1990) reported that *Aspergillus* spp are capable of initiating the degradation of n-alkanes by sub-terminal oxidation, hence their relative abundance in soil polluted by hydrocarbons.

Spent lubricating oil refers to any lubricating oil that has served its service properties and considered not fit for its initial purpose (Abdulhadi and Kawo, 2006). Khaled et al. (2012) reported that huge amount of spent lubricating oils are produced world wide. All types of lubricants become contaminated and lose their performance due to changes in some of their properties (Shakirullah et al., 2006). Hertzman et al. (1985) reported that 600,000 tons of lubricant is lost to the environment annually. This may constitute serious environmental hazard to the environment and also a potential hazard to the long-term health status of the population (Wright et al., 1993).

Some compounds in hydrocarbons may not be degraded by organisms (Atlas and Brag, 2009). Others may be degraded and broken down into carbon dioxide, water and cell mass (fatty acids) (Anene and Chika, 2011) while others may be transformed into other compounds. Hence, this study was undertaken to determine the potential of *Aspergillus niger* to degrade spent lubricating oil alone and the products of the degradation.

2.Materials and methods

Collection of isolate and lubricating oil:

Aspergillus niger was collected from stock culture from the Department of Microbiology, Kogi State University Anyigba while spent lubricating oil was collected from the mechanic workshop opposite First city Monument Bank, Anyigba, Nigeria. *Aspergillus niger* was inoculated into peptone broth for 24 hours. Mineral Salt Medium containing 2.0g of Na_2HPO_4, 0.17g of K_2SO_4, 4.0g of NH_4NO_3, 0.53g of KH_2PO_4, 0.10g of $MgSO_4.7H_2O$ was prepared in

1000ml of distilled water. 10 ml of Mineral Salt Medium was dispensed into ten test tubes. 2ml of spent lubricating oil was added and the solution sterilized by autoclaving. 2ml of overnight broth culture (peptone broth) of *Aspergillus niger* was seeded into five test tubes while the remaining five without *Aspergillus niger* served as the control. The test tubes were incubated at ambient temperature for 16 days without shaking. Degradation of the spent lubricating oil was monitored at 4 days interval for 16 days. Growth pattern of *Aspergillus niger* was determined by measuring the turbidity using turbidity meter (WGZ-113 Shanghi, China). pH was determined at ambient temperature using glass electrode pH and conductivity meter (Hannia, Italy). Nitrogen was determined by the micro Kjedahl method as described by Ibitoye (2006).

Gas Chromatography-Mass Spectrophometry:

The Gas Chromatographic –Mass Spectrophotometric analysis was carried out at day 0 (for control) and days 7 and 14 (for spent lubricating oil inoculated with *Aspergillus niger*). The mineral salt medium containing spent lubricating oil and *Aspergillus niger* was decanted into a 50 ml beaker using Whatmann filter paper. The oil on the filter paper was recovered by rinsing with 25ml of carbon trichloromethane (chloroform) in another 50ml beaker. The oil was placed in a water bath for 20 minutes to evaporate the solvent. The oil was then analysed using gas-liquid chromatography- mass spectrophotometer (GCMS Qp2010 plus, Shimadzu, Japan).

Statistical analysis:

Data obtained was subjected to T-test using MINITAB 14 Statistical software. Experimental precision achieved was reported at $p \leq 0.05$ level.

3.Results

The change in pH of the spent lubricating oil is shown in figure 1. Higher pH was observed in *Aspergillus niger* (ASP) inoculated medium than the control after four days. This continued till the end of the study. Significant difference ($p > 0.05$) was observed in the pH between the control (C) and the ASP medium.

The turbidity of the ASP medium was higher than that of the control (figure 2). The turbidity increased gradually after day 0 until it reached its peak at day 16. There was significant difference in the turbidity between the control and the ASP medium.

Nitrate utilization was profound in inoculated spent lubricating oil than the control (Figure 3). The nitrate level decreased greatly after 4 days. This decreased continued until day 16. Significant difference ($p<0.05$) was observed in the nitrate levels between the control and the inoculated sample.

Figure 4 shows the gas chromatographic tracing of the uninoculated spent lubricating oil (control). The compounds present were methylbenzene, ethylbenzene, o-xylene, propylbenzene, octane, pentadecane and hexane. The chromatogram showed that spent lubricating oil had more aromatic and cycloalkanes than straight chain alkanes.

Figure 5 shows the chromatogram of the spent lubricating oil inoculated with *Aspergillus niger* after one week (7 days). The branched aromatic compounds were degraded after one week. There was a reduction in the peak heights after one week. New compounds such as azulene (bicyclo (5,3,0) decapentene) was introduced as a result of the metabolism of the spent oil by *Aspergillus niger*.

The chromatogram of the inoculated spent oil after 14 days is shown in figure 6. The peak heights and compounds were further reduced compared to figures 4 and 5. Organic acids were more in figure 6 than 5. The compounds remaining in the spent lubricating oil after 14 days were benzene, azulene, dodecane, tetradecane, heptadecane, hexadecanoic acid, 9-octadecanoic acid and octadecanoic acid.

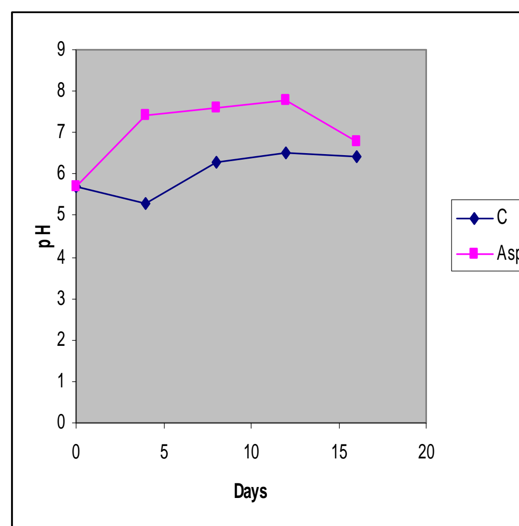

Fig 1: pH of spent lubricating oil undergoing biodegradation
C: control, Asp: *Aspergillus niger*

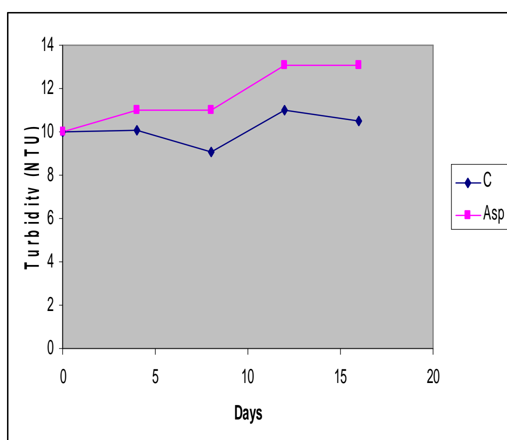

Fig 2. Growth pattern of *Aspergillus niger* in spent lubricating oil undergoing biodegradation
C: control, Asp: *Aspergillus niger*

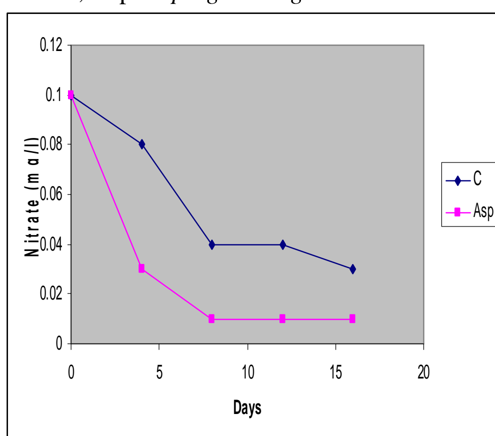

Fig 3:Nitrate utilization by *Aspergillus niger* in spent lubricating oil undergoing biodegradation
C: control, Asp: *Aspergillus niger*

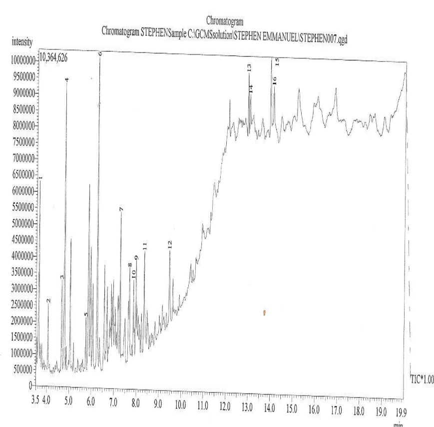

Fig 4: Gas Chromatographic analysis of Spent Lubricating Oil, SLO.

Peak sequences: 1: methylbenzene 2: octane 3: ethylbenzene 4: o-xylene 5: propylbenzene 6: ethylbenzene 7: benzene 8: 1-bromomethyl-4-isopropylbenzene 9: methyl-p-ethyltoluene 10: 1-phenyl-1-butene 11: cyclopentacycloheptene 12: 1,6-methanol 13: pentadecane 14: pentadecane 15: Hexadecane 16: hexadecane

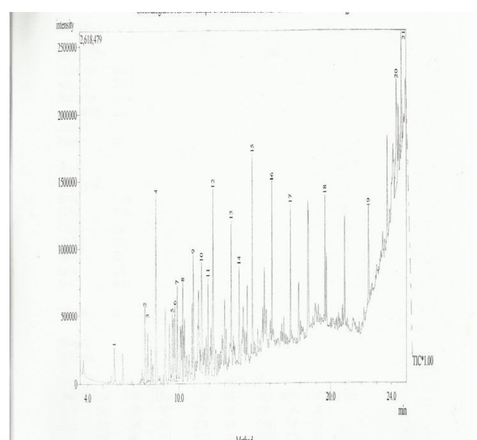

Fig 5: Gas chromatographic analysis of spent lubricating oil degraded by *Aspergillus niger* after one week.

Peak sequences: 1: benzene 2: benzene3: benzene 4: benzene 5: benzene 6: benzene 7: 4,7-methanoindene 8: 1,3-cyclohexadiene 9: benzene 10: 3-phenyl-1-butene 11: undecane 12: azulene 13: undecane 14: napthalene 15: tetradecane 16: pentadecane 17:hexadecane 18:heptadecane 19: heptadecane 20: tetracosanoic acid 21: tricontane

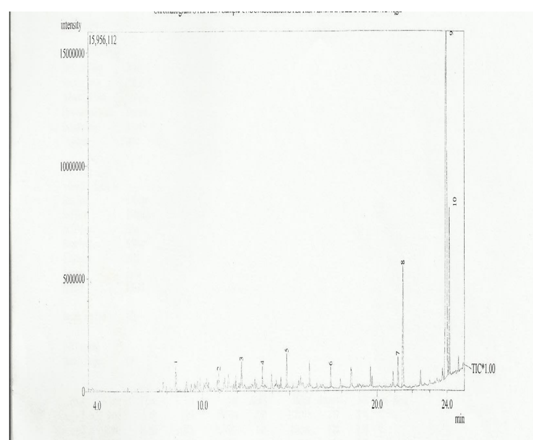

Fig 6: Gas chromatographic analysis of spent lubricating oil degraded by *Aspergillus niger* after two weeks.

Peak sequences: 1: benzene 2: benzene 3: azulene 4: dodecane 5: tetradecane 6: heptadecane 7: hexadecanoic acid 8: hexadecanoic acid 9: 9-octadecanoic acid 10: octadecanoic acid

4. Discussion

The pH of the results tends towards alkalinity (5.3-7.8). This range of pH has been reported to favour biodegradation of hydrocarbons (Bossert and Bartha, 1994, Stephen and Egene, 2012, Stephen et al., 2013). The higher pH in the inoculated spent lubricating oil than the control may be due to metabolism of the spent lubricating oil by the *Aspergillus niger*.

Turbidity was higher in the inoculated sample than the control. This indicates that *Aspergillus niger* was able to grow and utilize the lubricating oil as carbon source. The reason for the increased turbidity after 4 days till the end of the study may be due to the presence of nitrogen and phosphorus in the mineral salt medium which is necessary for biodegradative activity (Adesodun and Mbagwu, 2008) and also play a role in overcoming nutrient limitation during the biodegradation process (Sanyaolu et al., 2012).

Nitrate concentration was lower in the inoculated sample than the control. The decline in the concentration of nitrate between day 0 and 4 could be attributed to high metabolic activity and utilization of nitrate during the biodegradation process by *Aspergillus niger* (Sanyaolu et al., 2012).

Some of the compounds present in the control sample may be due to prolonged usage of the oil leading to its contamination by chemical impurities (Dominguez-Rosado and Pichtel, 2004). This observation is in line with the report of Diab (2008) that larger amounts of hydrocarbons are present in spent lubricating oil than normal alkanes.

The GCMS analysis of the inoculated sample after 7 days revealed the presence of more benzene than the control. This may be due to the cleaving of the methyl, ethyl, propyl branches as observed in the control. This also shows that benzene can not be degraded by *Aspergillus niger*. This is in agreement with Atlas and Brag (2009). These researchers reported that some aromatic hydrocarbons can not be degraded by some organisms.

The GCMS analysis of the inoculated sample after 14 days revealed reduced peaks and compounds. This may be attributed to increased biodegradation (Susarla et al., 2002). The chromatogram also revealed that benzene and azulene could not be degraded even after 14 days. The increased number of carboxylic acids (hexadecanoic acid, octadecanoic acid and 9-octadecanoic acid) is an indication of biodegradation of the alkanes found in the control (Meredith et al., 2000).

Conclusion

This study revealed that *Aspergillus niger* was capable of utilizing and growing in spent lubricating oil. Its metabolic activities increased the pH and turbidity of the growth medium. Methylbenzene, ethylbenzene, propylbenzene, hexadecane and octane were degraded into benzene, hexadecanoic acid, octanaoic acid respectively. The study also revealed that benzene and azulene were compounds that could not be degraded by *Aspergillus niger*.

Corresponding Author:
Dr. Stephen Emmanuel
Department of Microbiology,
Kogi State University, Anyigba,
Nigeria

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Applications of Electrochemical Elements in Systems of Artificial Intelligence

Mikhail Vaynshteyn[1], Aleksandr Lanis[2]

[1.] SciPeople Scientific Society, PhD, Saint Louis, USA
[2.] Mercy Hospital, MD, Saint Louis, USA
vamiz@yahoo.com

Abstract: Contemporary systems of artificial intelligence transduce information primarily by application of electronic, rather than nonelectronic, devices. Recently, numerous reports have been published about the research and development of electrochemical transducers, which are fully compatible with the technology of solid-state electronics and can provide substantial benefits in the circuit design. In nature, intellectual functions are carried out on the basis of electrochemical and chemical mechanisms of information transfer, such as occurs in the human brain. Artificial intelligence systems allow one to replicate and to amplify the various functions inherent in nature. This article describes the main results and the probable prospects of technologies which may permit one to carry out certain intellectual functions by means of technical solutions based on the application of electrochemical transducers of information.

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1. Introduction

Systems of artificial intelligence are the main goal in the development of modern computer technologies. Their construction requires application of different transducers, most of which are based on electronic mechanisms. In addition to electronic elements, there are also optical, thermal, electro-magnetic, electromechanical, and other elements that are based on physical mechanisms. However, there is a much less well known transduction element which employs an electrochemical mechanism using electrolyte-containing cells. The application of such elements in modern devices is slowly but steadily increasing. Recently, there also have been numerous reports on the research and development of electrochemical cells based on solid electrolytes, which are fully compatible with the technology of modern solid-state electronics and can provide substantial benefits in circuit design.

In nature, the intellectual functions of various organs are carried out on the basis of electrochemical and chemical mechanisms. The prime example of one such organ is the human brain. Artificial intelligence systems can potentially replicate and amplify various functions inherent in the brain. There is a common assertion that the brain is an electrochemical computer. For example, Bill Seaman and Otto E. Rossler [1] formulated three possible approaches to the creation of an electro-chemical computer that performs the functions of the brain and submitted an abstract scheme of the arrangement of different modules in such a computer. Also, a mathematical consideration of the simplified electrochemical model of a brain was presented in the publications of T. Triffet and H. S. Green [2]. Therefore, the question arises to what extent the level of current research and development in the field of electrochemistry allows us to come nearer to creation of more advanced computer models capable of performing brain functions.

This article describes the main results and the probable prospects of technologies which may permit one to carry out certain intellectual functions by means of technical solutions based on the application of electrochemical transducers of information.

2. Electrochemical Memory Elements in Artificial Neural Networks

The first time the idea of creating systems that use electrochemical elements of memory appeared, it was in connection with the task of modeling neural networks. The concept of an artificial neural network was formulated by McCulloch and Pitts in 1943 in the form of mathematical and electrical models of biological neural networks. On the basis of such networks, development of adaptive computer systems began, and the most attractive of these systems was the perceptron.

Learning ability is usually achieved by storing data changes in neural networks. For this purpose, it is necessary to apply elements in electrical circuits that have several requirements: a large number of storage levels, a reversible mechanism of their formation, a long memory retention time, and no consumption of energy during storage. To fulfill these requirements, Bernard Widrow in 1960 applied

a new electrochemical element, which he called a memistor.

The memistor, designed by B. Widrow and T. Hoff [3], was a sealed cell made of polymeric material in which the resistance of the graphite electrode reversibly varied in the range of 1 to 100 Ohms by electrochemical deposition or dissolution of copper. The size of resistance was achieved as a result of passing of multidirectional impulses in an operating circuit. Resistance was measured in a control circuit. The cell had three electrode terminals, one of which was common to both circuits. As described by the authors, their memistor allowed to achieve up to 1000 storage levels.

In the mid 1960's, due to the popularity of works on the creation of perceptrons, advanced models of memistors were developed. They differed from Widrow's memistor in that electrodeposition of copper was carried out on a cylindrical micro-electrode, which was a platinum wire 5 microns in diameter [4]. These microelectrodes were made on the basis of Vollaston platinum filaments. Theoretical calculations and experimental studies have shown that due to the cylindrical configuration of the diffusion layer such microelectrodes can increase the speed of electrodeposition and dissolution of copper by 10 times [5]. Additionally, it also allowed a linear dependence between measured resistance and quantity of the electricity forming storage levels. The mentioned works were carried out in a number of laboratories with coordination by the Institute of Electrochemistry of the Academy of Sciences where the scientific management was carried out by P. D. Lukovtsev [6].

In the 1960's, increased attention to electro-chemical memistors was given due to the fact that they allowed the construction of the most efficient artificial neural networks for perceptrons, in comparison with other circuit components. However, later publications showed the fundamental limitations of artificial intelligence based on percep-trons. Furthermore, it was discovered that memistors in which the resistive layer is formed by means of electrodeposition and electrodissolution of copper aren't completely reversible, since in the course of cycling the electrode accumulates a quantity of copper which does not participate in creation of the resistive layer [7]. As a result, work on memistors based on solutions of copper lost relevance. After that, in accordance with the general concept of solid-state microelectronics, the development of memistors was carried out on the basis of solid electrolytes.

The first sufficiently detailed report about creation of such a component appeared in 1990. The authors called this the solid-state thin-film memistor [8]. According to the authors' description, the adjustable resistive layer was formed by tungsten acid, which was obtained from tungsten oxide. The necessary hydrogen ions were delivered by means of reversible electrochemical transfer from a layer consisting of a hydrated oxide of hexavalent chromium. This process allowed the memistor to change resistance from 10^5 to 10^9 Ohms and provided a stable memistor lasting several months. The authors also point out the need for further research of their electrochemical system to avoid gassing at the electrodes. From the description of the experiment provided in their article, it follows that gas emission could arise owing to existence of ions of chromic and tungsten acids: i.e. the chosen system, despite the name, was not solid enough.

In 2005, V. Yerokhin,T. Berzina and M.P. Fontana published an article about the development of an electrochemical device on the basis of thin layers of polyaniline and polyethylene oxide [9]. The insulating substrate was coated with a 5-10 micron layer of solid electrolyte composed of polyethylene oxide and lithium chloride. Finally, above the coated layer, a very thin layer (~48 nm) of polyaniline was deposited. The component had three electrode terminals. Electric resistance of a polyaniline layer changed as a result of the transfer of lithium ions into this layer and the subsequent electrochemical redox reaction. According to the authors' assertion, the device showed stable and well reproducible electrical characteristics. The article points to the possibility of miniaturization of the device through the use of electron-beam processing for the purpose of creating a nanoscale configuration. In addition, it was noted that the resulting dependence of the conductivity on the exposure time of voltage can be the basis for the realization of adaptive polymer structures.

At present, most attention is given to the research and development of two-electrode electro-chemical elements functioning on the basis of reversible redox reactions and ion transport in the nanolayers of insulators and semiconductors. Several works have reported the studies of reversible changes in electrical resistance when thin (20 - 300 nm) layers of oxides of aluminum, silicon, titanium, and other fine-crystalline and amorphous insulators were exposed to an alternating voltage. These multiple works appeared in the 1960's, i.e., almost simul-taneously with the first information about memistors. They reported research of ion transition in various "metal-insulator-metal" systems. Changing of elec-trical resistance of an insulator resulted from injection or extraction of ions from adjacent metals.

In 1971, Leon Chua published an article which, proceeding from the principle of symmetry, theoretically showed a device which expresses dependence between charge and magnetic flux [10].

Along with the resistor, the capacitor and the inductor, he posited a "fourth fundamental circuit element" for complete characterization of circuit properties. He called it "memristor" as a result of reduction of the words "memory + resistor". In subsequent articles, he also introduced the concept of "memristance," defined as the property of an electronic element to remember its last resistance before being switched off. During the publication of his theoretical conclusions, L. Chua did not know about prior research and the memistor proposed by B. Widrow with a very similar name.

Memistor and memristor are similar elements, but not equivalent in their circuit design features [11, 28]. In the same article, L. Chua mentioned that creation of a memristor will allow the realization a number of unique properties which can't be reached in circuits consisting only of resistors, capacitors and inductors. However, in spite of the theoretical appeal of the memristor, the subsequent practical development of this element faced serious technological problems, which for a long time didn't even allow the creation of prototypes suitable for use in computer structures.

In the components considered earlier, memory process was carried out on the basis of an electrochemical mechanism. In memristors which are developed now, the process of memorizing is carried out on the basis of various mechanisms. According to the classification of R. Vaser [12], the process of memorizing can be performed using the following effects: ferroelectric, electrostatic, phase transitive, thermochemical, magneto-resistive, nanomechanical, by intramolecular reorganization, by changes in valence, or by electrochemical metallization. Thus, only the two last effects can be considered within the electrochemical mechanism. In particular, these two have attracted the largest amount of attention by researchers and developers as evidenced by the latest publications.

In 2008, D. B. Strukov, G. S. Snider, D. R. Stewart and S. Williams from authoritative computer firm Hewlett-Packard, reported about the development of a model of memristor on the basis of the Pt–TiO_2/TiO_{2-X}–Pt system [13]. They also suggested a more accurate mathematical model of the developed memristor, as compared to previously presented works of L. Chua. This model was in good consistence with their experimental results. In establishing their model, the authors proceeded from the fact that the memristance effect occurs in solid-state, nanoscale systems in which electronic and ionic transport is coupled under "external bias voltage." They note that the application of the external bias voltage produces a hysteretic behavior in the change of the current. According to the authors' proposal, this hysteresis requires atomic rearrangement that modulates the electronic current. The total memristor's resistance is defined by two components: a semiconductor layer with high dopant concentration of positive ions which has a low resistance (R_{on}) and the remainder layer with negligible ion concentration of the same ions and much higher resistance (R_{off}). Application of an external voltage causes the displacement of the boundary between the layers. On the basis of the accepted mechanism, the authors derived an equation defining the dependence of memristance from the total thickness of both layers, average mobility of dopants, and charge as a function of time. According to the derived equation the memristance becomes larger in absolute value for higher dopant motilities and for smaller film thicknesses of the semiconductor. Memristance becomes more important for understanding the electronic characteristics of any device as the critical dimensions shrink to the nanometric scale. Another team of developers of the same memristor explained that the role of dopants was carried out by positively charged oxygen vacancies [14]. In the subsequent publications about this development [15], one promising quality of the considered memristor is an exponential decrease of the energy spent during transition from high to low resistance, as current increases.

There are also other reports of memristor research using the transition metal oxides (VO, Ta_2O_5, Nb_2O_5, $SrTiO_3$) as a nanoscale layer and still other memristors based on the mechanism of charge transport, including migration, diffusion and ion valence change [16]. At the same time, there are a significant number of research and development proposals related to the "metal-insulator-metal" systems in which the mechanism of storing is caused by formation of subtle metallic filaments during electrochemical discharge of cations and their subsequent dissolution during the change of polarity [17]. Instead of insulators (such as SiO_2 or Al_2O_3) as the intermediate layer, some solid electrolytes (Ag_2S, Ag with dopants of $GeSe_2$) can be used. The cell of these memristors has two electrodes which are made from metals with different electrochemical properties. The first one, the active electode (AE), is made from electrochemically active metal with high conductivity Ag (for research) purposes or Cu (for broad consumption). The second electrode is an electrochemically inert counter electrode (CE) which is made from Pt (for research) or W (for broad consumption). Such memristors need a preliminary formative operation, during which porous channels are formed in the insulator. The existence of a predetermined pore structure provides further reproducibility of the formation and dissolution of

metallic filaments. Speed of these processes depends on what type of material (an insulator or solid electrolyte) is used for creation of the intermediate layer. In certain cases, ion mobility can make a more essential contribution to memristance than the rate of discharge at the electrodes.

Various authors report about stability of constructed samples and compliance of sample parameters to essential operating requirements. For example, achieved switching speed corresponded to the nanosecond range. Meanwhile, in devices of memory applied now, these speeds are two orders lower. The projected time of memory retention, calculated on the basis of imitating tests at temperatures of 70 - 130^{0}C is ten years.

Modern computers are created on the basis of the materials whose properties are described by solid-state physics. These materials possess stability of electric properties in a rather wide range of temperatures and possess good reproducibility of functional parameters. Unlike them, information systems in nature are constructed on the basis of liquid electrolytes. Electrical properties of liquid elements are more significantly temperature dependent than that of solids. In addition, the changes in their electrical parameters, which happen frequently in the course of functioning liquid systems, have significantly poorer reproducibility than solids. Despite this, many researchers believe that the use of chemical and biological media, based on aqueous solutions and other liquids, is promising, as they allow one to create information systems with new functionality (such as for example the molecular and biological computers). In this regard, publications about research and development of new electrochemical transducers based on aqueous solutions continue to appear.

In 2000, V. N. Ur'ev, B. M. Grafov, A. V. Dribinskii and V. P. Lukovtsev published an article in which they demonstrated the realization of a multi-bit cell with a liquid electrolyte, where data are written by a successive cathodic deposition of alternating metallic layers with different electrochemical properties [18]. The written data was read in two ways: on the basis of successive controlled anodic dissolution of the multilayer structures or by recording the potential difference between the electrodes. Record/reading speed on 20 µm diameter electrode in a copper sulfate electrolyte was 10 Kbps. By reducing the diameter of the electrode, this speed can be increased by several orders of magnitude.

A more long term outlook to create molecular memory elements based on the application of liquid electrolytes has been reported as well [19].

3. Electrochemical Transistors Based on Organic Polymers in Artificial Intelligence Systems

Transistors are one of the key elements of electronic devices. Modern industry offers a large selection of different types of transistors, most of which are made on the basis of solid-state technology. Electrochemical transistors can be made of inorganic or organic materials. In patent literature, the variety of electrochemical transistors based on inorganic materials is classified as H01G9/26. However, the electrochemical transistors developed so far on the basis of inorganic semiconductors, have no essential functional or technological advantages and therefore can't compete with the transistors produced by widespread methods of solid-state electronics.

Industrial samples and technologies of electrochemical transistors creating new functionality for systems of artificial intelligence appeared as a result of creation of organic semiconductor materials and the development of methods of their application to nanoscale products.

Targeted research to develop polymeric semiconductors began in the 1950's. The most important stage of these studies was the development of halide polyacetylene in 1977, which has electric conductivity two orders of magnitude higher than previously known organic polymers [20]. Currently, polymers are created in a wide range of conductivity, from values corresponding to semiconductors (10^0 - 10^{-2} S/cm) to values approaching characteristics of metals (10^4 - 10^5 S/cm).

In 1984, H. S. White, G. P. Kittlesen, and M. S. Wrighton reported the establishment of an electrochemical transistor made on the basis of an organic polymer film brought in contact with an electrolyte [21]. The electrochemical transistor has three electrodes, two of which conduct the operating current, while the third controls magnitude of this current. Electrodes are made in the form of inert metal films. These operating electrodes are deposited on an insulating base and separated by a polymer layer. The control electrode is located above them in a thin layer of electrolyte. Conductivity of the polymer varies depending on the degree of oxidation, which is defined by the size of the potential applied to the control electrode. As a result of electrochemical oxidation or reduction, the concentration of dopants in the polymer changes and switches the resistance between the conducting, semiconducting and insulating state.

Advantages of electrochemical transistors based on organic polymers over electronic transistors are numerous. Firstly, they can operate at lower voltages (< 1 V). They can also be used as integrators and elements of short-term memory because they

remain in the same state after the removal of voltage [22]. In addition, they can be manufactured by inkjet printing technology that is much simpler and cheaper than the methods of photolithography currently used in the manufacture of integrated circuits in solid state electronics [23]. Manufacture of such electro-chemical transistors can be performed under normal environmental conditions, i.e. they don't need vacuum requirements necessary for electronic transistors. Applicable polymers are dissolved in the corresponding organic solvent and then printed on the insulation substrates with inkjet printers. The electrochemical transistors created on the basis of organic polymers and inkjet printing technologies can be manufactured in a simultaneous cycle with other elements of printed circuit boards, such as for example, with resistors, inductors, or batteries. In particular, transistors (due to their multifunctionality) allow one to create a complete set of all elements of circuits placed on the printed boards.

In the future, the use of equipment for bulk printing (3D printers) will allow for creation of monolithic integrated circuits on multilayered printed boards and even finished technical devices. Considering that the programs for production of these devices can be created by computers, it opens a way to creation of self-replicating and self-learning systems of artificial intelligence.

The kinematic model of a self-reproducing automaton was first proposed in the middle of the last century by John von Neumann [24]. However, so far there is no real technology for implementation of this idea. From the stated, it follows that electrochemical transistors can become a basis of such technology.

4. Electrochemical Transducers as a Part of Artificial Organs of Smell and Taste

The input of information into artificial systems is fundamental for intellectual actions. In biological systems, the receivers of information are the sense organs. Traditionally, there are five types of human senses. Vision and hearing are the receivers of information which is transmitted in the form of waves. Touch perceives thermal and mechanical information. The senses of smell and taste are based on chemical processes and are, in their natural mechanism, electrochemical analyzers of information. In the wild, taste is detected through a combination of signals from receptors in the nose, tongue and other organs. A similar principle has been proposed for creation of artificial devices called the electronic nose and electronic tongue. Both of these devices can be considered as a necessary addition to various systems of artificial intelligence.

Technical predecessors of the artificial nose and tongue are well known methods of analysis of various gases and solutions. Currently, at least ten methods of gas analysis are widely applied in industry. The highest accuracy is achieved by isotopic and chromatographic methods, in which the minimum detectable concentration is 10^{-7} percent. Using the electrochemical method, gas is first dissolved in an electrolyte and then defined by potentiometric, conductometric, amperometric or polarographic measurements. The electrochemical method is second in terms of accuracy (10^{-6} percent), but it has the advantage in simplicity of execution. However, for creation of the device modeling a nose, it is also necessary to fulfill the requirement of selective sensitivity, which in biological systems is solved by a large number of different receptors.

The human nose possesses a staggering amount of sensitivity. It can distinguish the smell of a substance comprising an amount of several tens of molecules in a mixture of several thousand substances. Furthermore, it is known that some animals have more sensitivity than even humans. However, the human nose possesses the largest genome among all mammals (over 1000 genes), providing sensitivity to smells. Therefore, when modeling the human nose it is necessary, in addition to accuracy, to fulfill at least two conditions: first, to have many different sensor inputs, and secondly, to accurately process the data received from these sensors, just as it occurs in the neural networks of the brain. These conditions were first implemented in the work of K. Persaud and G. Dodd [25].

The electronic nose can be combined along with electrochemical sensors of other types (for example, chromatographic, piezoelectric, thermo-catalytic) capable of recognizing molecules in the gas state. At present, progress in development of an artificial nose device is being advanced by the appearance of new opportunities in the field of nanotechnology. In [26] there is report about the development of a multisensory element on the basis of the field transistor in the form of carbon nanotubes. In this device, a protein receptor layer is coated on the surface of each nanotube: the interaction between the protein and the analyzed component in a gas mixture changes the current value flowing through the transistor. Every crystal holds a large number of transistors, each of which, depending on the type of the protein applied onto it, is sensitive to a certain component of a gas mixture. As a result of the interaction between the protein and an analyzed component of a gas mixture, the value of the current passing through the transistor changes.

Publications about creating devices that simulate the functions of the tongue appeared in print 14 years after articles appeared on creation of an electronic nose. They were caused by the needs of the

pharmaceutical and food industries. The first "electronic tongue" device was proposed in 1998 [27]. It contained eight sensors, possessing cross sensitivity to several components with different tastes. Devices of this type are intended for measurements in solutions, and therefore, unlike the previously developed electronic nose devices, are constructed mainly on electrochemical methods. From the very beginning, their development was based upon knowledge gained by potentiometric measurements of glass and ion-selective electrodes. In addition, during the design process of the electronic tongue, principles successfully gained during the development of the electronic nose were also utilized: i.e. multisensory design and subsequent multivariate processing of entered data.

Vlasov Yu., Legin A., Rudnitskaya A. [28] together with other members of the Laboratory of Chemical Sensors at the University of St. Petersburg published a number of the fundamental works and theories which allowed for the creation and further development of more advanced electronic tongue designs. They proposed an empiric method for estimating cross-sensitivity and developed a flow-injection version of the electronic tongue that allows it to carry out multiple and repetitive measurements in automatic mode. Several hundred membrane materials for various sensors were investigated, including chalcogenide glasses, plasticized polymers containing active agents, and polycrystalline composites. The main principle behind their "massif of potentiometric sensors" was the serial measurement of the EMF of multiple electrochemical cells, each of which triggered a single sensor. Control of the measurement procedure was carried out by a computer. Methods of pattern recognition and multivariate calibration were used for interpretation of the received information. When aprioristic information about measured samples was absent, classification was carried out by means of self-learning methods.

In addition, in the above mentioned studies it is reported that the electronic tongue can identify multi-component liquids by a method similar to fingerprinting. This ability opens a new approach to the analysis of quality of many products and the determination of their compliance with the specified product taste.

From the considered results of development and research of devices for definition of smell and taste, it is apparent that their improvement occurs on the basis of application of electrochemical sensors. Processing of received multisensory information is carried out by methods of self-learning and pattern recognition by artificial neural networks. Furthermore, in sections 2 and 3 of this review it was demonstrated how essential progress in the field of creation of artificial neural networks and computing systems can be reached: this can be achieved by electrochemical information transducers such as memristors or transistors, which are based on organic polymers. These transducers allow the potential to carry out the most important functions of artificial intelligence, namely self-learning, self-improvement and even self-replication. As a result, they open a way to creation of intellectual systems with elements that will operate, mainly, on the basis of electrochemical mechanisms.

5. Conclusion

Electrochemical transducers of information developed thus far allow important advantages for technical implementation within various systems of artificial intelligence. The application of memristors and other similar electrochemical elements allow the creation of computers with new functionality, more compact layout of basic structures, longer retention time of stored information, data storage without consumption of energy, and higher ability for self-learning and pattern recognition. Electrochemical transistors based on organic polymers simplify manufacturing technology for a number of computer devices, allow them to operate at lower voltages, create the possibility of production by methods of 3D inkjet printing technology, and thus open the way for the creation of self-learning and self-replicating systems.

The considered trend in development of artificial intelligence systems brings their structure closer to the principles of organic intellectual systems founded on electrochemical and chemical mechanisms established in nature.

Corresponding Author:
Dr. Aleksandr Lanis
876 Wellesley Terrace Lane
Chesterfield, Missouri 63017, USA
E-mail: vamiz@yahoo.com, alexlanis@yahoo.com

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Histological and Ultrastructural studies On the Epididymis of Pigeon (Columba livia)

Abdel Aleem A. El- Saba and Mohamed I. Abdrabou

Department of Cytology and Histology; Faculty of Veterinary Medicine; Cairo University.
Abdo_1416@yahoo.com

Summary: The pigion's epididymis consisted of an extra testicular part of the rete testis, the proximal & distal efferent ductules, the connecting ductules & the epididymal duct. The extra testicular part of rete testis lined by squamous to cuboidal epithelium. The wall of the proximal efferent ductules was thrown into many longitudinal folds. Their epithelium formed of two main cell types, ciliated and non ciliated cells in addition to few basal cells. The ciliated cells appeared columnar with Long tuft of cilia projected from their luminal surfaces. Many cells showed large and small multi vesicular bodies, dense bodies associated with yellow Lipofuchsin pigment. The non ciliated cells were denser than the ciliated ones. They showed intracytoplasmic vacuoles, dens globules. Their apical cytoplasm might protrude into the ductular lumen to form bleb like projections with PAS +ve. The distal efferent ductule appeared smaller in diameter than the proximal ones. The epididymal duct had wide lumen. The lining cells consisted of non-ciliated columnar cells and basal cells. The columnar cells showed vacuolated cytoplasm.
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Key words: pigeon, Columba livia, epididymis, rete testis, efferent ductules, epididymal duct.

1. Introduction

In all avian species studied to date, the epididymal ducts was not uniform along its length. It consisted of a different number of regions with different histological and Cytological structure. The epididymal region was suggested to perform many activities. A Secretory activity was recorded by **(Tigari, 1972)** in fowl. Phagocytosis of the broken down germ cells and degenerated spermatozoa have been also speculated by **(Tingari & Lake, 1972 & Nakai et al., 1989)** in fowl & **(Aire 1979 & 1980)** in different birds.

The majority of studies have been established on the epididymis of domestic fowl **(Lake, 1957 & 1962; Tingari, 1971 & 1972; Tingari & Lake, 1972; Budras & Sauer, 1975; Aire, 1980 & 1982a & Nakai *et al*., 1989)**; Turkey **(Hess *et al*., 1976; Hess & Thurslon, 1977; Balah *et al*., 1989);** Japanes quail **(Aire, 1979a, b & 1982a & Rikihisa & Lin, 1988);** guinea Fowl **(Aire *et al*., 1979 & Aire, 1982a);** & Peking ducks **(Aire, 1982a & b & Tetez (off, 1977)** and **pigeon (Stefanini *et al*.,1999).**

However up till now little attention was directed to the epididymal region of pigeon. So the present study was directed to investigate the histological (Light & electron) structure of pigeon's epididymis.

2. Material and Methods:

The present work was conducted on 12 adult male apparently healthy pigeons. Samples from the the epididymal region of pigeon were taken and fixed in 10% buffered neutral formalin and Bouin's fluid for confirmation of the results. Fixed specimens were dehydrated, cleared and embedded in paraffin wax. Serial and step serial sections of 5-6 micrometers thick were obtained and stained with Harris Hematoxylin and Eosin, Weigert's elastic tissue stain, Gomori's reticulin method, Periodic acid Schiff (PAS) technique and Alcian blue pH 2.5 **(Drury and Wallington, 1987).** Crossmon's trichrome stain **(Crossmon, 1937).**

For transmission electron microscopy; small fragments from the epididymal region were fixed in Karnovesky's solution overnight and submitted to routine of transmission electron microscope**(Pearse, 1972)**. Semithen sections were stained with methylin blue were examined .Then ultrathin sections of 60-80nm were stained with uranyl acetate solution and lead citrate. The materials were examined and photographed in a Philips CEM-100 transimission electron microscope.

Results

The epididymal region of the pigeon macroscopically appeared as an elongated organ closely attached to the dorsomedial aspect of the testis and they were enclosed together within the tumica albuginea **(Fig.1).** Microscopically it consists of a mass of ducts and tubules. According to **Stefanini *et al*., (1999)** the ducts and tubules of pigion's epididymis could be differentiated into: An extra testicular part of the rete testis, the proximal, distal efferent ductules, the connecting ductules and the epididymal duct **(Fig. 2).** The reticular fibers formed the reticular lamina of the tubules basement membrane as well as the inter ductular framework **(Fig. 3).**

The extra testicular part of the rete testis:

This part appeared as an irregular thin walled channels located just outside the testicular capsule. It was lined by squamous to cuboidal epithelium **(Fig. 4).** As the rete tubules approached the proximal efferent ductules, their epithelium changes into high cuboidal, then to columnar type. The columnar epithelium continued with the higher columnar epithelium lining the proximal efferent ductules **(Figs. 5, 6).** These rete channels were supported by highly vascularized connective tissue containing lymphocytic aggregations. Some of these aggregations look the nodular form **(Fig. 4).** The lumina of the rete channels contained immature germ cells, macrophages, few spermatozoa and Some times parts of desquamated cells **(Figs 5, 6,7).** TEM of the rete tubules revealed overlapping low cuboidal lining cells joined apically with tight junctions. These cells contain irregular or oval nuclei, apical surface invagination could be observed in some cells. Phagosomes, residual bodies and cytoplasmic vacuoles were also detected. In addition, profiles of rER, and cytoplasmic dense bodies were seen **(Figs. 6, 7).**

The proximal efferent ductules:

The wall of the proximal efferent ductules was thrown into many longitudinal folds of variable height **(Fig. 8).** Their lumina were wide and contained few scattered spermatozoa and macrophage. The tubules epithelium showed two main cell types, ciliated and non ciliated cells. In addition, few basal cells with spherical or oval nuclei were observed between the bases of the main cell types **(Figs. 3, 8).**

The ciliated cells appeared columnar with ovoid to elongated lightly stained nuclei and acidophilic cytosplasm. Long tuft of cilia projected from their luminal surfaces. Many cells showed large and small multi vesicular bodies, dense bodies associated with yellow Lipofuchsin pigment **(Figs. 8, 8a).**

The ultra structure of these cells explained that the apical part showed cilia originated from clear basal bodies. Clear multi vesicular bodies, residual bodies, microvesicles, vacuoles, dense bodies, free ribosomes were observed in the ciliated cells **(Figs. 9, 10).**

The non ciliated cells were denser than the ciliated ones. They showed intracytoplasmic vacuoles, dens globules. Their apical cytoplasm might protrude into the ductular lumen to form bleb like projections. These projections contained acidophilic, PAS +ve bodies in other cells. Sometimes non ciliated cell appeared to be completely released into the tubular lumen **(Figs. 3, 8, 8a, 11)**.

At the ultrastrucutral level, these cells contained ovoid lightly stained nuclei. Multivesicular bodies, cytoplasmic vacuoles were also observed. In addition the luminal surface of these cells showed numerous microvilli, tight junctions were observed between them and with the adjacent ciliated cells **(Fig. 9).**

The distal efferent ductles:

The distal efferent ductule appeared smaller in diameter than the proximal ones. They showed little or no epithelial folding. Aggregations of closely packed spermatozoa were evident in their lumina **(Fig. 12).** Under the light microscope their epithelium showed ciliated and non ciliated cells in addition to few basal cells **(Figs. 12, 13).** The cells were high columnar at the initial part of the tubules then becomes lower near the connecting tubules. The ciliated cells as in the proximal tubules were lightly stained than the non ciliated ones. The non ciliated cells also showed many intracytoplasmic dense bodies. Apical long bleb like projections and some of these cells were completely shed into the lumen **(Figs.12a, 12b, 13).** Apical acidophilic, PAS +ve bodies were observed **(Figs. 12b & 14).** Basal cytoplasmic vacuoles were detected specially in the ciliated cells. **(Figs 13, 15).** Irregular or lobulated nuclei were characteristic in the lining tubular cells **(Fig. 15).** The ultra structure of the ciliated cells explained supra-nuclear numerous mitochondria, arrays of rER. Free ribosomes, cytoplasmic vacuoles and microvesicles, however the basal cytoplasm showed many dense bodies and large vacuoles. The vacuoles were also observed between the lining cells **(Figs. 16, 17).**

The connecting tubules:

It begins narrow, then they anastmosed together near the epididymal duct and became progressively wider. The initial region of these tubules resembles that of distal efferent ductules while its terminal part was lined with shorter columnar ciliated and non ciliated cells **(Fig. 18).** Many dense bodies were found in the non ciliated dark cells. Apical PAS +ve granules were observed in most cells **(Fig. 19).** Blebs were also projected from the non ciliated cells **(Fig. 18).**

The ultrastructure revealed progressive decrease in micrecovili length and number. The cilia disappeared toward the epididymal duct. The ciliated cells had lighter cytoplasm more irregular nuclei, less dense globules than in non-ciliated cells, the apical cytoplasm in both cell types contain mitochondria **(Fig. 20).**

Their cell membranes showed basal infolding with many hemidesmal junctions. Microvesicles and vacuoles were observed **(Fig. 21).** The cells showed large apical bleb-like projections with clear detached parts, cistermae of rER and free ribosomes were found in the supranuclear cytoplasm **(Figs. 22, 23).**

The epididymal duct:

It had wide lumen their wall supported with many layers of fibroblasts. The lumen of the duct was densely packed with spermatozoa. The lining cells consist of non-ciliated columnar cells and basal cells. The columnar cells showed spherical nuclei, vacuolated cytoplasm in some cells and acidophilic

less vacuolated cytoplasm in either cell. The heads of many spermatozoa appear to be embedded in the apical cytoplasm of some lining cells **(Fig. 24).** The TEM revealed apical invaginations. Tight junction between the cells, the cells contain many dense globules, mitochondria, apical cytoplasmic vacuoles **(Fig. 25).**

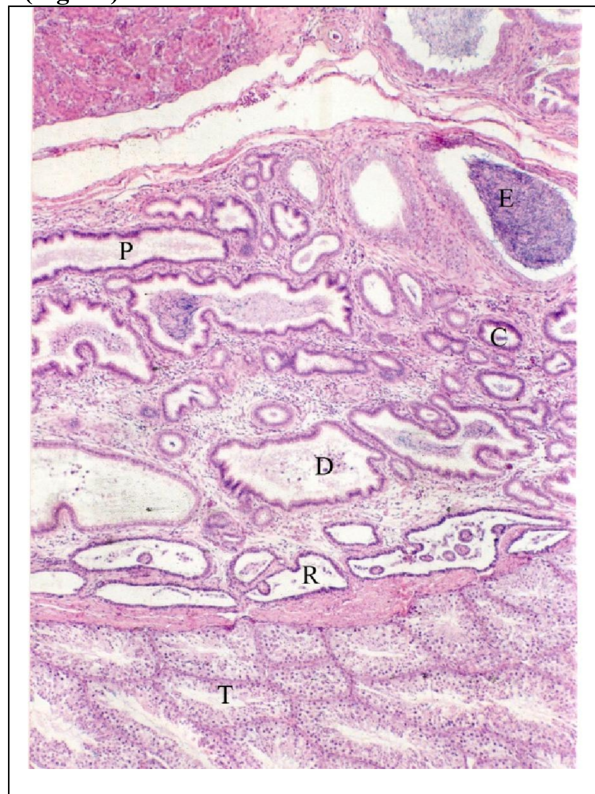


Fig. (1): Part of the testis (T) and epididymal region of the pigeonshowing the extratesticular rete (R), proximal efferent ductules (P), distal efferent ductules (D), connecting ductules (C) and the epididymal duct (E). (H&E stain X 65)

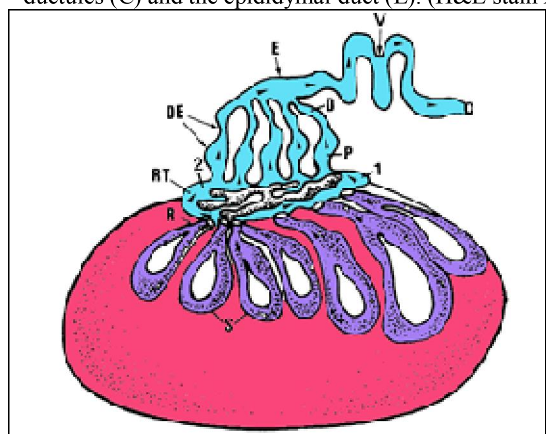


Fig. (2): Schematic drawing of the seminal pathway of pigeon Columba livia showing seminiferous tubules (S), tubuli recti (R), rete testis (RT), intratesticular segment of RT (1), extratesticular segment of RT (2), efferentductules (DE, roximal part 5 P, and distal part 5 D), epididymal duct (E), vas deferens (V), and seminal fluid course (arrowheads). **(Mairaa *et al.*, 1999).**

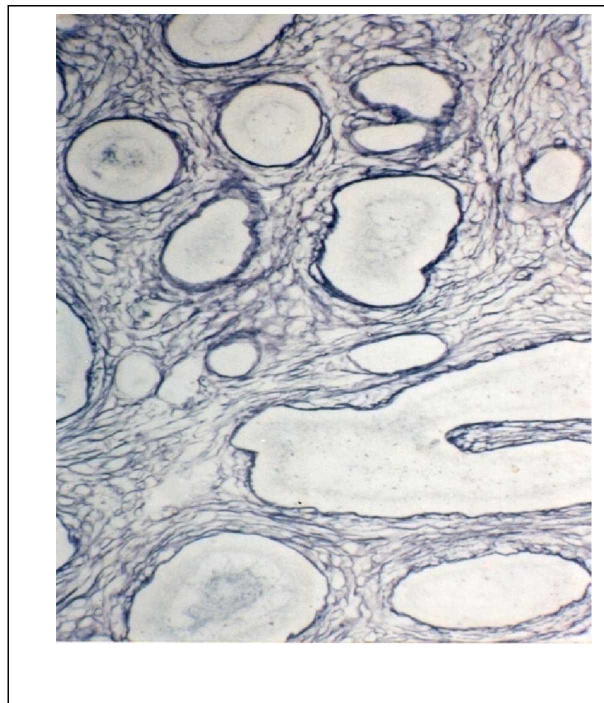


Fig. (3): Reticular fibers forming the reticular lamina of the basement membranes as well as net work between the epididymal ductules of the pigeon epididymis (Gomori's reticulin method X130)

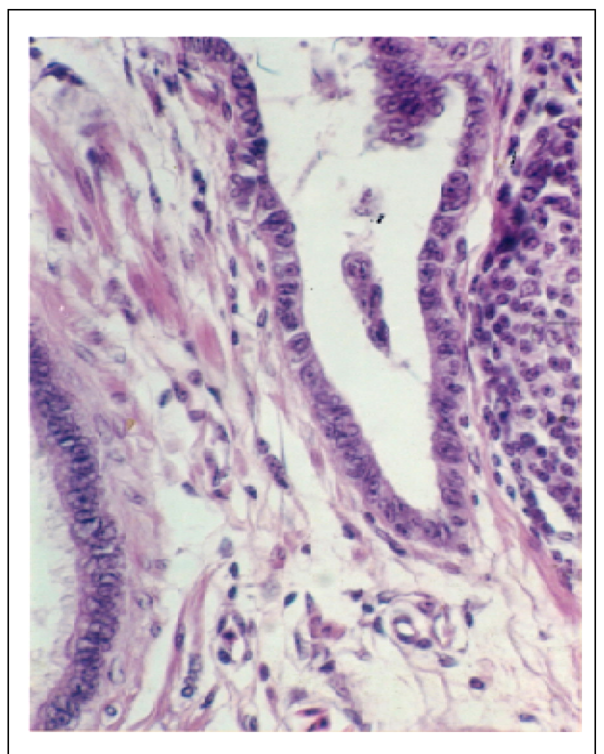


Fig. (4): Section of rete tubule of pigeon showing the cuboidal lining cells, notice the peritubular lymphoid aggregation & smooth muscle fibers in the interstitial tissue. (H&E X 320)

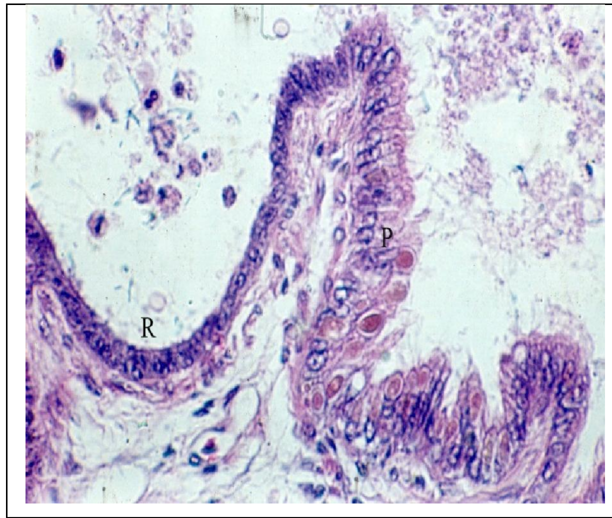


Fig. (5): section level showing the transition between the rete epithelium (r) & the proximal efferent ductules (P), Notice that the cuboidal rete epithelium increase in height to become high columnar epithelium at the proximal efferent ductile. (H&E X 1024)

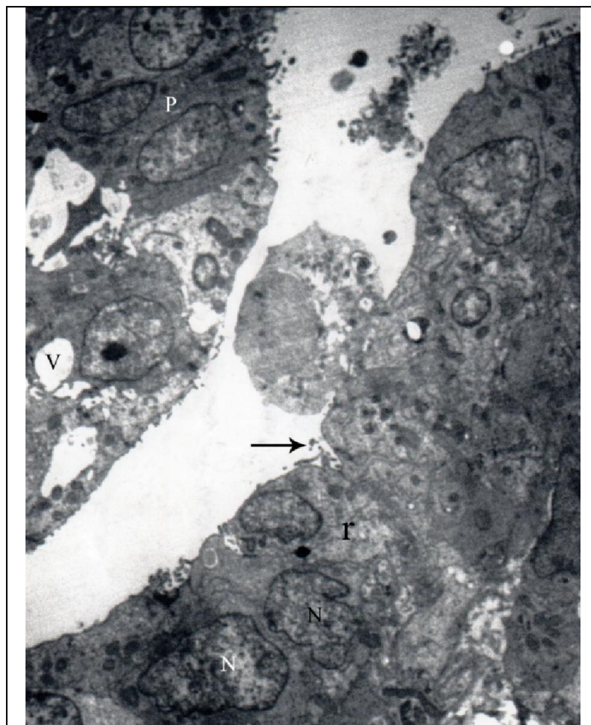


Fig. (6): TEM of the transitional region between the rete epith. (r) and the proximal efferent ductules epith. (P), Notice the overlapping low cuboidal epith. Of rete tubule changes into high cuboidal then columnar epith. Of the proximal efferent ductules. Some cells showing irregular nuclei (N), apical surface invaginations (arrow), intracellular Vacuoles (V), **phagosomes, residual bodies could be observed, luminal macrophage was found** (X5000)

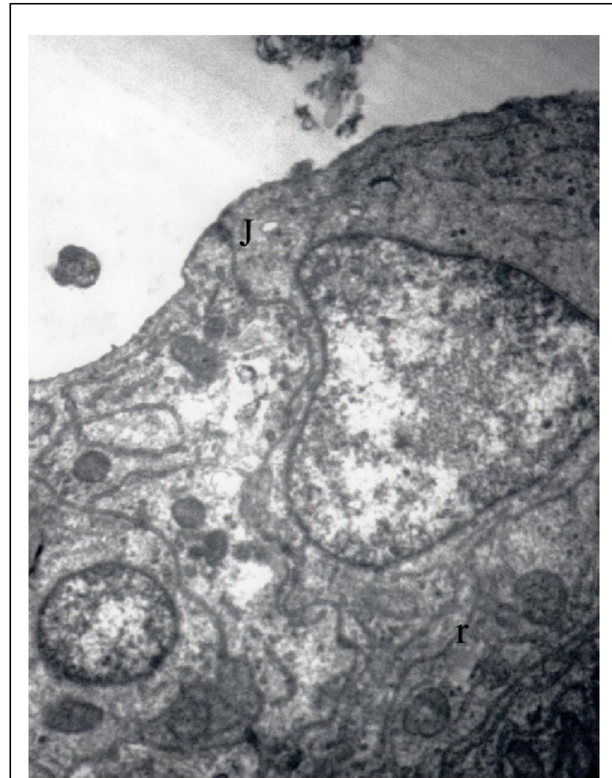


Fig. (7): Higher Magnification of the rete epith. Notice the overlapping cuboidal with oval nuclei, the apical tight junction between the cells (J), intercytoplasmic dens bodiofes & profiles rER (r) (X 10000)

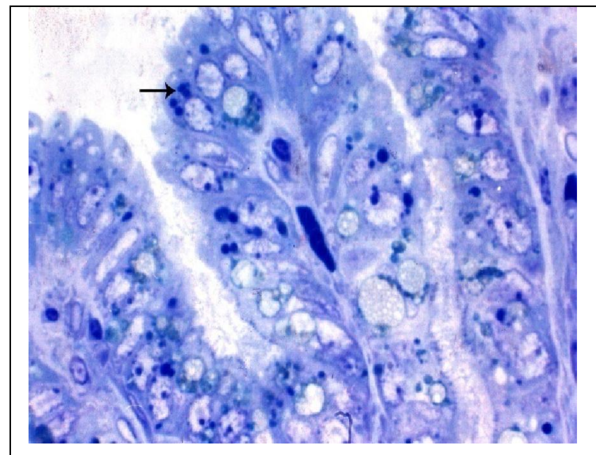


Fig. (8): Epithelium of proximal efferent ductile consisting of ciliated cells & nonciliated type I cells, Notice: the presence off vacuoles & dense globules in the nonciliated cells (arrow) which showed apical bleb like projection. Ciliated cells appear truncated lightly stained, Notice the presence of multivesicular bodies and yellowish lipofuchsin pigments in many cells. (T.B X 1024).

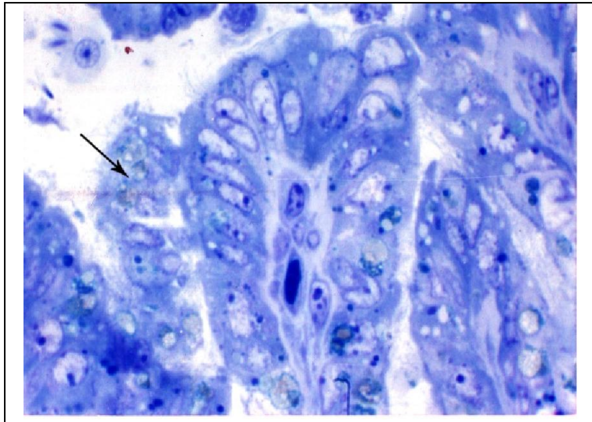


Fig. (8a): Epith. Of proximal efferent ductile showing ciliated & nonciliated cells. The nonciliated showing apical bleb like projectios & some of these cells were completely shed into the lumen (arrow), Luminal macrophage. (X 1024)

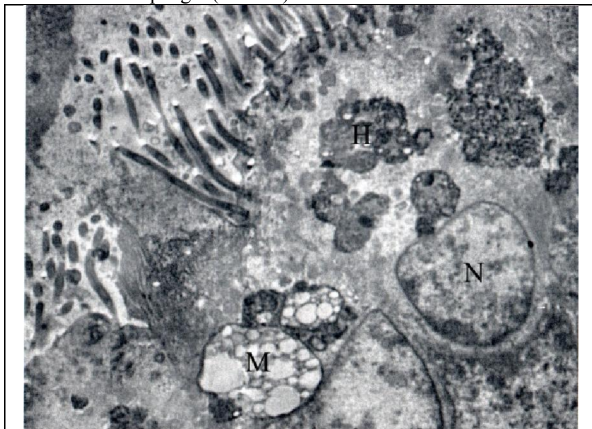


Fig. (9): TEM of proximal efferent ductile epith. Showing ciliated & nociliated lining cells with microvilli. The cells contains apical large heterogenous bodies, multivesicular bodies, ovoid nuclei, ciliated cell with free ribosomes basally. (X13000)

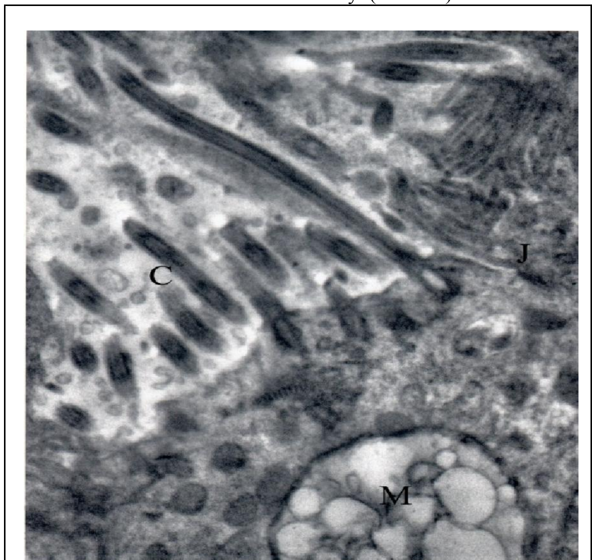
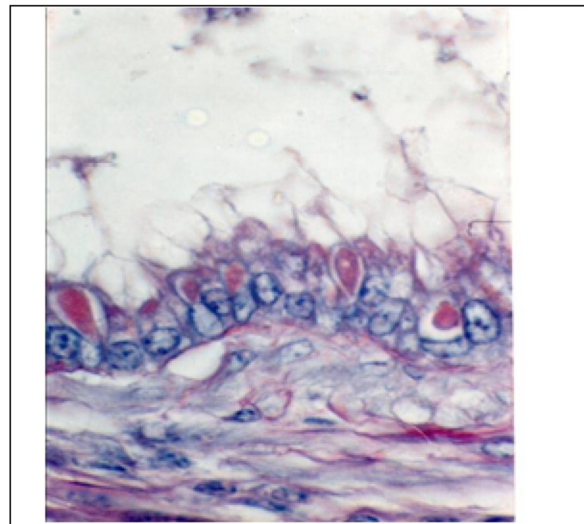


Fig. (10): Higher magnification of ciliated cells of the proximal efferent ductules, notice, large multivesicular body (B), Mitochondria (M), apical cilia (C), tight junction (J) & dense granules & vacuoles. (X15000)



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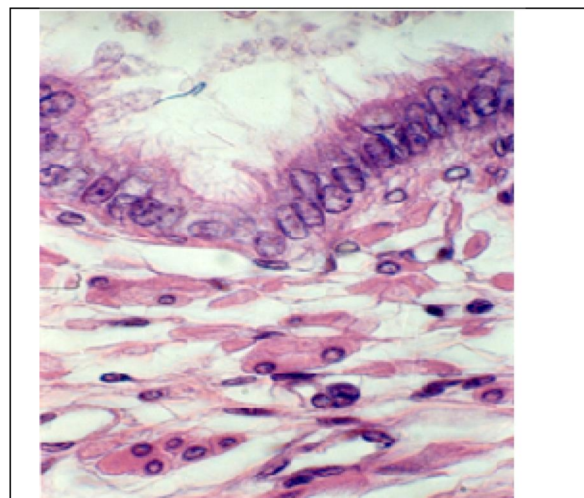


Fig. (12a): Distal efferent ductile showing apical bleb like projections of noncilated cells, notice the apical vacuolar & acidophilic bodies in many cells, supported by highly vascular stroma with may smooth muscle bundle. (H&E X 1024).

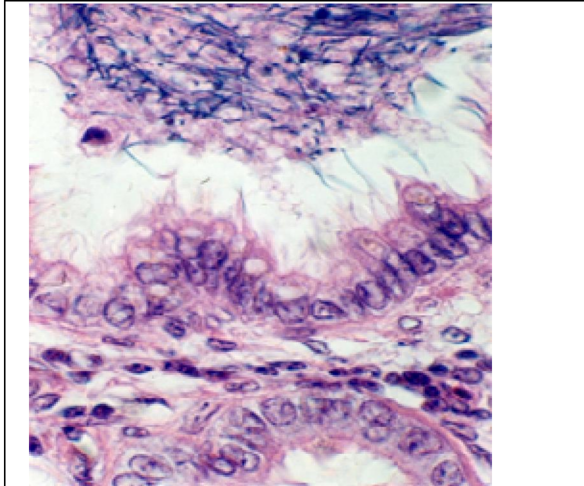
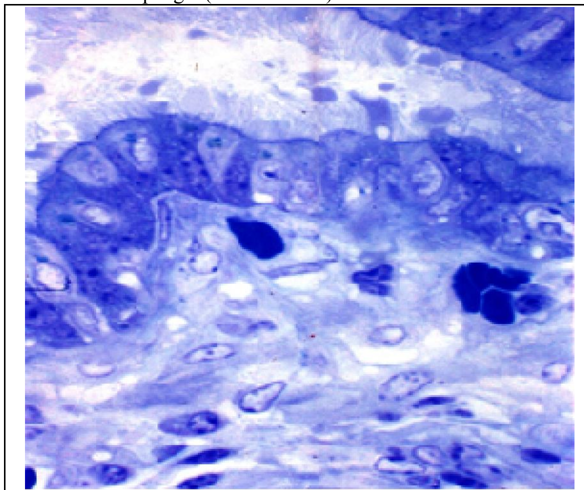


Fig. (12b): Distal efferent ductile showed apical acidophilic bodies & vacuoles. Notice the intraluminal aggregation of spermatozoa & luminal macrophage. (H&E X 1024)



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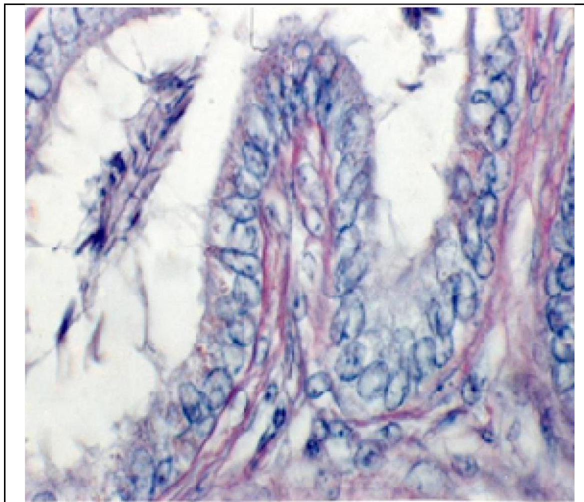

Fig. (14): Transverse section through distal efferent ductules showing PAS +ve granules and basal lamina. (PAS X1024)

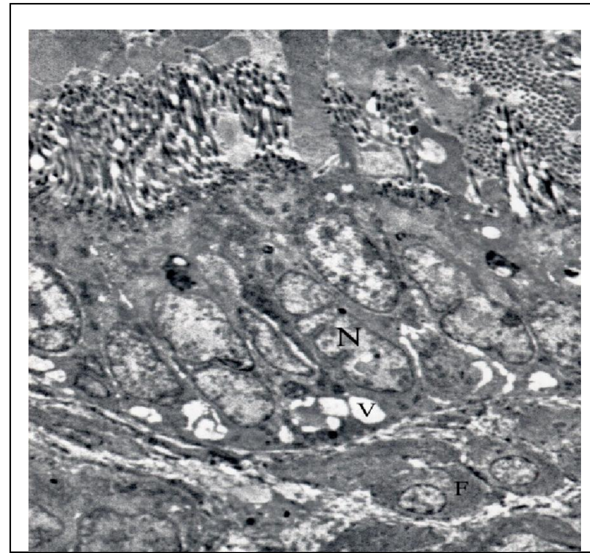

Fig. (15): TEM of the distal efferent ductulular epithelium showing tall columnar cells ciliated & nonciliated cells, Notice the highly irregular nuclei, basal vacuoles, apical mitochondria. Notice the peritubular fibroblasts & smooth muscle fibers. (x 6000)

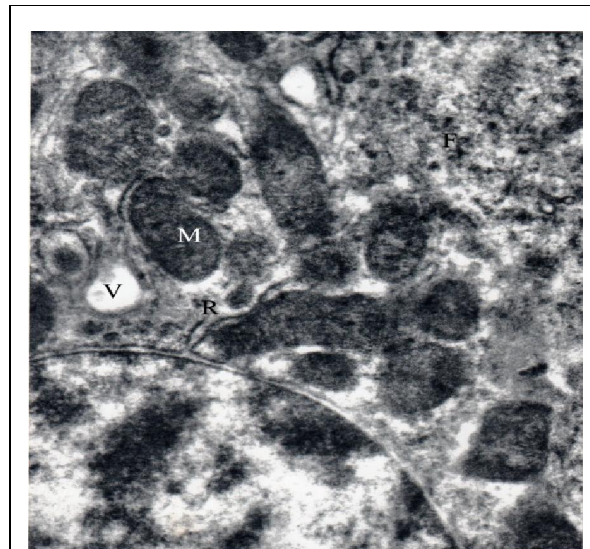

Fig. (16): Higher magnification of apical portion of distal efferent dutulular ciliated cell showing nucleus (N), many mitochondria (M), rER (R), Multiple vacuoles (V), free ribosomes (F). (X20000.)

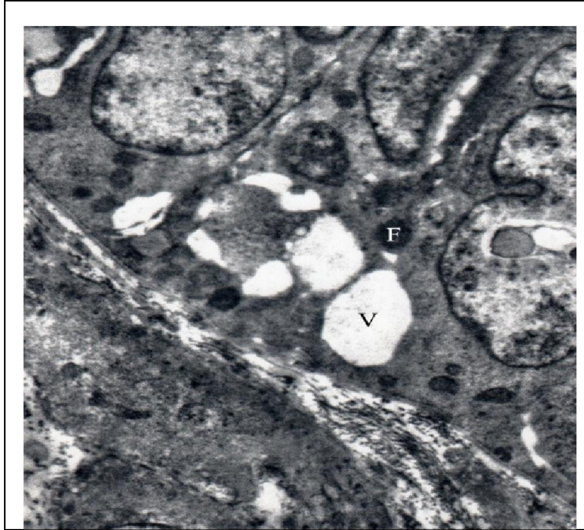

Fig. (17): Higher magnification of basal portion of distal efferent dutulular ciliated cell showing Multiple basal & intercellular vacuoles (V) & some dense bodies (F). (X20000)

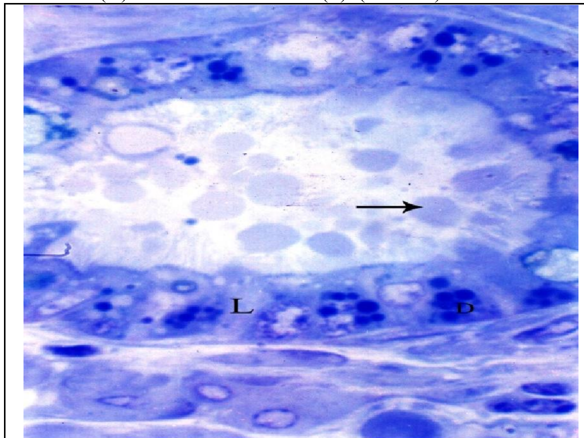

Fig. (18): Transverse section through the initial part of the connecting ductile, notice their regular outline, lined by light ciliated (L) & dark nonciliated cells(D), the dark nonciliated cells showing many dark granules & detached apical portion into their lumen (arrow). (T.B. X1024)

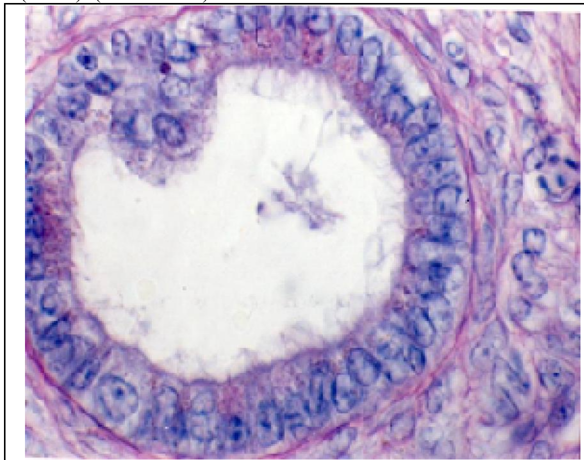

Fig. (19): Transverse section through the initial region of the connecting ductules showing their lining cells containing apical fine PAS +ve granules (PAS X 1024)

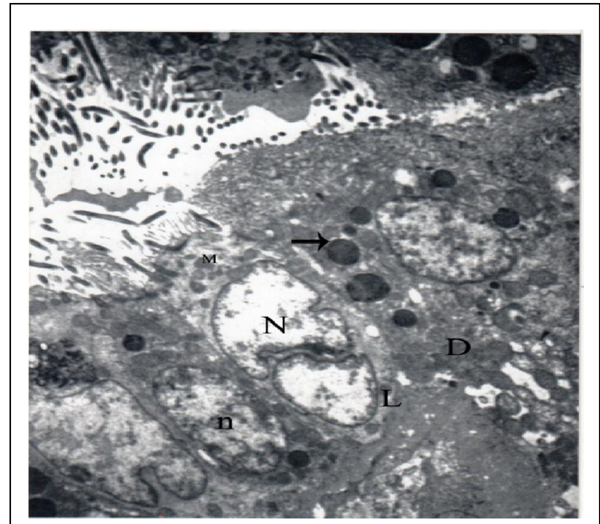

Fig. (20): TEM of the connecting ductules showing epithelial lining are dark nonciliated (D) & lighter ciliated cells (L), notice the highly irregular segmented nucleus (N) of ciliated cells & oval nuclei (n) in the nonciliated cells. Both types of cells contains multiple apical mitochondria (M), large electron dense granules (arrow), apical projection were seen in nonciliated cells. (X8000)Spermatozoa I the lumen some embedded at the apical surface of non ciliated cells

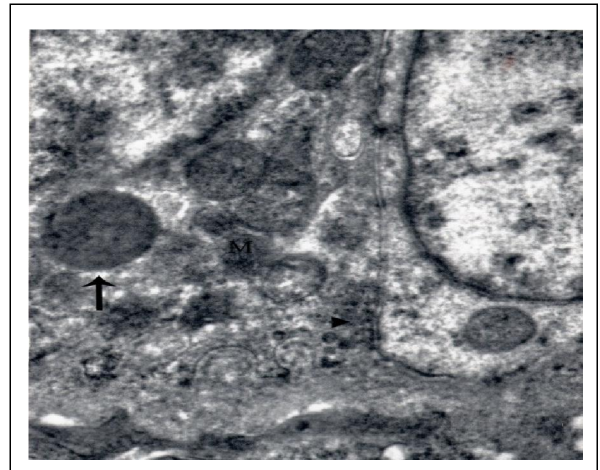

Fig. (21): TEM magnifying the basal part of the light & dark epithelial cells lining the connecting ductules. Notice the multiple electron dense granules (arrow) & mitochondria (M). Dark cells showing basal infolding with many hemidesmosomal junction (arrow head). (X 20000)

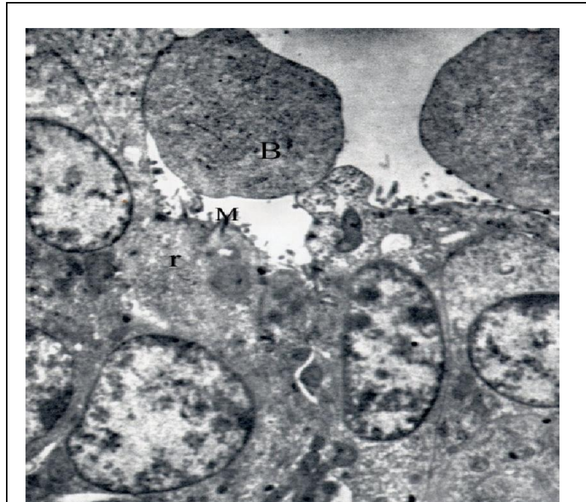

Fig. (22): TEM of the lining epith. Of terminal portion of connecting ductules showing absence of ciliated cells, the cells carry few microvilli (M), some cells showing apical bleb like projection (B), detached apical portion were seen in the tubular lumen, apical rER cistern (r) were present. (X 12000)

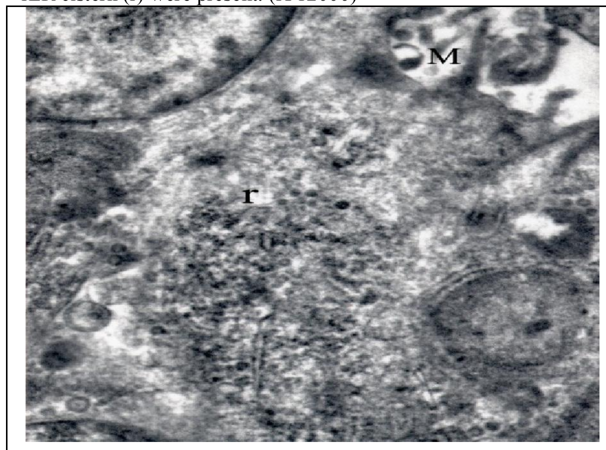

Fig. (23): Higher magnification of lining cell of connecting tubule, showing apical short microvilli (M) with microfilaments, multiple tubels pf rER (r), multiple microvesicles, dense bodies & dense granules. (X20000)

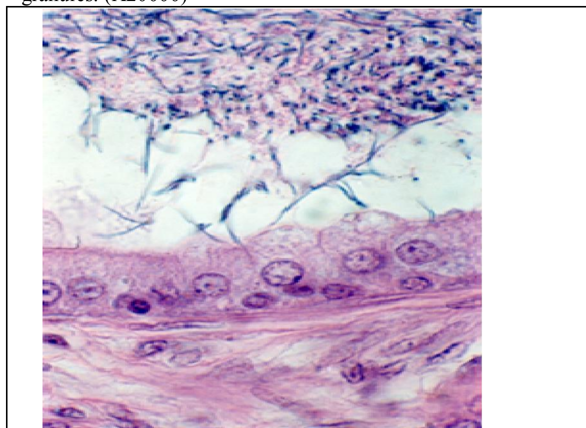

Fig. (24): section of the epididymal duct showing their lumen containing densely packed aggregation of spermatozoa (arrow), noncillated columnar cells with spherical nuclei, basal cells with more dark cytoplasm. (H&E 1024)

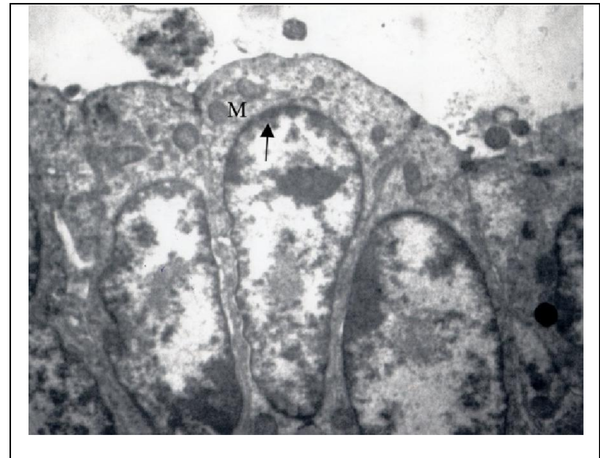

Fig. (25): TEM of the epididymal duct epithelium, all cells are columnar nonciliated with oval nuclei, contain many apical electron dense granules (arrow), mitochondria (M), dense bodies, apical tight junction, lighter intercellular space. (X 12000)

4.Discussion

The general structure of pigeon epididymal region was generally similar to that already described for domestic fowl **(Lake, 1957; Tingari, 1971; Budras and Sauer, 1975);** turkey **(hess *et al*., 1976);** japanese quail **(aire, 1979a);** guinea fowl **(Aira *et al*., 1979);** and duck **(Aire, 1982b and Sallam *et al*., 2000).**

Unlike the mammalian epididymis, it is not subdivided into head, body and tail since the efferent ductules arise throughout the entire length of the epididymis **(Lake, 1981).**

According to **Aire (1982a),** the extra testicular part of the rete testes of birds was termed the epididymal rete. The same author reported that the squamous rete epithelium changed into high cuboidal or columnar type before the typical epithelium of the proximal efferent ductules began, similar results were reported her in pigeon.

On the contrary **Tingari (1971)** and **Budras and Sauer (1975)** in fowl and **Aire *et al*., (1979)** in guinea fowl observed that this change was abrupt from squamous epithelium to the high columnar cells of the efferent ductules.

Most of studies dealt with the efferent ductules structure in different birds agreed with our results in that the efferent ductules consisted of two portions: the proximal and distal efferent ductules **(Aire, 1979a, 1980 &1982a; Budras and Sauer, 1975; Aire et al., 1979;).** On the other hand**, Tingari (1971)** did not describe such division in the fowl but he classified them as efferent ductules and narrow connecting ducts, respectively.

As described by **Aire (1979b)** in domestic fowl, japanese quail & guinea fowl, **Aire (1980)** in guinea fowl and **Aire (1982b)** in drake, that the proximal

efferent ductules revealed wider lumina and greater epithelial folding than the distal segment. In addition, the lumina of the first segment had scattered spermatozoa and desquamated immature germ cells, whereas that of the second segment were filled with a mass of closely packed spermatozoa. **Tingari (1971)** explained that the increased luminal contents of spermatozoa exert mechanical pressure on the folded wall of the ductules and might be regarded as a factor controlling the height of the ductalar epithelium as well as the luminal diameter.

Our study showed that mainly ciliated and non-ciliated columnar cells, in addition to few basal cells, lined both segments of the pigeon's efferent ductules. Similar results were recorded in fowl by **Budras and Sauer (1975a),** guinea fowl by **Aire *et al.* (1979),** Japanese quail by **Aire (1979a),** different birds by **Lake (1981)** and duck by **Sallam *et al.* (2001).**

Concerning the ciliated cells, **Aire (1980)** observed that such ciliated cells had a few short microvilli, vacuoles and flocculent content and small micropincytotic invaginations of the cell surface. So, our findings (presence of mulivesicular bodies, residual bodies, lipofuchsin pigment and the lysosome like dense bodies) strongly support the hypothesis of **Aire (1980)** in that the ciliated cells beside their principal role in the transport of the spermatozoa, they may also participate in the resorption of the seminal plasma and phagocytosis of the degenerated sperms. **Cooper and Hamilton (1977)** in rat assure that an extensive phagocytosis of spermatozoa occurs in the reproductive tract of intact males. The previous authors explained that the removal of these degenerated sperms before ejaculation cleared the lumen and permit the continual movement of the sperms along the tract.

The ultra structure of pigeon's epididymis followed **Aire *et al*., (1979) and Sallam *et al*., (2001)** in their classification of the non-ciliated cells of the proximal efferent ductules as type I and that of the distal efferent ductules as non-ciliated cells type II. Whereas, **Tingari (1972) and Hess & Thurston (1977)** described only one cell type, non-ciliated type i cell, for the efferent ductules in the fowl and turkey respectively.

The same point was discussed in guinea fowl **(Aire *et al*., 1979);** domestic fowl **(lake, 1981and Nakai *et al*., 1989)** and duck **(Aire 1982b)** that both the non-ciliated cells type II& I are characterized by microvillus projections of their luminal surfaces. However, the two cells, contained cytoplasmic vacuoles, pinocytotic vesicles, dense bodies or globules, lysosomes, fragments of spertmatozoa and tubular structures, but they were fewer and not so prominent in type II as those in type I. Moreover, **Aire (1980),** working on domestic fowl, japanese quail and guinea fowl recorded that the most striking characteristic of the type II as compared to type I cell was the absence of vacuoles and globules in the former cell. On the other hand, the type II cells had much more distended rER, a well-developed Golgi apparatus, smooth vesicles and electron dense secretory granules than the type I cell **(Lake, 1981).**

The present findings support the speculation of **Tingari and Lake (1972) and Nakai et al., (1989)** in fowl and **Aire (1979b and 1980)** in different birds that the morphological features of the ciliated cells indicated their positive participation in phagocytosis and digestion of broken down germ cells and degenerated spermatozoa as well as, pinocytosis of most of the fluid entering the epididymal region from the testes. **Aire (1980)** added that the activity of type I cell in resorption is more than type II cell. Such resorption may offer an explanation for the great concentration of spermatozoa in the distal efferent ductules and connecting ductules **(Tingr& Lake, 1981). El- rafey (1985)** suggested that the testicular fluid could be an unsuitable vehicle for sperm maturation in the epididymis and must be resorped for concentration.

As it was observed in the duck by **Aire (1982b)** and **Sallam *et al*., (2001),** that the apical cytoplasm of the non-ciliated cells may protrude into the ductular lumen to form blebs. These blebs represented a sign of apocrine secretion **(Tingari, 1971; Budras and Sauer, 1975; Hess *et al*. 1976; Hess and Thurston, 1977 and Bakest, 1980).** However, they were regarded as fixation artifacts by **Aire (1979a, 1980 7 1982b).** The last author reported that these blebs contained cell organelles such as mitochondria, dense globules (probably lysosomes) and rER. Also a demarcation zone between the blebs and the rest of the cell did not occur, as would be the case in apocrine secretion **(Kurosumi *et al*., 1961).**

The intra luminal released secretory cells have been observed in pigeon's efferent ductules were suggested to be mature holocrine secretory cells by **Martan & Risley (1963) and Martan & Allen (1964)** in mouse epididymis. The previous authors explained that these cells develop from the basal cells in a maturation cycle. They described the mature holocrine cells to be club-shaped with an expanded projecting apical part devoid of microvilli, central nuclei and a thin stalk attached to the basal lamina.

Martan & Risely (1963) added that mating shorten the cycle of holocrine cell maturation and the release of these cells from the epithelium. **Martan & Allen (1964)** assumed that the presence of holocrine cells in the epididymis establishes a secretory function of the organ these functions are likely related to the sperm maturation and maintenance in the epididymis. Moreover they reported increased holocrine secretory

activities with age and relate this finding to the increasing androgen levels with age.

Secretory capabilities have been also attributed to the efferent ductules of the fowl **(Tingari, 1972). Tingari (1973)** and **Budras and Sauer (1975)** showed that hormone synthesis occurred in the epididymal region of the sexually mature cockerel, especially in the proximal efferent ductules. **Aire (1980)** suspected a limited secretory activity in the non-ciliated type II and I cells. Meanwhile, **Lake (1981)** recorded that the non-ciliated type ii cells had ultra-structural features of typical protein secreting cells. Morphological evidence of secretory activities was indicated in the efferent ductules of the pigeon by the presence of vacuoles, PAS positive granules in the supra-nuclear cytoplasm, as well as rER and secretory vesicles. The secretory products of the epithelial cells of the efferent ductules might be needed for sperm nutrition.

Previous reports confirmed the presence of macrophages in the lumen of the rete channels and in the intraepithelial linning of the efferent ductules **(Tingari and Lake, 1972, Aire and Malmqvist, 1979b, Nakai *et al*, 1989 and Calvo et al, 1997).** These authors concluded that the spermiophagy by luminal and tissue macrophages are among the factors concerned in the disposal of unejeculated or degenerated spermatozoa in the epididymal region. Meanwhile, abundant macrophages were seen in the rete channels of fowl and duck by **Aire (1982)** and **Sallam et al. (2001).** In this respect, **Yeung *et al*., (1994)** postulated that the basal cells of the human epididymis might transform into macrophages.

The present study revealed that the connective tissue supported the rete channeles contained lymphocytic aggregations; some of these aggregations took the nodular form. **Aire, (1979b)** in domestic fowel and **Sallam *et al*., (2001)** in duck assured that lymphoid nodules were scattered erratically in the periductal region epididymis. Moreover, intra epithelial lymphocytes were also reported in the epididymal region of different species **(Aire & Malmquist, 1979a; Aire, 1980 and Calvo *et al*., 1997).** The presence of solitary non-encapsulated lymphatic nodules in the connective tissue of virtually all organs in the domestic fowl and wild birds was regarded as normal **(King and Mclelland, 1975).** These authors believed that these lymphoid cells were of the day-to-day immunological responses of the bird to its environment. **Balah *et al*., (1989)** suggested that these lymphocytes might add a more protective condition for the sperms inside the epididymal region of turkey. On the other hand, lymphocytic aggregation has developed a number of different immunological strategies including cell mediated one in fowl **(Sharma, 1997).**

Arie *et al*., (1979) in guinea fowl, **Lake (1981)** in birds and **Rikihsa and Lin (1988)** in japanese quail, **Sallam et al., (2001)** in duck and our study revealed that the epididymal duct possessed essentially the same structure as that of the connecting ductules, although it was larger in diameter. Both ducts were lined by a non ciliated columnar epithelium consisted of light and dark cells with fewer basal cells. Their lumina contained densely packed spermatozoa. **Aire *et al*., (1979)** reported that the connecting ductules in guinea fowl were hardly distinguishable from the epididymal duct. **Lake (1981)** found that the connecting ductules were at first narrow, but as they approached the epididymal duct began to anastomose with each other and thus became progressively wider. They end by joining the single epididymal duct.

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Inhibitory effect of *Psidium guajava* Linn. stem bark extracts on community acquired methicillin-resistant *Staphylococcus aureus*

Chibuike Ibe [1], Reginald Azu Onyeagba[1], Solomon Charles Ugochukwu[2], Venatius Chiamaka Ubah[3] and Chinenyenwa Joy Nduka[4]

[1]Microbiology Department, Abia State University, PMB 2000 Uturu, Abia State, Nigeria
[2]Plant Science and Biotechnology Department, Abia State University, PMB 2000 Uturu, Abia State, Nigeria
[3]Animal and Environmental Department, Abia State University, PMB 2000 Uturu, Abia State, Nigeria
[4]Environmental Health Technology Department, Abia State College of Health Technology, PMB 7016 Aba, Abia State, Nigeria
chibuike_ibe@yahoo.co.uk

Abstract: This study was conducted to investigate the inhibitory effect of *Psidium guajava* stem bark extract on methicillin-resistant *Staphylococcus aureus*. The plant materials were extracted and phytochemicals quantified using standard techniques. The agar well diffusion procedure and agar dilution method were used for the assessment of the antibacterial activity of the extracts, and quantitative determination of the bacteriostatic and bacteriocidal activities of the plant extracts respectively. The mean inhibition zone diameter (IZD) of the extracts ranged from 5-22 mm with ethanolic extracts exhibiting higher activities. The minimum concentrations range at which 50% and 90% of the isolates were inhibited for water and ethanolic extracts and their specific concentrations are: 1250->5000 µg/ml (MIC_{50}, 2500 µg/ml; MIC_{90}, 5000 µg/ml), and 625-5000 µg/ml (MIC_{50}, 1250 µg/ml; MIC_{90}, 2500 µg/ml) respectively. The phytochemicals estimated in mg/g dry body weight included: total phenol (111.82±0.47), tannin (141.98±0.51), flavonoids (3.31±0.04), vitamin C (1.59±0.03), saponin (285.79±0.81), and alkaloid (111.066±0.38). The methanolic extracts of the plant showed appreciable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical-scavenging ability of 0.056 mg/ml. Similarly, the plant extract had high trolox equivalent antioxidant capacity (TEAC) of 11.95±0.04 mM/gdw. The result of this study supports the use of *Psidium guajava* stem bark in folk medicine.

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Keywords: *Psidium guajava*; inhibitory effect; phytochemical analysis; community acquired methillin-resistant *Staphylococcus aureus*.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a vanguard for both nosocomial and community-acquired infections (Hossain et al., 2013). The antimicrobial resistance ability and extraordinary virulence of community acquired methicillin resistant *S. aureus* which allow it infect healthy persons are major medical issues worldwide. The term "methicillin-resistant *Staphylococcus aureus*" (MRSA) refers to those strains of *S. aureus* that have acquired resistance, whether in the community or in the hospital, to the antibiotics methicillin, oxacillin, nafcillin, cephalosporins, Imipenem, and/or other beta-lactam antibiotics (Hena and Sudha, 2011; Al-Anazi, 2009). *S. aureus* especially methicillin-resistance *S. aureus* (MRSA) is relatively ubiquitous and is the cause of many, community infections (Peters et al., 2013; McDougal et al., 2010; Okwu et al., 2012; Olowe et al., 2007; Yamamoto et al., 2006), endemic and epidemic nosocomial colonization (Hena and Sudha, 2011; Moussa et al., 2011) and infections (Al-Baidani et al., 2011; Olowe et al., 2007).

The two major strains of MRSA are known to be hospital-acquired (HA) MRSA and community-acquired (CA) MRSA (Milyani and Ashy, 2012). Community acquired MRSA (CA-MRSA) infections which were first described in small series of adult and paediatric patients presenting skin and soft tissue infections (SSTIs), pneumonia, or bacteremia have become a significant public health threat (Peters et al., 2013).

Since 1930, the epidemiology of *S aureus* has changed dramatically, and methicillin-resistant *S. aureus* (MRSA) has reached epidemic levels in both hospitals and community settings (Stenehjem and Rimland, 2013). Community acquired MRSA (CA-MRSA) has been increasingly reported from India (INSAR, 2013). These CA-MRSA strains cause serious skin and soft tissue infections, necrotizing pneumonia, and sepsis in healthy children (Saravanan et al., 2013). The epidemiological success of CA-

MRSA strain is believed to stem from combination of antibiotic resistance fitness at low cost with extraordinary virulence, allowing these strains infect otherwise healthy individuals and spread sustainably in the population (Perveen et al., 2013). The resistance to antibiotics in MRSA is due to the presence of *mecA gene* on the mobile genetic element, termed the staphylococcus cassette chromosome (SCC) (Corvaglia et al., 2010), which expresses a novel cell wall synthesizing enzyme, penicillin-binding protein 2A (PBP2A) with low affinity for all β-lactams (Al-baidani et al., 2011; Perwiaz et al., 2007; Brown et al., 2005).

The global emergence and increase of MRSA, also known as multidrug resistant or oxacillin resistant *S. aureus* have caused a shortage of effective beta-lactam antibiotics to MRSA based infections (Esimone et al., 2012). MRSA infections are very difficult to cure because MRSA strains are resistant to almost all clinically available antibiotics (Okwu et al., 2012).

Plants are the largest biochemical and pharmaceutical stores ever known on our planet. These living stores are able to generate endless biochemical compounds. In their living, human and animals are using only a small portion (1 to10%) of plants available on Earth (250,000 to 500,000 species) (Abdallah, 2011).

Psidium guajava (*P. guajava*) Linn. commonly known as guava is a plant of the family Myrtaceace. *P. guajava* is a low ever green tree or shrub 5 to 8 m high (Noudogbessi et al., 2013), with wide spreading branches and square downy twigs. It is a native of Tropical America (Esimone et al., 2012), but knows a good development in the tropical regions of Africa (Noudogbessi et al., 2013). *P. guajava* is one of the plants in folk medicine that has been used for the management of various disease conditions and is believed to be active. Various parts of the plant have been used in traditional medicine to manage conditions (Buvaneswari et al., 2011), extracts of root bark and leaves are used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wound, ulcers, toothache, cough, sore throat, inflamed gums and a number of other conditions (Esimone et al., 2012). The aim of the present study was to determine the inhibitory effect of *P. guajava* stem bark extracts on the growth of 10 community acquired methicillin-resistant *S. aureus* strains isolated from healthy individuals in Uturu rural communities, Abia State, Nigeria.

2. Materials and Methods

2.1. Cell cultures

Stock cultures of 10 methicillin-resistant *S. aureus* isolated from healthy individuals maintained on nutrient agar slants at 4°C at the Department of Plant Science and Biotechnology Laboratory, Abia State University, Nigeria were used in this study. These stock cultures were sub cultured on mannitol salt agar and incubated at 37°C for 24 hours to check for their purity and reidentification. The colonial morphology of the different bacterial isolates were observed and identified accordingly. After 24 hours of incubation of the organisms in a mannitol salt agar plates, a single colony of each bacterial isolate was picked and streaked on a fresh mannitol agar plate and incubated at 37°C for 24 hours. A pure colony of the pure culture was Gram stained and examined for Gram positive cocci in cluster. This was followed by testing the ability for DNase production on agar plates as well as the ability to produce catalase, and coagulase enzymes. Once the identity of each isolate was confirmed by Bergey's (Breed et al., 1957) manual according to Al-Jumaily et al. (2012), the isolate was inoculated onto nutrient agar slant, incubated overnight and then stored at 4°C until needed for further studies.

2.1.2. Oxacillin susceptibility testing

The oxacillin discs used was procured from Oxoid, Germany. The antimicrobial susceptibility testing of the isolates was determined using the disc diffusion technique assay as described by Orji et al. (2012). The discs were prepared by MAYO diagnostic laboratory, Nigeria according to the recommendation by Clinical and Laboratory Standard Institute (CLSI, 2007).

With a sterile wire loop, few colonies of each of the isolates were emulsified in 5 ml of sterile peptone water to a turbidity corresponding to 0.5 McFarland standards (corresponding to approximately 10^8 cfu/ml). Then 0.5 ml of each inoculum was dispensed unto the surface of dried Mueller Hinton agar plate using sterile Pasteur pipette. These were spread evenly on the agar surface with sterile swab sticks (one for each inoculum). The excess inocula were discarded into a disinfectant jar. The inoculated plates were kept on the bench for 3 minutes to dry. The oxacillin disc (1 µg) was then placed centrally on the inoculated plates aseptically using a sterile forceps.

The preparations were incubated aerobically for 24 hours at 35°C. The diameter of zone of inhibition produced by each of the disc was measured, recorded and the isolates were classified as resistant (≤ 10 mm) or sensitive (≥13 mm) based on the standard interpretative chart as described by the Clinical and Laboratory Standard Institute (CLSI, 2010; CLSI, 2007).

2.1.2. Plant collection and preparation

Fresh stem bark of *P. guajava* (guava) was collected from Uturu, Abia State, Nigeria. The plant

materials were identified and authenticated in the taxonomy unit of Department of Plant Science and Biotechnology, Abia State University, Nigeria. The plant materials were chopped into small pieces, air-dried at room temperature and ground into powder using a manual blender. Exposure to direct sunlight was avoided to prevent the loss of active components. The dried powder was stored at 4°C until further analysis.

Analytical grade ethanol, BDH and distilled water were used for extraction. Reagents for phytochemical screening of extracts were freshly prepared using standard methods.

2.1.2.1. Preparation of plant materials aqueous extract

This was done according to Soniya et al. (2013) with minor modification. Fifty grams of dried powdered plant (stem bark) material was macerated with 500 ml of distilled water and allowed to stand for 24 hours while agitating at regular time intervals. The extract was filtered through Whatman No. 1 filter paper, and evaporated to dryness at 40° C.

2.1.2.1. Preparation of plant materials solvent extract

Fifty gram of dried powdered plant (stem bark) material was soaked separately for 24 hours in 500 ml 50% (v/v) ethanol. The soaked material was agitated at regular time intervals. After 24 hours the soaked material was filtered using Whatman filter paper No. 1 on separate filtration setups. The final filtrates was collected in wide mouthed evaporating bowls and evaporated to dryness at 40° C.

The dried extracts were weighed to calculate the extractability percentage. The extracts were stored at 4°C until further use.

2.1.3. Sensitivity test: agar well diffusion assay

The assay was conducted using agar-well diffusion method described by Esimone et al. (2012). An 80 mg/ml concentration of both ethanol and water extracts of *P. guajava* was constituted by dissolving 0.16 g of the dry extract in 2 ml each of 20% v/v dimethyl sulfoxide (DMSO) and 2-fold dilutions made to obtain 40 mg/ml and 20 mg/ml. A single colony of the isolates each was suspended in 2 ml of sterile peptone water. The suspension of each isolate was standardized as stated previously and used to inoculate the surface of the Mueller Hinton agar and the excess fluid drained into disinfectant jar. The inoculated agar surface was allowed to dry and the plates appropriately labeled. Using a cork borer, two wells of 5 mm in diameter was bored in the inoculated Mueller Hinton agar. With a micropipette, 50 µl of each concentration of the test extracts was delivered into each well. The plates were left on the bench for 30 minutes to allow the extracts to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for inhibition zones around the wells. The diameter of the zones was measured with metre ruler to the nearest whole millimetre. Each test was carried out thrice and the mean inhibition zone diameter (IZD) recorded to the nearest whole millimetre.

2.1.4. Minimum inhibitory concentration (MIC) of plant extracts

This was carried out using agar dilution method following the procedure outlined by Esimone et al. (2012) and CLSI (2007). For each extract, 80 mg was weighed and dissolved in 2 ml of 20% v/v DMSO to get a stock solution with concentration of 40 mg/ml. Sterile test tubes were arranged on a test tube rack and 1 ml of sterile distilled water was dispensed into them. From the stock solution, 1 ml was transferred into the first test tube and dilution of the extract was carried out and the resultant concentrations in the test tubes were 40, 20, 10, 5, 2.5, 1.25, 0.625, and 0.3125 mg/ml.

One millilitre each of the extracts dilution was added to 19 ml of sterile molten nutrient agar, mixed thoroughly and poured into sterile Petri dishes. The plates were allowed to solidify and then labeled appropriately. The plates were kept overnight in the incubator to check for their sterility.

A single colony of each test isolate was picked from the sub cultured plates with wire loop and inoculated into 2 ml sterile peptone water to make a suspension of each test isolate. Each suspension was standardized as stated previously. Using a micropipette, a 10 µl of the standardized broth cultures was placed on the surface of the plates containing various concentrations of the extracts. Plain Mueller Hinton agar (that is, without the extract) was also streaked and served as negative controls. Inoculated plates were then incubated at 37°C for 24 hours and observed for any visible bacterial growth. MIC was taken as the lowest concentration of extract that resulted in no visible growth on the surface of the agar.

2.1.5. Minimum bactericidal concentration (MBC) of plant extracts

After completion of the MIC procedure, the agar plates showing no growth in the MIC test were used for the determination of the MBC. Blocks were cut out from the plates that showed no growth in the MIC test and transferred to a corresponding test tube of fresh nutrient broth, acting as the recovery medium. The newly inoculated broth medium was incubated for 24 hours at 32°C. At the end of incubation, microbial growth was ascertained by checking the turbidity of the medium. The absence of turbidity in the recovery medium was evidence of total cell death.

2.1.6. Phytochemical Analyses

These analyses determine the biologically active non-nutritive compounds that contribute to the flavour, colour, and other characteristics of plant parts. Quantitative analyses were done at the International Institute of Tropical Agriculture (IITA), Ibadan.

2.1.6.1. Preparation of methanolic extract

Methanolic extract of the guava powder (stem bark) was prepared following the method of Chan et al. (2007), by adding 25 ml of methanol to 0.5g of sample contained in a covered 50 ml centrifuge tube, and shaking continuously for 1 hour at room temperature. The mixture was centrifuged at 3,000 rpm for 10 minutes, and then the supernatant was collected and store at -4°C for further analysis.

2.1.6.2. Determination of total phenol content (TPC)

The total phenol content of sample methanolic extract was determined according to the Folin–Ciocalteu method reported by Chan et al. (2007). Briefly, 300 µL of extract was dispensed into test tube (in duplicates). To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na_2CO_3 solution (7.5% w/v). The reaction mixture was mixed, allowed to stand for 30 minutes at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 µL of distilled water instead of sample extract. TPC was expressed as gallic acid equivalent (GAE) in mg/g material.

2.1.6.3. Determination of tannin content

Tannin content of sample was determined according to the method of Padmaja (1989) as follows. Sample (0.1 g) was extracted with 5 mL of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The mixture was centrifuged at 3,000 rpm for 20 minutes. About 0.1 mL of the supernatant was added with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured at 760 nm. Blank was prepared with water instead of the sample. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material.

2.1.6.4. Determination of total flavonoid content (TFC)

Total flavonoid content was determined using aluminum chloride method as reported by Kale et al. (2010). About 0.5 ml of methanolic extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The reaction mixture was mixed, allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm. TFC was expressed as quercetin equivalent (QE) in mg/g material.

2.1.6.5. Determination of vitamin C content

The vitamin C content of the aqueous extract was determined using the method reported by Benderitter et al. (1998). Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 ml of 5 M H_2SO_4) was added to 500 µL reaction mixture (300 µL appropriate dilution of hydrophilic extract with 100 µL of 13.3% trichloroacetic acid and distilled water). The reaction mixture was subsequently incubated for 3 hours at 37°C, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the vitamin C content of the sample was subsequently calculated from the calibration curve prepared with ascorbic acid standard.

2.1.6.6. Determination of total saponin content

Total saponin was determined by the method described by Makkar et al. (2007). About 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 hours, after which contents of the tubes were centrifuged for 10 minutes at 3,000 rpm. In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 minutes. Then tubes were cooled in ice for 4 minutes and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a Uv/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

2.1.6.7. Determination total alkaloid content

The total alkaloid content of the sample was measured using 1,10-phenanthroline method described by Singh et al. (2004) with slight modifications. About 100mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000rpm for 10 minutes. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1 ml plant extract, 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCl and 1ml of 0.05 M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2°C. The absorbance of red coloured complex was measured at 510 nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of quinine (0.1 mg/ml, 10 mg dissolved in 10 ml ethanol and diluted to 100 ml with

distilled water). The values were expressed as mg/g of dry weight.

2.1.7. Antioxidant Activity Determination

2.1.7.1. Estimation of DPPH free-radical-scavenging ability

The free-radical-scavenging ability of the methanolic extract against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Cervato *et al.* (2000) with slight modification. Briefly, appropriate dilution of the extracts (1 ml) was mixed with 3 ml of 60 μM methanolic solution of DPPH radicals; the mixture was left in the dark for 30 minutes before the absorbance was taken at 517 nm. The decrease in absorbance of DPPH· on addition of test samples in relation to the control was used to calculate the percentage inhibition (%Inh.) following the equation: %Inh. = $[(A517_{control} - A517_{sample}) \div A517_{control}] \times 100$. The IC_{50}, which stands for the concentration of extract required for 50% scavenging activity, was calculated from the dose-inhibition linear regression curve of each extract.

2.1.7.2. Estimation of ABTS* radical-scavenging ability

The ABTS* radical-scavenging ability of extract was determined according to the method described by Sellappan and Akoh (2002). The ABTS* radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 hours at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 ml appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance was measured at 734 nm after 15 minutes The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated. The calibration equation for TEAC was $Y = -0.0505x + 0.1954$ ($R^2 = 0.9902$).

3. Results and Discussion

Methicillin-resistant *S. aureus* (MRSA) is primarily a nosocomial pathogen that emerged as a major cause of infection and colonization in hospitalized patients, whereas, community-associated MRSA infections are increasing in incidence and are said to be severe enough to cause fatality (Mandell et al., 2005). Strains of MRSA that cause infections have also developed resistance to antibiotics commonly used for therapeutic purposes (Milyani and Ashy, 2012). Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants (Milyani and Ashy, 2012). From the result of this study, the extractability percentage of *P. guajava* stem bark is 16.6% for water and 19.04% for ethanol. This indicates that the chemical components (antibacterial principles) of *P. guajava* were more soluble in ethanol than in water. The water and ethanolic extracts of *P. guajava* stem bark were active against all the 10 isolates of MRSA tested with a mean inhibition zone diameter (IZD) which ranged from 5-22 mm as shown in table 1. The ethanolic extracts had more activity than water extracts with a maximum IZD of 22 mm on 1 tested MRSA isolate. This result is in agreement with the work of Joseph and Priya (2011), who in related study reported a maximum inhibition zone diameter of 21 mm against *S. aureus* using methanolic extract of *Psidium guajava* stem bark. Water extract only recorded a maximum inhibition zone diameter of 18 mm on 2 MRSA isolates. The activity observed with ethanolic extract may be used to improve the medicinal value (enhance application) of the plant in common practice in traditional medicine to use the plant extracts prepared in form of infusion or decoctions.

The MIC results showed that the 10 isolates were inhibited by ethanolic extract with activities that ranged from 625-5000 μg/ml while only 9 isolates were inhibited by water extract with activities that ranged from 1250-5000 μg/ml (table 2). The MBC results showed 8 isolates to be susceptible to ethanolic extract within the range of 625-5000 μg/ml whereas 6 isolates were susceptible to the water extract with activity range of 1250-5000 μg/m (table 3). The results of well diffusion assay, MIC and MBC on the MRSA isolates agreed with the results of Anas et al. (2008) who in a related study reported higher activities in the methanolic extract of *P. guajava* leaves than in aqueous extracts. The results of this study is in contrast to the reports of Esimone et al. (2012) who in a similar study reported maximum activities with water extracts of *P. guajava* stem bark than methanolic extracts on MRSA isolates and also with that of Buvaneswari et al. (2011) who in a related study reported higher antibacterial activity with water extracts of *P. guajava* leaves than with 75% methanol extracts.

This study indicated that ethanolic extracts had lower and more active MIC_{50} and MIC_{90} concentrations than water extracts. The ethanolic extracts had MIC_{50} and MIC_{90} at 1250 μg/ml and 2500 μg/ml respectively, whereas, water extracts were at 2500 μg/ml and 5000 μg/ml (table 4).

Table 1: Susceptibility of MRSA to *P. guajava* stem bark extract

Extract (Conc. in (mg/ml)	Mean IZD of MRSA isolates (mm)									
	1	2	3	4	5	6	7	8	9	10
Water (40)	15	13	14	17	6	18	11	15	18	15
(20)	8	9	10	11	5	13	7	10	15	7
Ethanol (40)	17	13	18	20	6	20	12	16	22	16
(20)	9	10	10	13	6	14	8	10	15	9

Table 2: Minimum inhibition concentration (MIC) of *P. guajava* stem bark extracts on MRSA isolates

Extract	MRSA isolates (MIC (µg/ml))									
	1	2	3	4	5	6	7	8	9	10
Water	2500	5000	5000	2500	>5000	1250	5000	1250	1250	2500
Ethanol	1250	2500	1250	2500	5000	625	2500	1250	625	625

Table 3: Minimum bacteriocidal concentration (MBC) of *P. guajava* stem bark extract on MRSA isolates

Extract	MRSA isolates (MBC (µg/ml))									
	1	2	3	4	5	6	7	8	9	10
Water	5000	>5000	>5000	2500	>5000	2500	>5000	5000	1250	2500
Ethanol	2500	2500	2500	2500	>5000	1250	>5000	1250	625	2500

Table 4: MIC_{50} and MIC_{90} (µg/ml) of *P. guajava* extract for MRSA isolates

n = 10

Extract	MIC (µg/ml) MIC range	MIC_{50}	MIC_{90}
Water	1250–>5000	2500	5000
Ethanol	625–5000	1250	2500

The result of this study supports the findings of other workers on the antimicrobial studies of *P. guajava* extracts. The antibacterial activity of organic extracts and essential oils of *P. guajava* leaves was investigated by Goncalves et al. (2008), and the methanolic extract showed the highest inhibition against shrimp isolates and type strains of *S. aureus*, *E. coli* and *Salmonella* species.

The phytochemical contents of the stem bark are presented in table 5.

Table 5: Total phenol, tannin, flavonoids, vitamin C, saponin, and alkaloid expressed in mg/g dry body weight

Phytochemical	*P. guajava* (stem bark)
Total phenol	111.82±0.47
Tannin	141.98±0.51
Flavonoids	3.31±0.04
Vitamin C	1.59±0.03
Saponin	285.79±0.81
Alkaloid	111.066±0.38

Data represent the mean ± standard deviation of duplicate readings.

Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free-radical scavenging, lipid peroxidation and chelating of metal ions. They were reported to eliminate radicals due to their hydroxyl groups. In addition to these antioxidant effects, phenols were reported to inhibit alpha-amylase, sucrase, as well as the action of sodium glucose-transporter 1 (SGLUT-1) of the intestinal brush border, hence their antidiabetic action (Balakrishnan et al., 2010). They have also been reported to exert anti-inflammatory and anti-carcinogenic effects.

Tannin, the dietary anti-nutrients that are responsible for the astringent taste of foods and drinks, are known to cause browning or other pigmentation problems in both fresh foods and processed products. The presence of tannin in *P. guajava* stem bark suggests that it may have astringent properties and in addition, could quicken the healing of wounds and burns. The antibacterial action of tannins against *S. aureus*, is attributed to the antimicrobial mechanisms of their (I) astringent property (II) toxicity, and (III) complexation of metal ions (Esimone et al., 2012).

Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress, which has been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. The high flavonoid content of the stem bark may have contributed to the medicinal properties (Irondi et al., 2012).

The total saponin content of the plant was found to be quite high. The biological activities of saponins have been reviewed and include haemolytic, hypoglycaemic, antioxidant and hypolipidaemic activities, lowering of cancer risks, virucidal activity,

reduction of protein digestibility, antimicrobial activity, among others.

Alkaloids are a group of basic organic substances of plant and microbial origin, containing at least one nitrogen atom in a ring structure in the molecule. They were reported to be the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents due to their analgesic, antispasmodic and antibacterial properties (Oboh and Irondi, 2012).

As a water-soluble antioxidant, vitamin C is in a unique position to 'scavenge' aqueous peroxyl radicals before these destructive substances damage the lipids. These phytochemicals observed in the *P. guajava* stem bark may either individually or in combination be responsible for the antibacterial activity exhibited.

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts of the stem bark of *P. guajava.* As antioxidants donate protons to this radical, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging (Irondi et al., 2012; Oboh and Irondi, 2012). The IC_{50} value for the extract, defined as the concentration of extract causing 50 per cent inhibition of DPPH absorbance, is shown in table 6.

Table 6: DPPH IC_{50}, and Trolox equivalent antioxidant capacity (TEAC) of *P. guajava* stem bark methanolic extracts.

Antioxidant Activity	*P. guajava* stem bark
DPPH IC50 (mg/ml)	0.056
TEAC (mM/gdw)	11.95±0.04

Data for TEAC represent the mean ± standard deviation of duplicate readings.

P. guajava had IC_{50} of 0.056 mg/ml. Since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value is a reflection of greater antioxidant activity of the sample. Hence *P. guajava* displayed a high free radical scavenging ability.

ABTS* scavenging ability reported as the Trolox equivalent antioxidant capacity (TEAC) is presented in table 6. The results revealed that the ABTS* scavenging ability of *P. guajava* stem bark is high (11.95±0.04 mM/gdw). The ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS* which has a characteristic long wavelength absorption spectrum.

ABTS radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical which involve H atom transfer the reactions with ABTS radicals involve electron transfer process. From our results, there is a linear correlation between total phenol content and the antioxidant activity of *P. guajava* stem bark extract. The high reducing power of *P. guajava* extract is attributable to its high total phenol, tannin, total flavonoid and saponin contents, which are also reflected in its high DPPH free-radical-scavenging ability and TEAC.

In conclusion, the results of this study showed that *P. guajava* has promising medicinal properties due to the high content of phytochemicals which have been shown to exhibit antimicrobial characteristics and have validated the folkloric use of *P. guajava* in the treatment of bacterial infections especially those associated with *S. aureus*. Natural products still represent an important source of interesting leads for drug development. The plant could be exploited greatly in the development of phytomedicines for the control or management of resistant bacteria like CA-MRSA.

Corresponding author:
Chibuike Ibe
Microbiology Department
Abia State University
PMB 2000 Uturu
Abia State, Nigeria
Tel: +234(0) 805 9322 893
Email: chibuike_ibe@yahoo.co.uk

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Bacteriological Quality and Safety Evaluation of Raw Cow Milk in Ilorin, North Central Nigeria.

Laba, Sunday Ademola[1*] and Udonsek, Christiana Effiong[2]

[1]Department of Microbiology, University of Ilorin, P. M. B 1515, Ilorin 240003, Nigeria.
[2]Department of Microbiology, University of Ilorin. P. M. B 1515, Ilorin 240003, Nigeria.
labademola@yahoo.com

Abstract: Microbial contamination of raw milk has become a global health problem. Large number of people in Nigeria consume raw cow milk due to its cheap status. These study was undertaken to investigate the bacterial quality of of raw cow milk in Ilorin, and surrounding villages, Nigeria and the effect of pasteurization on the samples. Raw milk samples (n=12) were aseptically collected from the milking bowls from different locations. The samples were analyzed within three hours of procurement. Isolation, enumeration and identification of the prevailing bacteria were carried out following the standard procedure. The pH of the samples ranges between 6.3-6.8 while the TTA is from 0.87-1.98. Analysis of the Milk sample revealed high load of bacterial pathogens such as, *Listeria monocytogenes, Staphylococcus aureus, Salmonella spp., Escherichia coli, Klebsiella spp., Pseudomonas spp., Proteus spp.* and *Bacillus cereus.* Total viable count ranges from 1.16 x 10^6 to 2.60 x 10^6 while the pasteurization count ranges between 0.8 x 10^2 to 1.2 x 10^2. Results indicate the potential health risk of consuming raw cow milk under the current production and collection condition.

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Key words: Raw milk, Pasteurized milk, Escherichia coli, Coliforms, Staphylococcus aureus.

Introduction:

Raw milk has been a known vehicle for pathogens for more than 100years (Gillespie *et al*.,2003). Milk has been part of the human diet for thousands of years and it is a compulsory part of daily diet for expectant mothers as well as growing children. It contains lipids, proteins (casein, whey), carbohydrates (lactose), amino acids, vitamins and minerals (calcium), essential for growth(Haug *et al*., 2007; Javaid *et al*., 2009). It is a vital type of food for over 6 billion human zbeings all over the world and a major contributor to food security as it alleviates poverty and mitigates malnutrition (Belewu, 2006). Milk is predominantly (80–87%) water and protein in milk is predominantly (82–86%) casein with smaller amounts of globulins. Raw milk pH ranges from 6.4 to 6.8, with an average pH of 6.6 making it slightly acidic (William *et al.,* 2005). It is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining its quality (Karmen and Slavia, 2008). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits *et al*., 2008). Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008). The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health (Karmen and Slavia, 2008).

According to U.S. Food and Drug Administration (ICMSF, 2006), EEC directive 92/46(EC 2001), Beuvier and Buchin,2004 the principal pathogens of concern associated with milk and processed milk products are *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, pathogenic *E. coli*. Many of the common enteric pathogens such as *Salmonella*, *Escherichia coli* O157: H7 and *Campylobacter* are carried in the intestinal tract of ruminants, including domestic animals used in milk production, e.g. cows, sheep and goats(Baylis, 2009). Effective cleaning procedures, including removing faecal material from udders prior to milking and good manufacturing practices during cheese making process can reduce the risk (Baylis, 2009). Microbes may gain entry into raw milk directly from dairy cows experiencing sub clinical or clinical mastitis (Rodojcic-Prodaova *et al.,*1991). A number of bacteria including *S. aureus, Escherichia coli* and *Salmonella* have been recovered from raw milk (De Buyser *et al*., 2001) and some of these have been determined to be pathogenic and toxicogenic, and implicated in milk- borne gastroenteritis (Maguire *et al*., 1992). In recent year's *E. coli* 0157: H7 strain has become very important milk-borne pathogen and cattle are considered its main reservoir (Betts, 2000 ; Karmali, 1989).

Raw milk advocates claim that raw milk is healthier and has higher nutritional value than pasteurized milk, however research has shown no significant difference in the nutritional value of pasteurized and unpasteurized milk (Centers for Disease Control and Prevention, 1999; Hegarty et al., 2002). The coliform group of bacteria is defined as the indicator (faecal coliform) of suitability of milk for drinking (Chatterjee *et al*.,2006). Some isolates of S. aureus produce staphylococcal enterotoxins (SEs) that may cause food poisoning if food containing sufficient preformed SE is ingested. Symptoms typically have a rapid onset (1–6 h) and often include nausea, vomiting, diarrhoea and abdominal pain (Jablonski and Bohach 1997). Usually the condition is self-limiting and recovery is rapid. Minor outbreaks of staphylococcal food poisoning (SFP) are therefore likely to go unreported, and the true incidence of SFP is probably underestimated (Jorgensen *et al*., 2005). Outbreaks of foodborne illnesses following consumption of raw milk and products made from raw milk caused by Shiga toxin-producing *Escherichia coli* (**STEC),** (Proctor and Davis, 2000), *Salmonella* spp. (Mazurek *et al*., 2004), and *Listeria monocytogenes* (Centers for Disease Control and Prevention, 2001) have been reported. Gillespie *et al*. (2003) reported that between the years of 1992 and 2000, 52% of foodborne outbreaks in England and Wales were attributed to raw milk. Raw milk and products made from raw milk have been implicated in similar numbers of documented cases of foodborne illness in France (De Buyser *et al*.,2001). An estimated 1.4 million cases of salmonellosis occur annually in the United States (Mead *et al*., 1999). Sales of raw milk directly to the public have resulted in foodborne outbreaks of multidrug-resistant salmonellosis in California and Washington (Reed and Grivetti, 2000),

In Nigeria, raw milk is traditionally consumed at the small farms and in town where it is taken in addition with other food materials or process into soft cheese. The risk of contaminated and pathogen containing products could therefore be even greater than when the milk is processed at household level (FAO and WHO 1997). The importance of various etiological agents in milk borne disease has changed dramatically over time. The presence of these pathogenic bacteria in milk emerged as major public health concerns, especially for those individuals who still drink raw milk (Riser, E.T. 1998). *E. coli* 0157: H7 has become serious threat to the dairy industries ranging from mild diarrhoea to potentially fatal hemolytic uremic syndrome (HUS), hemorrhagic colitis and thrombotic thrombocytopenic purpura (Coia, *et al* 2001).

The intent of pasteurization of milk is to eliminate pathogenic microbes, also to lowers microbial numbers, which prolongs milk's good quality and shelf life under refrigeration. However, spores are not affected by pasteurization **(**Montville and Matthew, 2005). This study investigate the bacteriological quality, safety and effects of Pasteurization on raw milk samples.

Materials and Methods

Sample Collection

Twelve raw milk samples were collected from the milking bowl from Fulani cattle settlement in sterile bottles in Ilorin and surrounding villages, Nigeria. The samples were collected in the morning and transported to the laboratory on ice maintaining sterile condition and analyzed within 2 hours of sampling.

Microbiological Analysis.

Isolation and enumeration of microbes were performed using serial dilution of samples carried out up to 10^{-6} in peptone water(OXOID, Unipath Ltd., Basingstoke, Hampshire, England). Samples were plated in duplicate using pour plate technique. 0.5ml of diluted samples was delivered by pipette into 19.5ml of enriched agar. The plates were incubated at 37^0C for 24-48 hours. Total viable count were carried out on plate count agar. The number of colony forming unit(CFU/ml) were recorded after the incubation period. The presence of specific microorganism were done through pour plate technique on selective media. De Mann Rogosa and Sharpe(MRS-agar)(OXOID, Unipath Ltd., Basingstoke, Hampshire, England). for Lactic acid bacteria, Salmonella Shigella agar(SS-agar) for salmonella colonies, Coliform count was carried out with MacConkey Agar and Eosine Methylene Blue agar(EMB-agar).

Identification and characterization of microbial isolates.

Following incubation, the isolated colonies were pure cultured and Gram stained. Biochemical characterization of the isolated colonies was carried out using standard protocols (Kannan, 2002). Identification was carried out according to Bergey's Manual.

Laboratory Pasteurization Count(LPC).

10ml of the raw cow milk samples were dispensed into sterile MacCartney bottles and heated at a temperature of 62.9^0C for 30 minutes using the water bath. The samples were then inoculated on agar plates and incubated at 37^0C for 24 hours after which the plates were observed

RESULTS

Table 1 shows the physico-chemical analysis of the raw milk samples. The colour of the samples are white, light yellow and yellowish white. The pH of the samples ranges between 6.3 to 6.8 whose average is 6.6. The titratable acidity(TTA) of ranges from 0.87 to 1.98. As the pH reduces, the TTA increases.

Table 1: **Physico-chemical analysis of samples.**

Sample	Colour	pH	TTA
M-1	Light yellowish	6.8	0.87
M-2	White	6.6	1.30
M-3	Yellowish White	6.5	1.55
m-4	Yellowish white	6.6	1.36
M-5	White	6.5	1.56
M-6	Yellowish white	6.3	1.98
M-7	White	6.7	0.99
M-8	Yellowish white	6.6	1.37
M-9	Light yellowish	6.4	1.87
M-10	Yellowish White	6.5	1.59
m-11	Yellowish white	6.6	1.33
M-12	Light yellowish	6.4	1.86

Key: TTA – Titratable Acidity.

Table 2 shows the enumeration of microorganism of the different milk samples using the standard method. The results shows that the standard plate count on plate count agar ranges from 1.16×10^6 to 2.60×10^6 while the laboratory pasteurization count reveals that the count ranges from 0.7×10^2 to 1.2×10^2.

Table 2. Enumeration of microorganism in different milk sample by standard plate count method.

Sample	SPC	LPC
M-1	2.60×10^6	1.2×10^2
M-2	2.49×10^6	1.2×10^2
M-3	1.16×10^6	1.5×10^2
M-4	1.94×10^6	0.8×10^2
M-5	2.34×10^6	0.9×10^2
M-6	1.76×10^6	0.8×10^2
M-7	2.59×10^6	1.1×10^2
M-8	2.50×10^6	1.2×10^2
M-9	1.18×10^6	0.7×10^2
M-10	1.92×10^6	0.8×10^2
M-11	2.31×10^6	1.0×10^2
M-12	1.76×10^6	0.9×10^2

Key;
SPC - Standard Plate Count
LPC - Laboratory plate Count

Figure 1 shows the frequency of occurrence of bacterial isolate in the samples. E. coli and S. aureus was discovered in all the sample making them the highest while the lowest occurrence is recorded in *Salmonella spp.* and *Klebsiella Spp.*

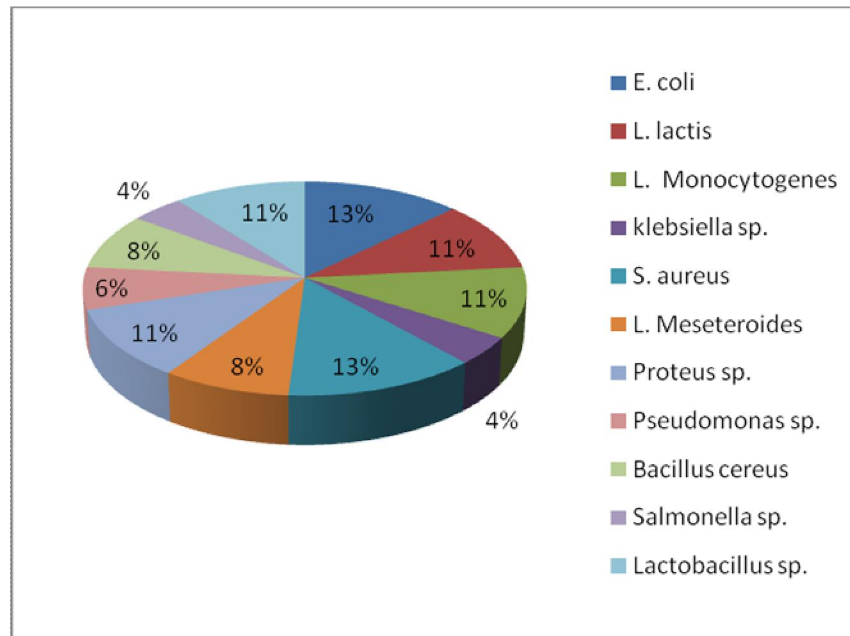

Figure 1. Frequency of occurrence of the isolates.

DISCUSSION

Pathogenic bacteria have been a major concern to the public all over the world. The fact that milk contain a lot of nutrients made it havens for growth and development of host of microorganisms including the pathogenic ones(Saeed *et al*., 2009). Pareke and Subhash,(2008) asserted that, animal health, milking utensil and the environment are contributors to contamination of fresh raw milk.

Also, the unclean or unsterilized teat can introduce a lot of microorganism into the raw milk sample. Table 1 shows the physico-chemical analysis of the raw milk sample collected in diverse location in Ilorin, Nigeria. From all the raw milk sampled, 50% were yellowish white in colour, 25% white and 25% light yellow which agrees with Judkins and Mach (1995) who reported normal milk are yellowish white in colour due to the presence of casein, fat colouring matters. Also, Khan et al.,2008 stated that the difference in colour of milk may be due to nature of feeds or breed of the cow. The physicochemical properties of the milk were also noted to have significantly favored the microbial growth i.e. adequate temperature within mesophilic range and pH close to neutrality (Oladiji *et al.,* 2004). Most bacteria especially in milk normally grow within the neutrophilic pH. The pH of raw milk therefore plays an important role as it has an effect on the distribution and growth rate of micro organisms in the milk. The pH ranges between 6.4 and 6.8 in which the average was 6.6, this agrees with William *et al*., 2005. Also the titratable acidity ranges from 0.80 to 1.76 which shows that as the pH of the samples reduces the titratable acidity increases. From this study, eleven microbial flora were isolated, they include *Lactococcus lactis, Psuedomonas spp, Bacillus cereus, Salmonella spp., Staphylococcus aureus and Esherichia coli.* Others are *Proteus spp., Lactobacillus spp., Leuconostoc meseteroides, Klebsiella spp. and Listeria monocytogenes*. The presence of these large number of microflora suggests the extent to which the milk is contaminated by the animal, environment and the milking utensils(Murphy and Boer, 2002).

The Fulani herdsmen do not disinfect the teats and udders prior to milking despite the fact that the cow lie in muddy barnyard and dirty environment which inevitably contaminate the milk and increase the microbial load. Bramley and McKinnon(1990) reported that organism associated with the beddings materials which contaminate the surface of teats and udders includes Staphylocci, Spore formers, coliforms, Streptococci and other Gram negative bacteria.

The sampled raw milk has high microbial load due probably to the insanitary condition of the environment and the season (raining).The bacterial count far exceed the EC Regulation(No. 853, 2004) of the European Parliament and of the Council (EC) which sets down the hygienic limit ≤ 100,000 CFU/ml milk for the total bacteria count (TBC) in cow's raw milk. TBC is one of the main indicators of hygienic quality of cow's raw milk that is also used to set the purchase price of milk(Cempirkova, 2007). Jayarao and Henning, 2001 reported that operational conditions mainly a failure to observe the hygienic rules of milking process contributes to the impairment of microbial quality of bulk samples of cow's raw milk.

From the study, the potential pathogenic bacteria isolated includes *Staphylococcus aureus, Listeria monocytogens, Salmonella spp., Escherichia coli, Proteus spp., Pseudomonas spp. Klebsiella spp. and Bacillus cereus.* The incidence of Salmonella spp. was in the raw milk sample was high which pose health risk for the consumers if taken without pasteurization. De- Buyser (2001)reported that Salmonella is one of the most etiological agent response for several outbreaks associated with the consumption of raw milk and milk products. All salmonellae are of public health concern having the ability to produce infection ranging from a mild self-limiting form of gastroenteritis to septicemia and life threatening typhoid fever (Oliver *et al.,* 2005). Thus, their presence in the raw milk sample pose a health risk to consumer that consumed it without any heat treatment. This problem is particularly evident in developed countries like England and Wales, where the most frequently reported out-breaks were salmonellosis associated with the consumption of raw milk and products (De -Bayser 2001). The presence of Listeria monocytogenes which was known to cause listeriosis, that is, a major cause septicemia, meningitis and encephalitis in the infected person. In case of pregnant women, it may cause intrauterine or cervical infections which may result in an abortion or still birth (Oliver *et al*., 2005 ;Cheesebrough, 2007)). Hence, the presence and consumption of this pathogen in raw milk is highly dangerous. *Staphylococcus aureus* and *Escherichia coli* was discovered in all the collected samples(Figure 1). An overview of annual reports of food borne diseases from several country indicated that S. aureus was far the most frequent pathogen associated with these fooborne outbreak followed by Salmonella. S. aureus is considered the third most important cause of disease in the world amongst the reported food-borne illnesses (Zhang *et al*., 1998). S. aureus poisoning is a mild, generally self-limiting disease, with symptoms that include vomiting with or without diarrhea (Dinges *et al.,* 2000), hospitalization is required in approximately 10% of the cases (Holmberg and

Blake, 1984). As a consequence food products may originally become contaminated during the milking processes or after due to the fact that is can be found on the body surfaces of animals and man. Tamarapu *et al*., (2001) reported that *S. aureus* has been isolated from several foods such as Chicken, meat, milk and dairy products, fermented food items, etc

The standard set maximum amount of coliform bacteria in raw milk is not more than 10 bacteria per milliliter of raw milk. This level is consistent with both national and international public health and food safety requirements (FAO/WHO, 2002). This theory however, was not in correlation with the count shown in the result as the total coliform count was too high for all the samples. This may signify contamination from the grazing environment. Thus, poor herd hygiene, contaminated water, unsanitary milking practices and improperly washed milking bowl mayl have led to elevated coliform counts in raw milk. The presence of *Escherichia coli* in the milk is an indicator of fecal contamination and could be dangerous as the strain isolated may be either toxigenic or enteropathogenic, causing major public health hazard (FAO/WHO, 2002). *E. coli* O157:H7 strain has been associated with a number of food-borne outbreaks and is the cause of bloody diarrhea, frequently associated with dairy cattle, microbial contamination of raw milk and soft cheeses can result in disease. Drinking of raw milk in rural areas could be of health concern due to the presence of *E. coli* 0157:H7 *species* in the raw milk (Fook *et al.,* 2004).

Different measures can also be taken to reduce microbial contamination of raw milk, these includes the animal health which may be a vehicle for infecting the consumer. The environment should also be worked on to reduce contamination of the animals. The sanitary state of the milk handler is of paramount importance. The milking bowl should be washed with detergent and disinfected after use. Before milking, the teat and other breast area should be disinfected.

The occurrence of both *Staphylococcus spp.* in all the milk samples can be attributed to the fact that they are both normal flora of the human body as well as animals. They have been implicated as agents of nosocomial infection in hospitals (Okpalugo *et al.,* 2008). *Bacillus cereus* isolated from the raw milk samples, are aerobic, rod shape bacteria that are ubiquitous in nature especially in the soil. These characteristics accounted for their resistance to heat and presence in the milk after the raw milk was pasteurized (Goff and Griffiths, 2006).

The presence of *Pseudomonas Spp.* and its ability to survive laboratory pasteurization is probably due to the fact that Some strains of this organism particularly, dominate the micro flora of refrigerated raw milk and secrete heat-stable extracellular enzymes (proteases and lipases), which survive pasteurization and even ultra-heat treatments (UHT) and degrade the casein and fat components of raw milk causing a reduction in cheese yield, gelation of UHT milk and off flavors in many dairy products (Dunstall *et al*., 2005).

The result of pasteurization is shown in Table 2. After the raw milk was pasteurized, the microbial load was drastically reduced although some bacteria were still found present resisting the thermal application of heat on the raw milk samples. Organisms found includes *Bacillus cereus, Pseudomonas spp.* and *Lactobacillus spp* as reported by Jay, (1996). According to Jay, (2000), this may be due to several factors such as product water activity, pH, quantity of protein and number of physiological status of organisms in the total population. From the result of pasteurization, it is pertinent that pasteurization of raw milk is the antidote to preventing and reducing food borne infection as a result of consuming raw milk. This research along with previous work on consumption of raw cow milk indicated that raw milk consumer stand a high risk of exposure to foodborne pathogen. The consumption of this raw milk is a preventable cause of foodborne illness which support the call for drinking pasteurized milk in the interest of public health. All the sample tested were exposed to differs microbial contamination and they were handled under unhygienic conditions which pose health risk to consumers. Based on these findings, it is strongly recommended that people should desist from taken raw milk and their products. Also, the Fulani herdsmen should be educated on proper personal and environmental hygiene. Disinfection of the teat and mammary gland area closer to the where milking is done should be encouraged. Corresponding Author

Corresponding Author
Laba Sunday Ademola
Department of Microbiology,
University of Ilorin,
P. M. B 1515.
Ilorin, 240003
Nigeria.

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Parameters Optimization of Cellulase Zymosynthesis by *Aspergillus flavus* NSPR017 Grown on Pretreated Orange Peels

Akinyele Bamidele Juliet, Ekundayo Temitope Cyrus and Olaniyi Oladipo Oladiti

Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.
cyruthm@yahoo.com

Abstract: The potential of *Aspergillus flavus* NSPR016, *Aspergillus flavus* NSPR017 and *Aspergillus flavus* NSPR019 for overproduction of industrial cellulase; adopting cheap and readily available agrowastes as sole carbon substrates under submerged fermentation was investigated. Cellulase production was considerably heightened via physicochemical and nutritional optimization. The effects of several parameters such as carbon and nitrogen substrates, incubation period and temperature, pH and substrate concentration were evaluated. The isolates were screened for cellulase production in mineral medium with carboxymethylcellulose (CMC) supplemented as the sole carbon source. All the tested isolates proved to be cellulase producers with varied rates of enzyme production. However, the highest cellulase production was found with *Aspergillus flavus* NSPR017 and was therefore selected for further optimization studies. Utilization of agrowastes as carbon subtrates instead of CMC for cellulase production was also evaluated. Among tested carbon sources (yam peels, orange peels and wheat bran), orange peels at 5% was found to be the most suitable carbon source. By optimizing the fermentation conditions, maximum cellulase activity was attained at 96 hours, pH 6.5 and temperature 28°C of incubation, 5% orange peels and 0.2% soybeans. The results obtained suggest that lowcost system for hyperproduction of cellulase is achievable for industrial application.

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1. Introduction

Fungal cellulases remained indispensable bio-tools in conversion of agrowastes into value-added products. The cellulase complex secreted by filamentous fungi consists of three major enzymes components, an endo-1,4-β-D-glucanase (EC 3.2.1.4) which attacks β-linkages at random on the amorphous parts of cellulose, a 1,4-β-D-cellobiohydrolase (EC 3.2.1.4) which releases a cellobiose from nonreducing or reducing end, generally from the crystalline parts of cellulose and a 1,4-β-D-glucosidase (EC 3.2.21) which release glucose from cellobiose and short chain cellooligosaccharides, which act synergistically during the conversion of cellulose to glucose (Bhat, 2000; Sun and Cheng, 2002; Xu *et al*., 2011). Cellulases and hemicellulases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture (Bhat, 2000; Sun and Cheng, 2002; Lynd *et al*., 2005; Gilna and Khaleel, 2011).

Agrowastes on the other hand, are food processing wastes, crop wastes and agro-allied industry wastes and other lignocellulosic wastes. Large amounts of lignocellulosic "waste" are generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro industries and they pose an environmental pollution problem. Sadly, much of the lignocellulosic waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon (Mabrouk and Ahwany, 2008; Arijit *et al*., 2010). However, efficient utilization of agro-industrial residues such as cassava, sugar beet pulp, coffee pulp/husk, and apple pomase has being increasingly advocated. Several processes have been developed that utilize these as raw materials for the production of bulk chemicals and value-added fine products such as ethanol, single cell protein (SPC), mushroom, enzymes, organic acids, amino acids and biologically active secondary metabolites (Howard *et al.,* 2003; Lynd *et al*., 2005; Ibrahim, 2008). Applications of agro-industrial residues in bioprocesses on the one hand provide alternative substrates, and on the other hand help in solving pollution problems, which their disposal may otherwise cause. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have opened for their utilization (Howard *et al*., 2003; Mabrouk and Ahwany, 2008).

With such advancement in biotechnoresearches, agricultural wastes are no longer

environmental issue but resources for energy production. 'Waste-to-Wealth' perception of agricultural wastes is a tremendous potential in improving the general state of sanitation, positive environmental actions to reduce green house gas emissions and significantly improve soil fertility, crop yield and reduce global dependence on chemical fertilizers and fossil fuels (Agamuthu, 2009).

Nevertheless, up to date, the production of cellulase, which is one of the key enzymes for agrowaste biodegradation, has been found to be the most expensive step (Kotchoni *et al*., 2003). Thus, there are needs for researches channeled at making biosynthesis of cellulases economically feasible, cost effective, and sourcing of cheap and efficient raw materials and utilization of agrowastes. Various parameters that affect cellulase production require optimization to encourage local production and local industrial development in area of enzyme production.

2. **Materials and Methods**

Fungi isolates

Aspergillus flavus NSPR016, *A. flavus* NSPR017 and *A. flavus* NSPR019 were obtained from the culture collection of the Nigerian Stored Products Research Institute Ilorin, Kwara State, Nigeria and maintained on Potato Dextrose Agar (PDA) plates. These were subcultured once in a month by incubation at 30 ±2°C until the entire plates were covered by active mycelium and then stored at 4°C in refrigerator on agar slants.

Chemicals and lignocellulosic substrates

All the chemicals used for this study were of analytical grade unless otherwise stated and produced by Fluka (France), Merk (Germany) and Sigma Chemical Co., (USA). Orange peels, yam peels and wheat bran were procured from farm fields, domestic source and market in Akure, Ondo State, Nigeria and were prepared according to Hafiz *et al.* (2010).

Pretreatment of lignocellulosic substrates

Lignocellulosic substrates (10g) were treated separately with 1000 mL of 4% solution of sodium hydroxide for 24 h in Petri dishes at room temperature prior to autoclaving. The substrates were washed with distilled water until it is neutral to litmus paper and dried at 70°C (Model DHG Heating Drying Oven) to constant weight. The effect of sodium hydroxide was further neutralized with diluted hydrochloric acid and they were autoclave at 121°C for 15 min (Muthuvelayudham and Viruthagiri, 2006).

Media preparation and enzyme production

Medium composition described by Mandles and Weber as reported by Acharya *et al.* (2008) was used for submerged fermentation. The media contained (per liter of distilled water): Urea 0.3 g, $(NH_4)_2SO_4$ 1.4 g, KH_2PO_4 2.0 g, $CaCl_2$ 0.3 g, $MgSO_4.H_2O$ 0.3 g, peptone 1.0 g, $FeSO_4.H_2O$ 5.0 mg, $MnSO_4.H_2O$ 1.6 mg, $ZnSO_4.H_2O$ 1.4 mg, $CoCl_2$ 2.0 mg and carboxymethylcellulose (CMC) 10g. pH of the media were adjusted to 6.5 with pH meter (Denver Instrument, Model 20 pH/ Conductivity meter) prior sterilization. Then, 100 mL of the liquid medium was dispensed in 250 mL Erlenmeyer flask and sterilized by autoclaving 121°C for 15 min. This was cooled and inoculated with 10 discs of 8 mm diameter of the organism from PDA culture plates using sterile cup borer. The flasks were incubated at 30 ±2°C for 5 days on a rotary shaker (Gallenkamp) at 120rpm. Sterile basal medium supplemented with carboxymethylcellulose without organism served as the control. Crude enzyme preparation was obtained by centrifugation at 5000rpm for 10mins at 4°C using refrigerated ultracentrifuge (Centurion Scientific Limited). The supernatant was used as the crude extracellular enzyme source (Gautam *et al*., 2010).

Optimization of culture conditions

Culture conditions are an integral aspect of enzyme production. The present study investigated the effect of nutritional conditions on the production of cellulase by *Aspergillus flavus* NSPR017, adopting different carbon sources (Orange peels, yam peels and wheat bran), organic nitrogen sources (cotton seeds and locust beans) at 0.2%, substrate concentrations (1%, 2%, 3% and 5%), pH ranges (4.5 to 7.5), and temperature (28, 32 and 37°C) according to Gautam *et al*. (2010). The flasks were kept on a rotary shaker (Gallenkamp) at 120rpm at 30 ± 2°C for 4 days of cultivation (Hafiz *et al*., 2010).

Cellulase Assay

Cellulase activity of supernatant collected at the end of each optimization step was determined using a reaction mixture containing 0.5 mL of 0.5% of carboxymethylcellulose as substrate prepared in 0.5 M sodium acetate buffer pH 5.5 according to Acharya *et al.* (2008). The control tube contained the same amount of substrate and 0.5 mL of the enzyme solution heated at 100°C for 15 min. Both the experimental and control tubes were incubated at 50°C for 30 min. At the end of the incubation period, tubes were removed from the water bath (Lamfield Medical England Model DK-600), and the reaction was terminated by addition of 3 mL of 3, 5-dinitrosalicylic acid reagent per tube (Shazia *et al*., 2010). The tubes were incubated for 5 min in a boiling water bath for color development and were cooled rapidly. The activity of reaction mixture was measured against a reagent blank at 540nm. The concentration of glucose released by enzyme was determined by comparing against a standard curve constructed similarly with known concentration of

glucose. Unit enzyme activity was defined as the amount of enzyme required for liberating 1µM of glucose per milliliter per minute and was expressed as µM/mL/min.

Protein and reducing sugar estimation

Protein in the medium was determined by the method of Lowry *et al.* (1951) with Bovine Serum Albumin (BSA) as standard while the amount of reducing sugar in culture filtrate was determined according to Miller (1959).

Statistical Analysis

Data presented on the average of three replicates (±SE) are obtained from there independent experiments.

3. Results

Screening of fungal isolates for cellulase production

The selected fungal strains (*Aspergillus flavus* NSPR016, *A. flavus* NSPR017 and *A. flavus* NSPR019) showed the ability to produced cellulase enzyme, protein and liberate reducing sugar on carboxymethylcellulose incorporated into minimal salt medium (Figure 1-3) but with varied rate of enzyme production. The highest cellulase activity (0.110 µmol/min/mL), protein content (0.701 mg/mL) and reducing sugar (0.029 mg/mL) was observed with *A. flavus* NSPR017 after 5 days of incubation on rotary shaker. While, the lowest cellulase activity (0.081 µmol/min/mL) and protein content (0.585 mg/mL) was observed with *Aspergillus flavus* NSPR016. In term of reducing sugar liberation from incorporated substrate, *A. flavus* NSPR019 gave the lowest amount of 0.09 mg/mL.

Effect of different agrowastes on cellulase production

Different agricultural by-products (orange peels, wheat bran and yam peels) supplemented with mineral salt medium for cellulase production and protein content estimation (Figure 4 and 5) showed that, orange peels were the most substrate for the production of cellulase, which gave maximum yield of cellulase activity and protein content of 0.322 µmol/min/mL and 15.76 mg/mL respectively. The cellulase activity of orange peel was observed to be almost 3.0 fold increase compared to carboxymethylcellulose (control). All the tested substrates proved to be novel carbon sources for cellulase production.

Effect of incubation period on cellulase production

The flasks were incubated at different time duration; 24, 48, 72, 96 and 120 h and cellulase activity expressed in terms of percentage relative activity were 25.93 %, 69.1%, 80.28%, 100%, 25.1% respectively (figure 6). Thus, at 96 h of incubation, maximum degradation was obtained. The production of enzyme increased with increase in fermentation period and beyond the optimum incubation period (96 h), a decline in enzyme production was observed. Time course profile of protein production by *A. flavus* NSPR017 is shown in Figure 7. Protein production progressively increased with increase in incubation period until an optimum production (2.394 mg/mL) was attained at 72 h. Subsequent increase in incubation time beyond 72 h resulted into a decline in production of protein.

Effect of incubation temperature on cellulase production

The effect of incubation temperatures (28°C, 32°C and 37°C) on cellulase biosynthesis and protein production by *A. flavus* NSPR017 on pretreated orange peels under submerged state fermentation is shown in Table 1. There was gradual decrease in cellulase activity and protein content with increase in incubation temperature. However, optimum cellulase activity and protein content was obtained at 28°C.

Table 1: Optimization of temperature

Temperature (0°C)	Cellulase activity (µmol/min/mL)	Protein content (mg/mL)	Specific activity (µmol/min/mg)	Percentage relative activity (%)
28^{0}C	0.322±0.01	2.043±0.02	0.158	100
32^{0}C	0.281±0.03	1.985±0.01	0.142	0.116
37^{0}C	0.149±0.01	1.284±0.01	0.116	46.27

(Values are means of three replicates, ± = standard deviation)

Effect of pH on cellulase production

The specific activity of cellulase by *A. flavus* NSPR017 was studied by varying the pH of the fermentation media from 4.5 to 7.5 (Figure 8). Maximum specific activity of cellulase (0.393 µmol/min/mg) was achieved when the pH of basal medium was kept at 6.5. At pH 7.5 the specific activity dropped to about 22.65% of that obtained at pH 6.5.

Effect of different concentrations of orange peels on cellulase production

Cellulase activity and protein content estimation was studied by varying the concentration of orange peels (Fig.9 and10). Different

concentrations of orange peels were used for the enzyme production ranging from 1 to 5% with the exclusion of 4%. Of these, 5% orange peels were optimized for maximum production of cellulase (0.967 µmol/min/mL) and protein content (2.86 mg/mL). Thus, the optimum substrate concentration for maximum production of cellulase was obtained at 5%. It was observed that protein content increased progressively with increase in substrate concentration until optimum protein content (3.269 mg/mL) was recorded at 3% and above this, a decline was recorded. However, all substrate concentrations utilized in this study gave appreciable yield of cellulase activities and protein contents.

Effect of organic nitrogen sources on cellulase production

In this present work, different organic nitrogen sources such as cotton seeds, locust beans and soybeans were added separately to the fermentation medium at 0.2% concentration replacing ammonium sulphate (inorganic) from mineral salt medium. Among all the organic nitrogen sources tested; soybeans gave maximum production of cellulase (0.337 µmol/min/mL) (Figure 11). However, the lowest cellulase production was obtained in ammonium sulphate $(NH_4)_2S0_4$. The cellulase activity obtained from soybeans was almost 2.011 higher than the ammonium sulphate (control). The use of ammonium sulphate as inorganic nitrogen source caused a reduction in enzymatic activity to about 45.10% of that obtained with soybeans. The biosynthesis of protein by *A. flavus* NSPR017 was evaluated (Figure 12). The highest protein content was observed in cotton seeds (1.634 mg/mL) followed by locust beans (1.284 mg/mL) while the lowest was recorded in ammonium sulphate.

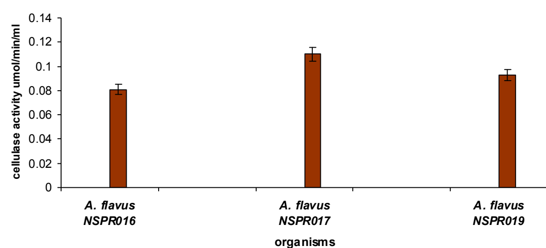

Figure 1. Screening of fungal isolates for cellulase production on carboxymethylcellullose (CMC). They were grown on mineral salt medium with tested substrate (10 g/L) and incubated at 30±2°C for 5 days.

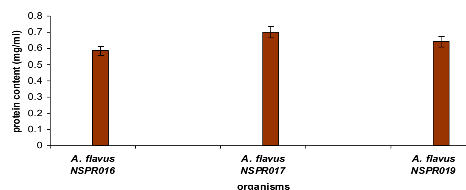

Figure 2: Protein content in culture filtrate inoculated with different fungal isolates at 30±2°C for 5 days.

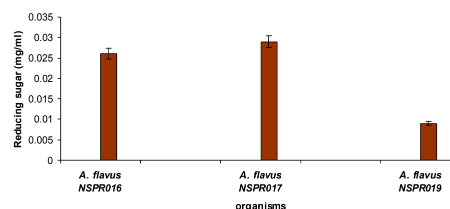

Figure 3. Amount of reducing sugar liberated in culture filtrate of different fungal isolates at 30±2°C for 5 days.

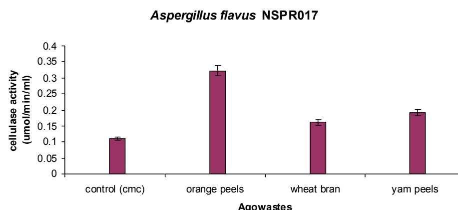

Figure 4 : Effect of different agriculture by-products as carbon source on the production of cellulase by *Aspergillus flavus* NSPR017. Y-error bars indicate the standard deviation among the three replicates.

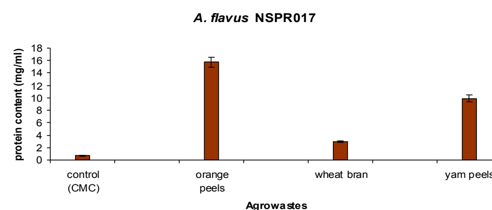

Figure 5: Protein content in culture filtrate inoculated with *Aspergillus flavus* NSPR017 at 30±2°C for 5 days.Y-error bars indicate the standard deviation among the three replicates.

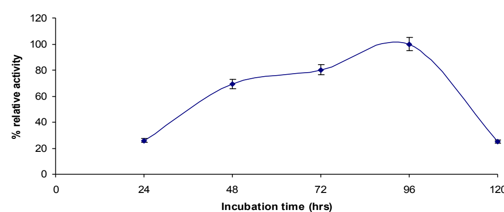

Figure 6: Time course of the cellulase production by *Aspergillus flavus* NSPR017 using orange peels as carbon source. Y error bars indicate the standard deviation among the three parallel replicates.

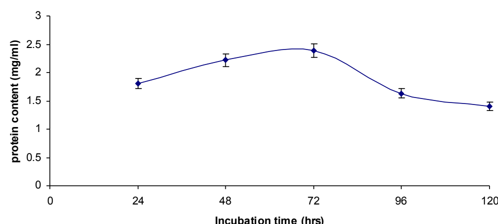

Figure 7: Time course profile of the protein production by *Aspergillus flavus* NSPR017 using orange peels as carbon source. Y error bars indicate the standard deviation among the three parallel replicates

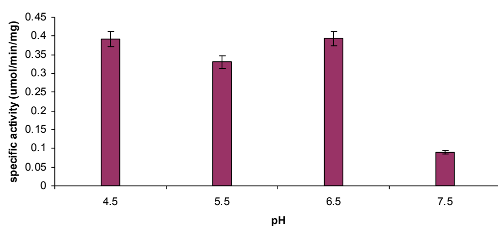

Figure 8: Effect of initial pH on cellulase production

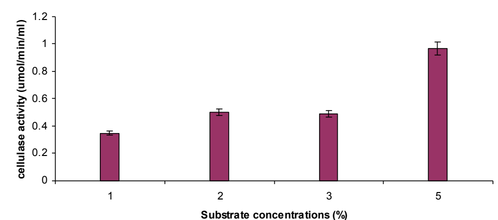

Figure 9: Effect of varying substrate concentrations on cellulase production by *A. flavus* NSPR017

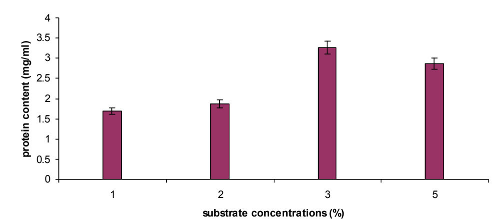

Figure 10: Protein content from different substrate concentrations

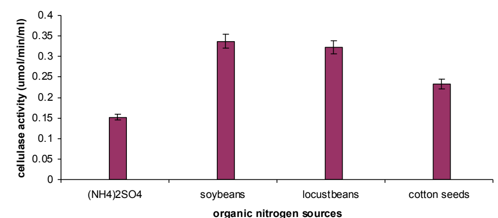

Figure 11: Effect of different nitrogen sources on the cellulase production by *A. flavus* NSPR017 in shake flasks. Y error bars indicate the standard deviation among the three parallel replicates

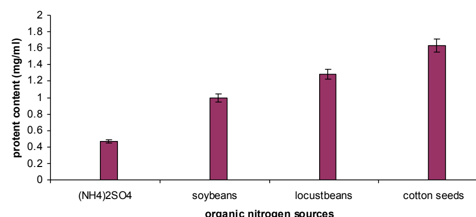

Figure 12: Protein content from different nitrogen sources. Y error bars indicate the standard deviation among the three parallel replicates

4. Discussions

Screening of fungal isolates for cellulase production

The differences obtained in the enzyme synthesis suggest that the rate of cellulase produced depends on the genetic composition of the microorganisms (Gautam *et al*., 2010). *Aspergillus flavus* NSPR017 was therefore selected for further studies because of its high cellulase activity. A capacity to degrade cellulose is a character distributed among a wide variety of aerobic, facultative aerobic, anaerobic bacteria and fungi. The characters are restricted to a few species among several major taxa (Hafiz *et al*., 2010). The important cellulolytic fungus like *Trichoderma* sp. (Jamal and Alam, 2010; Gautam *et al*., 2010), *Penicillium* sp. (Han *et al*., 2009); *Sporotrichium* sp (Sukumaran *et al*., 2005); *Aspergillus* sp (Hafiz *et al*., 2010) have been reported to have cellulolytic activity.

Effect of different agrowastes on cellulase production

For optimum production of cellulase, the selected agrowastes were given alkaline pretreatment with 2N NaOH at 4%, autoclaved at 121°C for 15 min, in an Erlenmeyer flask to ensure proficient deprivation of lignocellulosic content of wastes to get optimum cellulase production (Hafiz *et al*., 2010). The pretreatment of lignocellulosic wastes has been reported by many researchers (Hafiz *et al*., 2010; Gilna and Khaleel, 2011). The enzymatic conversion of cellulosic materials requires some form of pretreatment to improve cellulose accessibility and digestibility. It was apparent that the most effective pretreatment was one which allowed the substrate to be hydrolysed partially and completely, using a minimum amount of enzyme (Godliving, 2009). It was observed that pretreatment causes the removal of lignocellulosic contents including lignin and hemicelluloses successfully, which at the same time induces the loosening in structure of lignin and destroy the crystallinity of cellulose that improves the porosity characteristics of substrates

(Muthurelayedham and Viruthagiri, 2006; Gilna and Khaleel, 2011).

Agricultural by-products such as corn cob, wheat straw, rice straw, bagasse and so on were used in previous studies for cellulase production (Ojumu *et al.,* 2003; Ikram *et al*., 2006; Omojasola *et al*., 2008). Although, the raw materials are cheaper, pretreatment is generally required to improve the utilizability of lignocellulosic materials and the cost is considerable (Mahro and Timm, 2007; Foyle *et al.,* 2007; Godliving, 2009). In view of the above facts, in the present study, the natural waste materials were utilized effectively as major carbon substrate for the production of cellulase by selected fungal isolate. The substitution of CMC in the culture medium with different agrowastes resulted in a maximum cellulase activity. There was variation in the amount of cellulase produced when agrowastes were substituted in culture medium. The large variation in cellulase yield may be due to the nature of cellulose or hemicellulose, presence of some components (activators or inhibitors) in these materials and variations in the substrate accessibility (Mabrouk and Ahwany, 2008). Orange peels, yam peels and wheat bran were the agrowastes utilized in this study. Of all the substrates tested, orange peels were found to be the most suitable substrate for the production of cellulase which gave maximum yield of cellulase activity. The selection of orange peels might be due to the fact that its provided adequate amount of nutrients like proteins, carbohydrates, fat, fibres, ash, trace elements, various amino acids and porosity for oxygen supply (Bakri *et al*., 2003; Ikram *et al*., 2006).

Effect of incubation period on cellulase production

The effect of incubation period on cellulase production was estimated for 120 h. The enzyme was found to increase steadily with increase in incubation time. Maximum production was observed after 96 h and beyond this, the enzyme production substantially decreased, probably due to the depletion of essential nutrients in the media and/ or accumulation of toxic secondary metabolites produced by the fungus itself (Gautam *et al*., 2010).

Effect of incubation temperature on cellulase production

The cultivation temperature has a marked influence on the growth rate as well as on the level of cellulase production (Arijit *et al*., 2010). Hence, the optimum temperature depends on whether the culture is mesophilic or thermophilic (Ahmed *et al*., 2009; Gautam *et al*., 2010). Among the fungi, most cellulase production studies have been done with mesophilic fungi within the temperature range 25 to 37 ^{o}C (Lu *et al*., 2003; Gautam *et al*., 2010). In the present investigation, 28°C was an optimum temperature. The optimum temperature obtained from this study correlated with the finding of Narasimha *et al*. (2006), who reported maximum cellulase activity at 28°C when *Aspergillus niger* was cultured on pretreated sawdust. Similar result was reported by Acharya *et al*. (2008) when pretreated sawdust was optimized for cellulase production by a strain *Aspergillus niger*. As the temperature increased, there was a gradual reduction in the enzyme production. This may be due to the fact that higher temperature denatures the enzymes. High temperature may also lead to inhibition of microbial growth (Shazia *et al*., 2010). Many workers have reported different optimal temperatures for cellulase production either in shake or in fermentor studies using *Aspergillus* spp. suggesting that the optimum temperature for cellulase production also depends on the differences within the same genus of the same fungus (Hafiz *et al*., 2010).

Effect of pH on cellulase production

The enzyme is very sensitive to pH. Therefore, the selection of optimum pH is very critical for the production of enzymes (Gupta *et al*., 2010). A pH regulatory system may be especially important. Apart from the regulatory effect on gene expression, cultivation pH can also affect fungal morphology greatly (Gupta *et al*., 2010). Thus, development of an optimal pH control strategy is helpful in obtaining higher protein productivity. Here in the present study, it was found that pH 6.5 is optimum in case of *Aspergillus flavus* NSPR017 as an organism for cellulase production. Similar result was reported by Gautam *et al*. (2010) they found that the cellulase production was optimum at pH 6.5 for *Trichoderma viride* under submerged fermentation. Contrary to this, Pham *et al*. (2010) showed that the optimum pH for cellulases production from strain of *Aspergillus niger* VTCC-F021 was 5.0. Acharya *et al*. (2008) reported pH optimum that fall between 4.0-4.5 for cellulase enzyme from *A. niger*. Coral *et al*. (2002) reported pH optimal for a cellulase production by an *A. niger* strain was 4.5 and 7.5.

Effect of different concentrations of orange peels on cellulase production

The carbon is an important factor affecting cell growth and product formation of microorganisms. Carbon source may have either repressing or inducing effect on enzyme production (Gupta *et al*., 2010). A dynamic influencing feature that affects the yield and initial hydrolysis rate of cellulase is substrate concentration (Hafiz *et al*., 2010). Low substrate concentration results in an increase in yield and reaction rate of the hydrolysis while, high substrate concentration can cause substrate inhibition, which substantially lowers enzymes formation (Liu and Yang, 2007; Singhania *et al*., 2007). In this present

study, orange peels at 5% proved to be the best for cellulase production by *Aspergillus flavus* NSPR017. This result matched with other reports that the optimum substrate concentration for cellulase production by a strain of *Trichoderma* spp. was 5% (Gautam *et al*., 2010) and that of *Aspergillus* spp as reported by Abo-State *et al*. (2010). Although, different optimal substrate concentrations had been reported by many researchers and this could be attributed to the chemical nature and nutrient availability of the used substrates (Gautam *et al*., 2010).

Effect of organic nitrogen sources on cellulase production

Most industrially used microorganisms can utilize inorganic or organic nitrogen sources. Inorganic nitrogen may be supplied as ammonia gas, ammonium salts or nitrates and as amino acids, protein or urea. It was found that the growth was faster with the supply of organic nitrogen, and a few microorganisms also were found to have absolute requirement for amino acids (Ray *et al*., 2007). However, amino acids are more commonly added as complex organic nitrogen sources which are not homogenous, cheaper and readily available. In the present study, soybeans at 0.2% level proved to be the best organic nitrogen source for cellulase production by *Aspergillus flavus* NSPR017. It was due to the fact that soybeans provided both the ammonium as well as sulfate ions for conidial cell growth and enzyme production (Mekala *et al*., 2008). Hence, it should be used in proper concentration for an optimum production of cellulase.

Based on the results obtained, we were able to established that the optimized culture conditions (96 h, pH 6.5 and temperature 28°C, 5% orange peels and 0.2% soybeans) for *Aspergillus flavus* NSPR017 could be exploited in lowcost system for hyperproduction of industrial cellulase at bioreactor level for commercialization. Thus, recommended that the isolate should be subjected to strain improvement studies to fully harness its potential to scale up production of cellulase.

Corresponding Author:
Ekundayo Temitope Cyrus. Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.
E-mail: cyruthm@yahoo.com

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A Formula for Calculating the Critical Load of the Needles Used in the Garment and Apparels Sewing Technology: Part 1: Pucarenko Technique

El Gholmy S. H., I. A. Elhawary

Department of Textile Engineering, Faculty of Engineering, Alexandria University, Alexandria, Egypt
sh_gholmy@yahoo.com

Abstract: The sewing machines needle is an important and vital machines member. The general objectives of sewing needle is to penetrate the sewn materials either single layered or multiple layered fabrics and to carry the sewing thread via the sewn fabrics for loop formation during the penetration by the sewing needle, a resisting force at the free end of the needle is built up that subjects the needle to an axial compressive force this force can lead to the needles buckling in the elastic or plastic region of the needles metal [steel]. In both cases the sewing needle may be bent this will lead to produce a defective readymade garment or a downgraded quality clothing [1]. In the present work a Pucarenko technique has been applied for calculating the industrial sewing needle critical load $P_{cr.}$It was found that $P_{cr.}$ for an industrial sewing machine is 62 N (newton). The working resisting force during sewing process must be less than $P_{cr.}$ to avoid needles buckling this must be controlled by safety factor of elastic stability (m) where $m=P_{cr.}/P_W$, for jeans (denim) fabrics, where P_W =120 c N (woven fabric, 120 g/mt^2) when the needle free length changed from 45mm to 12mm, the critical load increased from 62N to 90N.The Pucarenko formula calculates only the stability factor **η** due to the different needle cross- section. The value of **η** changed about 0.0284% which can be neglected.

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1. Introduction:

The sewing machine needle is a major part of the sewing machine and its functions in general are: producing hole in the material, carrying the thread through the material and passing the needle thread through the loop **[1].**

The kinematics of the sewing needle during the process of stitch formation is important to obtain a good formed stitch. Increasing stitch velocity from 1000rpm to 4000 rpm the needle bar and the take-up lever velocity changed linearly, but the acceleration performed a non-linear function **[2].** Thus the needle penetration velocity was reduced by replacing the conventional slider-crank mechanism with a link drive mechanism. This reduced the needle velocity in the needle penetration area by 25% in comparison to the conventional slider –crank mechanism**[3]**.

A sewing machine needle is precision item and if it becomes misshapes in any way it will fail to form stitch properly. A bent needle may cause slip stitching. The reason why the needle

became bent could be related to incorrect needle size for the fabric weight being sewn or to faulty operator handling. A needle where the point has become burred or damage in some way will almost certainly cause damage along the stitch line. Needles become deflected (buckled) during sewing by fabric that is too thick for the size of the needle. If the deflection is too severe, a larger size needle may be necessary. Needle can also become damaged as result of impacting a harsh material over a period of time, such as that used for denim jeans which are made up in a stiff state and then washed in garments form can require that the needles sewing it are changed every two hours. The ways in which the needles can strike and break fabric yarns and burst the loops in the knitted fabrics, have considered as one of the main reasons for the much critical damage. There for it is preferable to use needles with small size as it could be possible [1].Needle defection during sewing may cause uneven seam or thread breakage. It was measured by placing two gauges on the lateral sides of the needle, which was found correlated to needle eccentricity **[4].**

Needle heating, is another problem in stitch formation, which occurs as a result of friction between the needle and the sewn fabric. In high speed sewing of dense materials temperatures as 300̊C - 350̊C can be reached, it is possible that needle may suffer damage and lose hardness can easily be buckled. **[5].** A lot of suggestions have been written to reduce the friction between the needle and the material such as blowing an air current during the machine stitching; the production of jeans in cotton fabrics is typical of situation where high speed machines are used on a natural fiber and fabric and where considerable needle heat can be generated particularly on the long seam **[1, 5].**

Finally, we can say that to obtain a good looking seam, we have to measure forces acting on the needle during this process. Therefore, previous researches modeled the forces acting on the needle and gave some accurate results **[6-8].**

2- Review of literature:

Pucarenko et al. [9] has written the buckling formula of Euler for a bar subject to an axial compressive load:

$$y = A \sin n \pi \frac{x}{\ell} \quad , n = 1,2,3 \ldots etc \, .$$

Number of waves [single wave, double waves or triple waves ……etc],

$x = \dfrac{\ell}{2n}$ displacement of the compressed bar axis and A-amplitude of the waves constantly, the critical load Pcr formula is:

$$P_{cr} = \frac{\pi^2 E I}{(\gamma \ell)^2} = \eta \frac{E I}{\ell^2} \quad \ldots\ldots\ldots\ldots \quad \ldots\ldots(1)$$

Where:-

γ - Coefficient of the equivalent length, ℓ - Length of the bare (beam) between supports γ = 1 for simply supported beam, γ = 2 for cantilever, η - ideal stability factor & equals = $\frac{\pi}{\gamma}$, η =9.8696 for both simply supported beam and for a cantilevers .

In the same work Pucarenko, Yakovlevand Matveev, **[9]**, it has been stated that the elastic stability factor η for n variable cross – sections of machine member is:

$$\eta = 2.467 \left[\left(1 - \frac{I_2 - I_1}{I_1} \times \frac{(\ell - a_1)^2}{\ell^2} \right) \left(1 - \frac{I_3 - I_2}{I_2} \times \frac{(\ell - a_2)^2}{\ell^2} \right) \ldots\ldots\ldots \left(1 - \frac{I_n - I_{n-1}}{I_{n-1}} \times \frac{(\ell - a_{n-1})^2}{\ell^2} \right) \right] \ldots(2)$$

The critical load is calculated by formula:-

$$P_{cr} = \eta \frac{E I_1}{\ell^2} \quad \ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots \ldots\ldots\ldots(3)$$

Where:

Inertia of the beam cross section at the completely fixed end.

Mutunskiand Movnin [10] have reported that the critical load for a machine member subjected to axial compressive load is:

$$P_{cr} = \frac{\pi^2 . E I_{\min}}{(\gamma \ell)^2} = \eta \left(\frac{E I_{\min}}{\ell^2} \right) \ldots.(3-a)$$

The critical stresses due to the critical load could be calculated by either formula.

$$\sigma_{cr} = \frac{\pi^2}{(\gamma \ell)^2} . \frac{E I_{\min}}{A} \quad \ldots\ldots\ldots\ldots \quad \ldots\ldots\ldots\ldots \quad (4)$$

$I_{\min} = i^2_{\min} \quad , A \; , \; i_{\min}$

Radius of gyration or inertia radius of cross-section area A, or by formula:

$$\sigma_{cr} = \frac{\pi^2 E}{\lambda^2} \quad \ldots\ldots\ldots\ldots \quad \ldots\ldots\ldots\ldots \quad \ldots\ldots\ldots\ldots \quad \ldots\ldots(5)$$

$\lambda = \dfrac{\gamma \ell}{i_{\min}} - beam$ Elasticity

imin = Imin / A,A – area of buckled beam cross-section.

Belyaev [11] has stated that the elastic line equation for the buckled beam axis depends on the number of wave in the beam, for single wave

$$y = a \sin \frac{\pi x}{\ell} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6-a)$$

for double waves

$$y = a \sin \frac{2\pi x}{\ell} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6-b)$$

$y = a \sin \dfrac{3\pi x}{\ell}$ for triple waves, or general formula is

$$y = a \sin \frac{\pi x}{\ell} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6-c)$$

Where n=1, 2, 3…………..,and a is the wave amplitude. In the same work Belyaev**[11]**, it was written that for conical machine member subjected to an axial compressive load, the critical load.

$$P_{cr} = \frac{\pi^2}{(\mu \ell)} . \frac{EI}{\ell^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

where, $n = \dfrac{I_1}{I_2}$

μ -is calculated by special table, I1- inertia of the machine membercross- section at its free-end while I2 – the inertia of cross- section area at the fixed end. **Belyaev [11]** has mentioned that the critical stress in the buckled beam is:

$$\sigma_{cr} = \frac{\pi^2 E}{\lambda^2} \quad \ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots (8)$$

Where, E-mong's module and λ - beam elasticity. The critical stress σ_{cr} must be less than yield stress σ_y for steel 5. $\lambda \geq 85$ For cast-iron $\lambda \geq 80$

Ponomarev et al. [12] have found the critical load for a beam [cantilevers type] with a conical shape with inertia I1 at the free end and I2 at the fixed end . the

formula is:

$$P_{cr} = 13.370 \frac{EI_2}{\ell^2} \ldots\ldots(9)$$

Where, L -the actual length of the cantilever. They applied the energy technique for the calculation where general equation of the total potential energy u_T for a buckled beam under an axial compressive load is calculated by formula

$$u_T = u_\circ + u - w \ldots\ldots\ldots\ldots(10)$$

where: UT – total potential energy, u0- potential energy up to the buckling, w- the work done by external applied compressive forces during buckling and u- potential energy due to buckling. The potential energy due to buckling u is

$$u = \frac{1}{2EI}\int_\circ^\ell M^2 dx \ldots\ldots\ldots\ldots\ldots\ldots(11)$$

M-bending moment

$$y''.dx, \quad y = \sum_k a_k \sin\frac{\pi x}{\ell} \ldots\ldots\ldots(12)$$

k- Section number of the buckled beam, and the work w is calculated by formula

$$w = P.\frac{1}{2}.\int_\circ^\ell y^2.dx \ldots\ldots\ldots\ldots\ldots\ldots\ldots(13)$$

p- is the external force.

Elhawary [13] has introduced a simplified formula for calculating the critical load of the needles used in the needle punching machine. The derivation of these formula is based on the energy method .It was found that the critical load for the compound needle was 8.76N where as it was 14.75 for the simple needle, while the factor of safety of the elastic equilibrium (stability) of the needles either compound or simple was running from 1.6 to 2.7 that was considered to be a safe range of values.

Hussien, Nahrawyand Arafa**[14]**measured the needle penetration force on the fabric handle tester, using a modified jaw. It was found that the fabric weight and the use of a softener affect the needle penetration force. Also, the technology of needle manufacture had significant effect, especially, in knitted fabrics. **Ujevic et al. [15]** measured the needle force; the blade of the sewing needle was used as a sensor. It was found that sewing needle penetration forces increase proportionally with needle sizes.

Finally the critical stress σ_{cr} in the buckled needle as written by **Timoshenko [16]** is:

$$\sigma_{cr} = \frac{\pi^2 E}{\lambda^2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(14)$$

Where: σ_{cr} - critical stress, E- young's modulus and λ - sewing needle elasticity = $\gamma\ell / i_{\min}$ (see nomenclature). The critical stress must be less than both of σ_y - yield stress & [σ]- design stress = σy: F.s c factor of safety:

$$\sigma_{cr} \le \sigma_y \le [\sigma] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(15)$$

In the workof Ponomarev,Buderman, Klikharev,Makyshin, Malinin and Foedosef**[12]** it has mentioned a formula for calculating a coefficient φ of decrease the design stress [σ] due to buckling where:

$$\left.\begin{aligned}\varphi &= 1 - 0.8\left(\frac{\lambda}{100}\right)^2 \quad \ldots\ldots \lambda \le 75 \\ &= \frac{3100}{\lambda^2} \quad \ldots\ldots \lambda > 75\end{aligned}\right\}\ldots\ldots\ldots(16)$$

Nomenclature:

η - Ideal stability coefficient $= \pi^2 / \gamma^2$

μ or γ - Coefficient of the equivalent length

$\gamma\ell$ or $\mu\ell$ - Equivalent length.

ℓ - Actual span length of the beam or of the machine member.

Pcr – critical load $= \dfrac{\pi^2 EI}{(\gamma\ell)^2} = \eta\dfrac{EI}{\ell^2}$

E- Young's modulus or modulus of elasticity in compression

I- Iinertia of the cross- section area of the beam

$\lambda = \gamma\ell / i_{\min}$ - beam elasticity, imin – min. radius of inertia of beam cross–section or radius of gyration.

$i^2_{\min} = I_{\min} / A, A$ - Beam cross- section area

σ_{cr} - Critical stresses in the cross- section of the buckled beam

3- Mathematical approach:

3-1 Pucarenko et al. [9] technique:

According to Pucarenko et al. [9] technique the following formula will be applied for the industrial sewing needle of the sewing machine of the clothing technology manufacture:

3-1-1 The axial compressive critical load Pcr:

$$P_{cr} = \eta\frac{EI_1}{\ell^2} \ldots\ldots\ldots\ldots\ldots\ldots(17)$$

Where:Pcr – critical load, η - stability factor, E- young's modulus = 206 Gpa for steel sewing needles material, I1- inertia of the needle cross- section and ℓ - is the total free length of the needle

3-1-2 The stability factor η general formula is:

$$\eta = 2.467\left\{1-\left(\frac{I_2-I_1}{I_1}\right)\times\left(\frac{(\ell-\ell_1)^2}{2}\right)\right\}.\left\{1-\left(\frac{I_3-I_2}{I_2}\right)\times\left(\frac{(\ell-\ell_2)^2}{2}\right)\right\}$$

$$.\left\{1-\left(\frac{I_4-I_3}{I_3}\right).\left(\frac{(\ell-\ell_3)^2}{2}\right)\right\}\times\ldots\ldots\ldots\left\{1-\left(\frac{I_n-I_{n-1}}{I_{n-1}}\right)\times\frac{(\ell-\ell_{n-1})^2}{2}\right\}\ldots\ldots\ldots\ldots 18$$

Where, I1, I2, I3 & I4 – cross sectional sewing needle area's inertias respectively from section1 to 4. $\ell_1, \ell_2, \ell_3, \ell_4 \,\&\, \ell$ - Lengths of the different sewing machine needle sections and the total length of the needle respectively.

3-2 Critical load Pcr calculation:

3-2-1 Actual configuration of sewing machines needle: [four sections]

As shown in fig. (1), the sewing needle has four sections: shark, blade, scarf with eye and tip. Therefore the general formula of **Pucarenko [9]** will be applied with needles data bases from table (1):

$$\eta = 2.467 : \left\{1-\left(\frac{1.0417\times10^{-14}-2.4850\times10^{-13}}{2.4850\times10^{-13}}\right)\times\left(\frac{(0.045-0.015)^2}{2}\right)\right\}.$$

$$\left\{1-\left(\frac{7.8125\times10^{-16}-1.0417\times10^{-14}}{1.0417\times10^{-14}}\right)\times\frac{(0.045-0.040)^2}{2}\right\}.$$

$$\left\{1-\left(\frac{1.9175\times10^{-16}-7.8125*10^{-16}}{7.8125\times10^{-16}}\right)\times\frac{(0.045-0.04)^2}{2}\right\}$$

Using formula (2):

$$\eta = 2.467 : \{1+0.9581\times4.05\times10^{-7}\}.\ \{1+0.925\times1.25\times10^{-5}\}$$
$$.\ \{1+0.754546\ \times5\times10^{-7}\}$$
$$= 2.467 : \{1.000000388\}.\ \{1.000012\}.\ \{1.000000373\}$$
$$= 2.467\ \times 1.000016$$
$$= 2.467 / 1.000016$$
$$= 2.46696$$

∴using formula (1)

$$P_{cr} = 2.46696\times\frac{206\times10^9\times2.4850\ \times10^{-13}}{(0.045)^2}$$
$$= 62\ N.$$

3-2-2 Modified configuration of the sewing machine's needle [3- sections]

By using formula (14):

$$P_{cr} = \eta\frac{E\ I_1}{\ell_4^2}$$

By using formula (2):

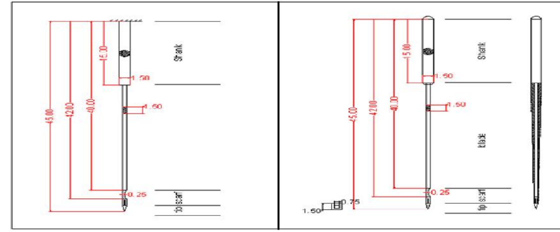

Figure (1) four parts of sewing needle

Table (1) geometrical characteristic of sewing needle:

Section	I (m4)	ℓ
Shark	$I_1 = 2.4850\ \times10^{-13}\ m^4$ $[\varphi = 1.5\ mm]$	$\ell_1 = 0.015\ m$
Blade	$I_2 = 1.0417\ \times10^{-14}\ m^4$ $= \frac{w\times t^3}{12}\left[, w = 1.0\ mm,\ t = 0.5\ m\right]$	$\ell_2 = 0.040\ m$
Scarf	$I_3 = 7.8125\times10^{-16}\ m^4$ $= \frac{w\ t^3}{12}\left[w = 0.6\ mm,\ t = 0.25\ mm\right]$ $I_3^{'} = w_1t_1^3/12\ - w_2t_2^3/12\ (eye) - negle$	$\ell_3 = 0.042$
Tip (point)	$I_4 = \frac{\pi\ \varphi^4}{64},\ \varphi = 0.25\ mm$ $= 1.9175\ \times10^{-16}\ m^4$	$\ell_4 = 0.045\ m$
		total $\ell = 0.045\ m$

$$\eta = 2.467 : \left\{1+(0.9581)\frac{(0.044-0.015)^2}{2}\right\}.\left\{1+0.925\times\frac{(0.044-0.040)^2}{2}\right\}$$
$$= 2.46598$$
$$\therefore P_{cr} = \eta\frac{206\ \times10^9\times2.485\ \times10^{-13}}{(0.044)^2}$$
$$= 2.46598\ \times 26.442$$
$$= 65\ N.$$

3-3-3 Modified configuration of the sewing machine's needle [2-sections]

by applying formulas (14) &(15) then:

$$\eta = 2.467\left\{1+0.9581\times\frac{(0.042-0.015)^2}{2}\right\}$$
$$= 2.467\{1.0003492\}.\left\{1+0.925\frac{(0.042-0.040)^2}{2}\right\}$$
$$= 2.467 / 1.00034939$$
$$= 2.466138$$
$$\therefore P_{cr} = 2.46614\times\frac{206\times10^9\times2.48125\times10^{-13}}{(0.042)^2}$$
$$= 72.0N$$

3-3-4 Modified configuration of the sewing machine's needle [one section]:

By applying both of formulas (14)&(15):

$$\eta = 2.467 \ :\left[1 - \frac{I_2 - I_1}{I_1} \times \frac{(\ell - \ell_1)^2}{2}\right]$$

$$= 2.467 \ :\left[1 + 0.9581 \times \frac{(0.040 - 0.15)^2}{2}\right]$$

$$= 2.467 \ / \ 1.000299$$

$$= 2.46626 \ .0$$

$$P_{cr} = 2.466261 \times \frac{206 \times 2.850 \times 10^{-4}}{(0.040)^2}$$

$$= 90 \ N$$

The summary of calculations that concern the critical load of the sewing machine's needle are tabulated in table (2). It is shown from the table that the actual sewing needle has the minimum critical load due it's more length as explained by equation or formula (1). On the contrary for the virtual sewing needle with one section (shank), the critical load Pcr is the highest because it has the shortest free length, as shown by Euler formula (1).The high critical load Pcr for the short sewing machine needle will enhance the value of the safety elastic stability factor m where:

$$m = \frac{P_{cr}}{P_{w0}}$$

Table (2) summary of calculation for Pcr values

	Type of sewing needle	The critical load Pcr .(N)*
1	Actual needle (4- section)	62
2	Virtual sewing needle (3 section)	65
3	Virtual sewing needle (2-section)	72
4	Virtual sewing needle (one section)	90

N- new tons - force unit due to S.I organization

Where, Pcr – critical of the sewing needle i.e. the load after which the sewing needle will lose its straight configuration and Pw is the working load, established during needle penetration the sewn fabric. Therefore the modern clothing technology manufacturers recommended the application in the industrial sewing machines, the short needle [1]. And the overlock machine needle is the shortest needle as this type of machine has the maximum speed. According to the high stability elastic safety factor m, the probability of elastic or plastic buckling of the needle is too negligible. These will lead to less bent or defective needle that will enhance the sewn fabric quality and sew ability.

From table (2) it will be noted that decreasing the sewing machine needle from 45 mm to 15 mm will increase the critical load Pcr from 90 N to 62 N i.e. by about 45% i.e. the critical load Pcr is too sensitive for the free length (ℓ) of the sewing needle.

The formula (2), concerns the stability elastic factor (η), gives different values for (η) with the change of sewing machine's needle configuration. For example for the longest sewing needle, $\eta = 2.46696$ while for the virtual shortest needle $\eta = 2.46626$ i.e. the difference is about 0.0284% that means it could be neglected. Therefore the main item in calculating Euler formula of Pcr is the free length of the sewing needle. By the way, the highest resisting force on the sewing needle tip [conical] part penetrating the sewn layered fabric takesplace for jeans [denim] cloth during garments production [1].

4-Conclusions& Recommendations

From the above mathematical approach, the following conclusions can be drawn for actual & virtual sewing industrial machine's needles.

1-The coefficient of the equivalent length of the sewing needle $\gamma = 2$. The elastic ideal stability factor η of Euler formula Pcr for sewing needle $= \frac{\pi^2}{\gamma^2}$ i.e $\eta = 2.4674$ where it is assumed the sewing needle has a constant cross- section.

2-The elastic stability factor η is 2.46696 for the actual sewing machine needle, [the longest needle 4 sections], while η is 2.46626 for the shortest virtual needle [one section] only. The difference between them is too negligible.

3-The main factor in calculating **Pucarenko et al. [9]**Pcr is the free length of the sewing machine needle.

4-In **Pucerenkoand Yakovlev and Matveev.[9]** formula (2) for calculating the elastic stability factor η , the second part of the formula that decrease or increase the value of η ideal for sewing needle with constant cross-section, has a title effect on the value of η ideal and could be neglected.

5-Seemingly, the **Pucarenko et al. [9]** formula (2) for calculating the critical load on the actual sewing machine needle [5- sections: shank, blade, scarf, eye & point] is enough for the industrial applications. The eye section is neglected.

It is recommended to try with another techniques, for calculating the critical load Pcr. such energy techniques **[9]** also, it is recommended to carry out an experimental work for finding or exploring the actual critical load for actual sewing machine needle and then calculating the safety factor of the elastic stability m.

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Effects of Ethanolic Purslane Shoot and Seed Extracts on Doxorubicin-Induced Hepatotoxicity in Albino Rats

Osama M. Ahmed[1, 3]; Walaa G. Hozayen[2]; Haidy Tamer Abo Sree [3]; Mohamed B; Ahmed[4]

[1]Physiology Division, Zoology Department, Faculty of Science, Beni- Suef, Beni-Suef University Egypt
[2]Biochemistry Division, Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt
[3]Biochemistry Division, Faculty of Oral and Dental Medicine Nahda University, Beni-suef, Egypt
osamamoha@yahoo.com; walaahozayen@hotmail.com; haidyalshafeey@yahoo.com

Abstract: Doxorubicin (DOX), an anthracycline antibiotic, is a broad-spectrum antineoplastic agent, which is commonly used in the treatment of uterine, ovarian, breast and lung cancers, Hodgkin's disease and soft tissue sarcomas as well as in several other cancer types. The effect of doxorubicin (4 mg/kg b.w.week) without or with oral administration of ethanolic purslane *(Portulaca oleracea*) shoot (leaves and stems) extract (50 mg/kg b.w.day) or ethanolic purslane seeds extract (50 mg/kg b.w.day) co-treatments for 6 weeks was evaluated in adult male rats. Serum ALT, AST, ALP, GGT, total bilirubin,total protein and albumin levels were assayed. Lipid peroxidation (indexed by MDA) and antioxidants like hepatic glutathine, glutathione transferase, peroxidase, SOD, CAT were assessed. There was an increase in serum levels of ALT, AST, ALP, GGT and total bilirubin. In addition, hepatic glutathine, glutathione transferase, peroxidase, SOD, CAT activities were decreased while lipid peroxidation in the liver was increased. Co-administration of ethanolic purslane and seed extracts successfully improved the adverse changes in the liver functions with an increase in antioxidants activities and reduction of lipid peroxidation. In coclusion, it can be supposed that dietary purslane extract supplementation may provide a cushion for a prolonged therapeutic option against DOX hepatopathy without harmful side effects. However, further clinical studies are required to assess the safety and efficacy of these extract in human beings.

[Osama M. Ahmed; Walaa G. Hozayen; Haidy Tamer Abo Sree; and Mohamed B; Ahmed. **Effects of Ethanolic Purslane Shoot and Seed Extracts on Doxorubicin-Induced Hepatotoxicity in Albino Rats.** *Nat Sci* 2013;11(10):94-101]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 15

Key words: Doxorubicin, purslane, hepatotoxicity and antioxidants.

1.Introduction

Doxorubicin (DOX) obtained from soil actinomycetes *Streptococcus peucetius* is used for the treatment of solid tumors such as those arising in the breast, bile ducts, endometrial tissue, esophagus and liver, osteosarcomas, soft-tissue sarcomas and non-Hodgkin's lymphoma (**Tikoo *et al*., 2011)**. DOX is known as a powerful anthracycline antibiotic widely used to treat many human cancers, but significant cardiotoxicity **(Kuznetsova *et al*., 2011)**, hepatptoxicity **(Patela *et al*., 2010)**, nephrotoxicity **(Mohana *et al*., 2010)** and testicular toxicity **(Trivedi *et al*., 2011)** limits its clinical application**.** Mitochondria are considered to be one of the primary targets of DOX through mitochondria-mediated apoptosis, remarkable modification of mitochondrial membranes (e.g. *via* binding with cardiolipin), which is also associated with changes in various mitochondrial functional parameters and activities of respiratory chain complexes **(Trivedi *et al*., 2011)**. Moreover, doxorubicin significantly damages energy-transferring and -signalling systems like creatine kinase and AMP-activated protein kinase **(Kuznetsova *et al*., 2011)** Doxorubicin causes disturbances in the balance between oxidative stress and antioxidant defence system leading to tissue injuries **(Saad *et al*., 2001; Karaman *et al*., 2006)**.

A number of studies were conducted for antioxidants screening from the natural medicine aiming to minimize oxidative injury by DOX. Several natural antioxidants have been shown to alleviate the DOX-induced cell damage without compromising its anti-tumor efficacy in the animal studies **(Xin *et al*., 2011)**. *Portulaca oleracea L*, is commonly known as purslane. It is a warm climate, annual, green shoot **(Al-Quraishy *et al*., 2012)**. Recent research indicates that purslane offers better nourishment than the major cultivated vegetables due to its shoot that is a rich source of X9-3-fatty acids, α-tocopherols, ascorbic acid, β-carotene and glutathione. Its seeds also contain a high percentage of α-linolenic acid (LNA) **(Al-Quraishy *et al*., 2012).** These features contribute to the anti-oxidative properties of purslane which derive from the following pharmacologically active substances, including: 28% flavonoids, that are nearly exclusively flavonol-O-glycosides; 8% terpenoids (principally ginkgolides A, B, C and bilobalide); 6–12% organic acids; and >0.5% proanthocyanidins defined as flavonoid-based polymers. Purslane is effective as an antioxidant agent **(Dkhil *et al*., 2011)** as well as providing nourishment for the liver, kidneys and testes. Experimental evidence has also shown that purslane has an anti-oxidative effect in heart tissues in mice by increasing superoxide dismutase activity **(Al-Quraishy**

***et al*., 2012)**. Other outhors reported that the purslane contains many compounds, including alkaloids, omega-3 fatty acids, coumarins, flavonoids, polysaccharide, cardiac glycosides, anthraquinone glycosides. and containing β-sitosterol **(Mohamed *et al*., 2011)**.

Based on these issues and concerns, the present study was designed to investigate the preventive effect of ethanolic extract of purslane shoot parts and seeds on liver dysfunction and oxidative stress in doxorubicin-administered rats.

2.Material and methods

I- Experimental animals:

Male Wistar albino rats weighing about 140-180g were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Opthalmology, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature (25±5°C) as well as 12 hours daily normal light periods. Moreover, they were given access of water and supplied daily with standard pellet diet *ad libitum*. All animal procedure are in accordance with the recommendations of the Canadian committee for care and use of animals (**Canadian council on Animal care [CCAC], 1993**).

2. Chemicals and drugs

Doxorubicin was purchased from EBEWE pharma, Ges. m.b.H. Nfg. KG A-4866 Unterach, Austria., Purslane shoot parts and seeds were purchased from Harraz Medicinal plant company, Cairo, Egypt (WWW.harrazegypt.com).Total billirubin and ALP (alkaline phosphatase) kits were obtained from SCICO Diagnostic Company and ALT (alanine aminotransferase), AST (aspartate aminotransferase) and GGT (gamma-glutamyl transferase) kits were purshased from Quimica Clinica Aplicada S.A. Company, (Spain), Albumin and total protein kits were obtained from Diamond Diagnostics, Egypt. Chemicals used in measurement of antioxidants were obtained from Sigma Chemical Company, USA.

3. Shoot and seed extract

The shoot parts of the plant and seeds were dried in the shade.They were powdered by an electric grinder then, they were exhaustively extracted with 80% ethanol. The solvent was removed by evaporation under reduced pressure using Bucchi Rotary Evaporator **(Wang *et al*., 2012)**.

4. Experimental Animal grouping and experimental design:

The animals of the present experiment were allocated into 4 groups:

1-Normal control: The rats of this group were given the equivalent volume of vehicle (0.9% NaCl) for 45 days

2-Doxorubicin–administered control: The rats of this group was administered intraperitoneally a dose of 4 mg/Kg b.w.week for 6 weeks **(Trivedi *et al*., 2011)**.

3-Doxorubicin-administered group treated with purslane shoot extract: This group was orally treated with purslane shoot extract at dose level of 50 mg/kg b.w.day for 6 weeks **(Ali and Bashir, 1994; Fayong Gong *et al*., 2009)**.

4- Doxorubicin-administered group treated with purslane seeds extract: This group was orally treated with purslane seeds extract at dose level of 50 mg/kg b.w.day for 6 weeks **(Ali and Bashir, 1994; Fayong Gong *et al*., 2009)**.

5. Preparation of blood and tissue homogenates

By the end of the experimental periods (6 weeks), rats were scarified under mild diethyl ether anesthesia at fasting state. Blood samples were collected and allowed to coagulate at room temperature. The clear, non-haemolysed supernatant sera were quickly removed divided into four portions for each individual, and stored at - 20˚ C for subsequent analysis. Liver was quickly excised, weighed and homogenized in a saline solution (0.9 %NaCl) (10% w/v) using Teflon homogenizer (Glas-Col, Terre Haute, USA), The homogenates were centrifuged at 3000 r.p.m. for 15 minute and the supernatants were kept at -20 C$^{°}$ for the assay of biochemical parameters related to oxidative stress and antioxidant defense system.

6. Assay of liver function:

ALT and AST activities in serum was determined according to the method of **Reitman and Frankel (1957)** using reagent kits purchased from Quimica Clinica Aplicada S. A. Company (Spain). GGT activity was measured according to the method of **Szasz (1969)** using reagent kits obtained from Quimica Clinica Aplicada S. A. Company, (Spain). ALP activity was measured according to the method of **Kind and King (1954)** by using reagent kits obtained from SCICO Diagnostic Company, Egypt. Total bilirubin concentration in serum was determined in serum according to the method of **Jendrassik and Grof (1938)**, using the reagent kits purchased from SCICO Diagnostic Company, Egypt. Albumin concentration was determined in serum according to the method of **Doumas *et al*. (1971)**, using the reagent kits purchased from Diamond Diagnostics, Egypt. Serum globulin concentration and albumin/globulin ratio were calculated according to **Rojkin *et al*. (1974).** Serum total proteins concentration was determined according to the method of **Henry (1964)**, using reagent kits purchased from Diamond Diagnostics, Egypt.

7. Assay of Lipid peroxidation and antioxidant parameters

Liver oxidative stress and antioxidant defense parameters were estimated using chemicals purchased from Sigma Chemical Company (USA) and using Jenway Spectrophotometer (Germany), Glutathione activity in homogenates was determined according to the chemical method of **Beutler *et al.* (1963)** with little modification. Lipid peroxidation concentration in homogenates was determined according to the chemical method of **Preuss *et al.* (1998)**. Peroxidase (POX. EC 1.11.1.7) activity in homogenates was estimated according to the modified chemical method of **Kar and Mishra (1976)**. Superoxide dismutase (SOD EC 1.15.1.1) activity in homogenates was determined according to the chemical method of **Marklund and Marklin (1974)**. Glutathione-s-transferase (GST. EC 2.5.1.18) concentration in homogenates was determined according to the chemical method of **Mannervik and Guthenberg (1981)**. Catalase (CAT, EC 1.11.1.6) activity in homogenates was assayed according to the chemical method of **Cohen *et al.* (1970)**.

8-Histological examination:

After sacrifice and dissection at specific time intervals, pieces of liver from all groups were immediately removed from each animal, fixed in 10% neutral buffered formalin and transferred to Department of Histopathology, Faculty of Veterinary medicine, Beni-Suef University, Egypt for preparation, sectioning and staining with haemotoxylin and eosin (H&E) **(Bancroft and Stevens, 1982).**

9. Statistical analysis

The data in the present study were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) followed by LSD test to compare various groups with each other. Results were expressed as mean ± standard error (SE) and values of $P > 0.05$ were considered non-significantly different, while those of $P < 0.05$ and $P < 0.01$ were considered significantly and highly significantly different, respectively.

3.Results

Biochemical effects

The doxorubicin-administered rats showed a highly significant increase ($P < 0.01$) in serum ALT, AST, GGT and ALP activities as compared to normal control group. The treatment of doxorubicin-administered rats with purslane shoot and seed ethanolic extracts induced a highly significant decrease of the elevated serum of ALT, AST, GGT and ALP ($P < 0.01$) activities as compared to doxorubicin-administered rats (Figures, 2 & 3). The doxorubicin-administered rats showed a highly significant increase ($P < 0.01$) in serum level of total bilirubin concentration as compared to normal control group. The treatment of doxorubicin-administered rats with purslane shoot and seed ethanolic extracts induced a highly significant decrease of the elevated serum total bilirubin ($P < 0.01$) level as compared to doxorubicin-administered rats. (Figures 4 & 5). The doxorubicin-administered rats showed a highly significant decrease ($P < 0.01$) in serum level of total protein, albumin and globulin levels as compared to normal control group. The treatment of doxorubicin injected rats with purslane shoot ethanolic extract induced a significant increase of the serum total protein, albumin and globulin ($P < 0.05$) level. The treatment of doxorubicin-administered rats with purslane seeds ethanolic extracts induced a highly significant increase ($P < 0.01$) of serum total protein, albumin and globulin as compared to doxorubicin-administered rats (Figures, 7 & 8). The liver antioxidants levels of glutathione, catalase, SOD, peroxidase and glutatione-S-transferase in doxorubicin-administered rats showed a highly significant decrease ($P < 0.01$) recording percentage decreases of -68.49, -86.70, -168.50, -39.7 0 and -47.30% respectively as compared to normal control group. Lipid peroxidation exhibited a highly significant increase in doxorubicin-injected rats as compared to normal rats. The treatment of doxorubicin-administered rats with purslane shoot ethanolic extract induced a highly significant increase of the serum glutathione, catalase, SOD, peroxidase and glutatione-S-transferase levels ($P < 0.01$); the recorded percentage increases were 83.89, 222.6, 98.30, 30 and 44.30% respectively as compared to doxorubicin-administered rats. The treatment of doxorubicin-administered rats with purslane seeds ethanolic extracts induced a highly significant increase ($P < 0.01$) in serum glutathione, catalase, SOD, peroxidase and glutatione-S-transferase activities ($P < 0.01$) recording percentage increases of 141.89, 548.60, 121.90, 58.90 and 75.80 % respectivily as compared to doxorubicin-administered rats. Liver peroxidation was profoundly ($P < 0.01$) improved in doxorubicin-administered rats treated with purslane shoot and seed extracts. In general, the seed extract seemed to be the most potent in improving liver function and antioxidant defense system (Figures 9-14).

Histological changes

The normal liver histological architecture is shown in figure 15. The hepatocytes are arranged in hepatic strands radiating from the central vein. The hepatic sinusoids are found between hepatic strands.

The liver of doxorubicin-administered rats showed congested or hyperemic hepatic portal and central veins as well as the hepatic sinusoids. There was mild perivascular fibrosis, fatty change, kupher cell multiplication and hemorrhages.

A larg numbers of hepatocytes showed vascular degeneration and had a peripheral distribution within the hepatic lobules and there was hyperplasia of the bile ducts.

The treatment of doxorubicin-administered rats with purslane shoot and seed extracts produced marked improvement of liver histological changes. Mild vascular changes and kupffer cell multiplication were noticed in liver of doxorubicin-administered rats which were treated with purslane shoot ethanolic extract (Figures 17a & 17b). Also, congested hyperemic central vein and kupffer cells polifiration were observed in liver section of doxorubicin-administered rats treated with purslane seed extracts (Figures 18a & 18b).

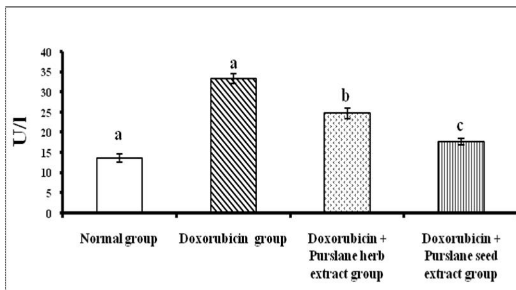

Figure 1: Effect of purslane shoot and seed ethanolic extracts on serum ALT activity in doxorubicin-administered rats.LSD at the 5% level: 3.235; LSD at the 1% level: 4.413.

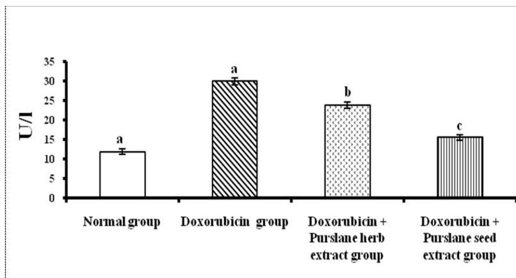

Figure 2: Effect of purslane shoot and seed ethanolic extracts on serum AST activity in doxorubicin-administered rats. LSD at the 5% level: 2.345; LSD at the 1% level: 3.202

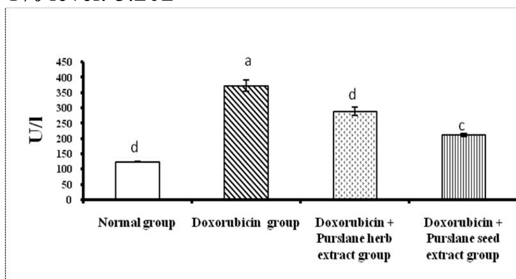

Figure 3: Effect of purslane shoot and seed ethanolic extracts on serum Alkaline Phosphatase activity in doxorubicin-administered rats. LSD at the 5% level: 35.753; LSD at the 1% level: 48.762

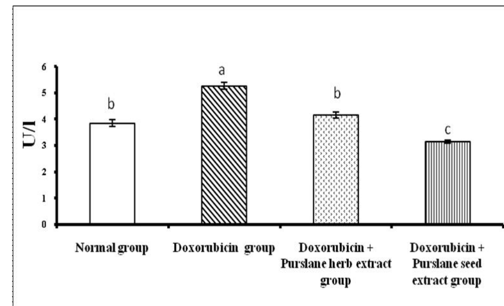

Figure 4: Effect of purslane shoot and seed ethanolic extracts on serum GGT activity in doxorubicin-administered rats. LSD at the 5% level: 0.330; LSD at the 1% level: 0.450.

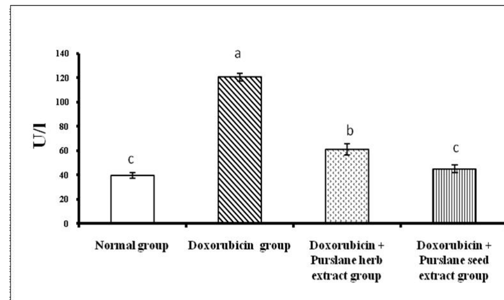

Figure 5: Effect of purslane shoot and seed ethanolic extracts on total bilirubin level in doxorubicin-administered rats. LSD at the 5% level: 10.214; LSD at the 1% level: 13.931

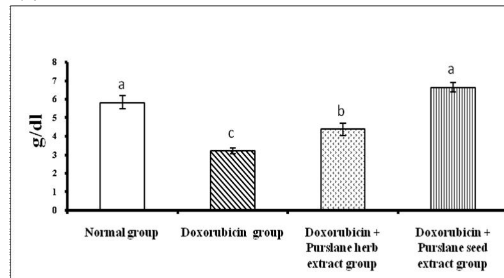

Figure 6: Effect of purslane shoot and seed ethanolic extracts on serum total protein level in doxorubicin-administered rats. LSD at the 5% level: 0.839; LSD at the 1% level: 1.145.

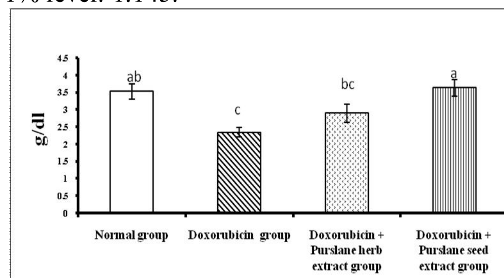

Figure 7: Effect of purslane shoot and seed ethanolic extracts on serum albumin level in doxorubicin-administered rats. LSD at the 5% level: 0.648; LSD at the 1% level: 0.883.

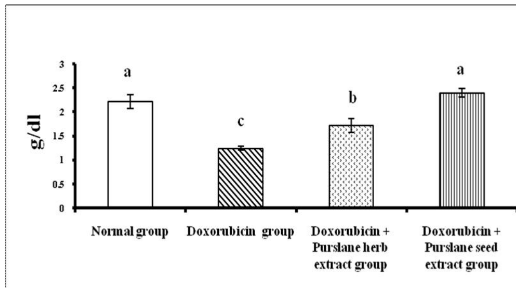

Figure 8: Effect of purslane shoot and seed ethanolic extracts on serum globulin level in doxorubicin-administered rats. LSD at the 5% level: 0.348; LSD at the 1% level: 0.474.

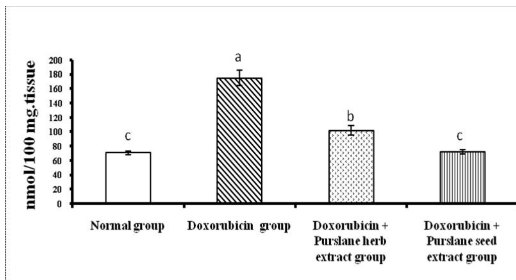

Figure 9: Effect of purslane shoot and seed ethanolic extracts on hepatic MDA in doxorubicin-administered rats. LSD at the 5% level: 19; LSD at the 1% level: 25.91.

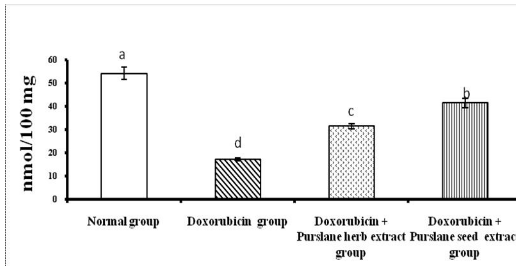

Figure 10: Effect of purslane shoot and seed ethanolic extracts on Hepatic glutathione in doxorubicin-administered rats. LSD at the 5% level: 5.388; LSD at the 1% level: 7.348.

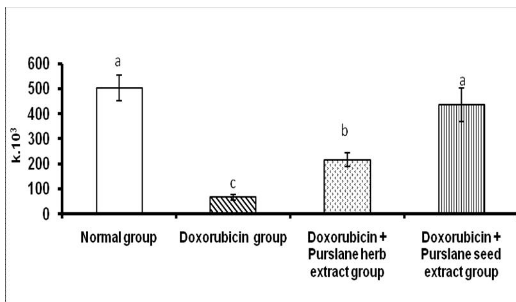

Figure 11: Effect of purslane shoot and seed ethanolic extracts on Hepatic catalase in doxorubicin-administered rats. LSD at the 5% level: 131.558; LSD at the 1% level: 179.427.

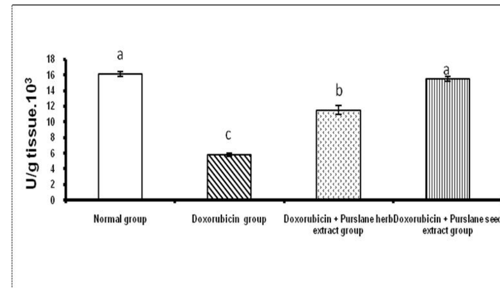

Figure 12: Effect of purslane shoot and seed ethanolic extracts on hepatic SOD activity of doxorubicin-administered rats. LSD at the 5% level: 1.120; LSD at the 1% level: 1.528.

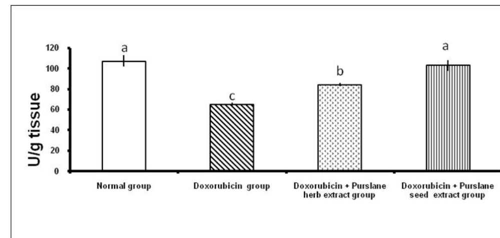

Figure 13: Effect of purslane shoot and seed ethanolic extracts on hepatic peroxidase activity of doxorubicin-administered rats. LSD at the 5% level: 12.317; LSD at the 1% level: 16.799.

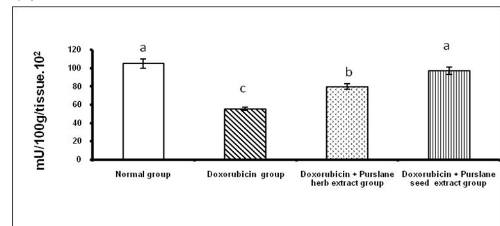

Figure 14: Effect of purslane shoot and seed ethanolic extracts on hepatic glutathione transferase activity of doxorubicin-administered rats. LSD at the 5% level: 10.744;LSD at the 1% level: 14.653.

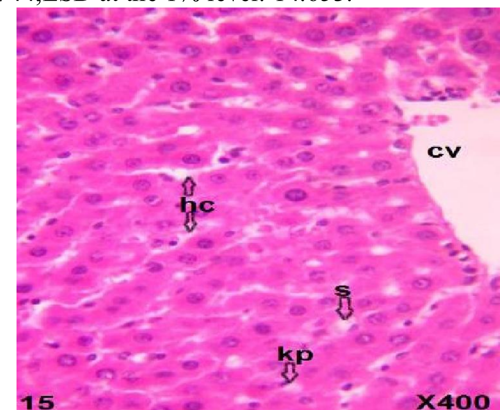

Figure 15: Photomicrograph of liver section showing normal rats histological architecture. Central vein (CV), hepatic strands (hs), sinusoids (s) and kupher cells (kp) are noticed.

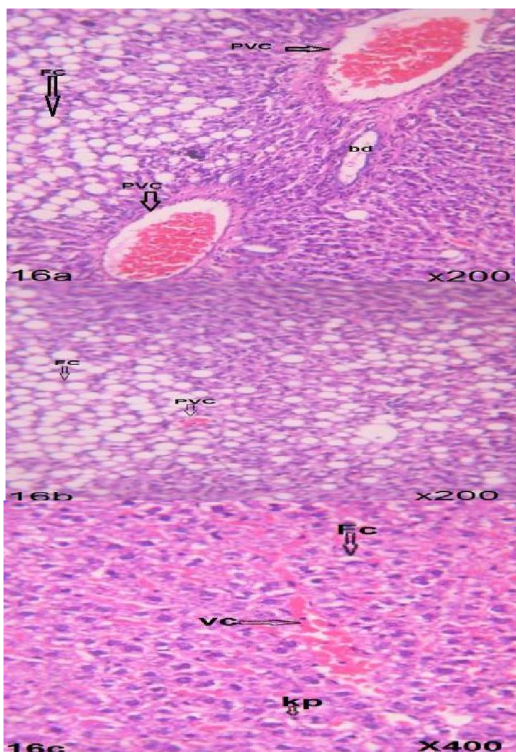

Figure 16: Photomicrograph of liver section of doxorubicin administered rats showing fatty change (FC), perivascular fibrosis (PVF), congested portal vein (CPV) and bile ductules (bd) proliferation as well as hemorrhage (hr),vascular degeneration (VD) and kupffer cells (kp) multiplications (Figures 16 a,b and c).

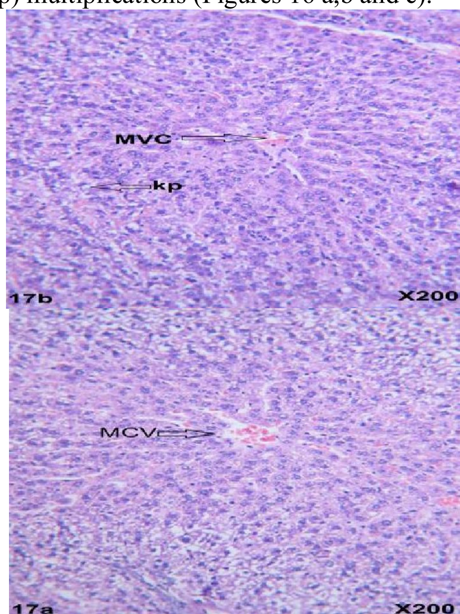

Figure 17: photomicrograph of liver section doxorubicin administered rats treated with purslane shoot ethanolic extracts showing moderate vascular changes (MVC) and kupffer cells (kp) proliferation (Figures 17 a and b).

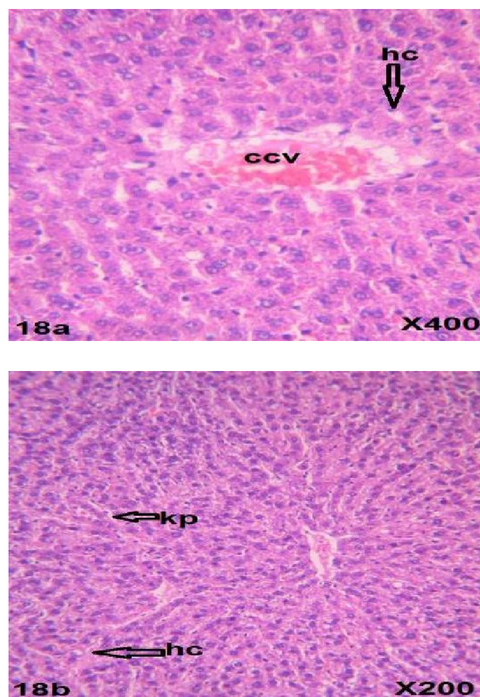

Figure 18: photomicrograph of liver section doxorubicin administered rats treated with purslane seed ethanolic extracts showing congested central vein (ccv) (figure 18 a), kupffer cells (kp) multiplication and hydropic cells (hc) (Figures 18a and 18b).

4.Discussion

Doxorubicin, a quinone-containing anthracycline antibiotic, is an important agent against a wide spectrum of human neoplasms. However, its toxicity limits usage in cancer chemotherapy **(Singal *et al*., 1987; Fadillioglu *et al*., 2003)**. It has been shown that free radicals are involved in doxorubicin-induced toxicities **(Yagmurca *et al*., 2004)**. The chemical structure of doxorubicin causes the generation of free radicals and the induction of oxidative stress that correlates with cellular injury **(*Saad et al*., 2001)**. Doxorubicin causes an imbalance between free oxygen radicals (ROS) and antioxidants. The disturbance in oxidant–antioxidant systems results in tissue injury that is demonstrated with lipid peroxidation and protein oxidation in tissue **(Karaman *et al*, 2006)**.

The present study revealed that intraperitoneal injection of 4 mg doxorubicin/ kg b.w. for 6 weeks induced hepatoxicity manifested biochemically by a significant increase of serum ALT, AST, ALP and GGT activities and bilirubin concentration in addition to a significant decrease in serum total protein, albumin and globulin levels. These results are in accordance with **Injac *et al*. (2008)** who attributed the increase in the serum enzyme levels to their increased leakage from damaged and necrotic hepatocytes as a result of toxicity. **El-Maraghy *et al*. (2009)** attributed alteration

in serum protein to changes in protein and free amino acids and their synthesis in the injured liver cells and/ or increased protein degradation.

The increase in serum total bilirubin may be owing to blockage of bile ducules as the inflammation and fibrosis in the portal triads and/ or due to regurgitation of conjugated bilirubin from the necrotic hepatocytes to sinusoids **(Ahmed, 2001).**

The previous deleterious biochemical alterations of the present study were associated with a marked elevation of liver lipid peroxidation and a significant decrease of non-enzymatic antioxidant (glutathione) content and enzymatic antioxidants (catalase, superoxide dismutase, peroxidase and glutathione- S – transferase) enzyme activities. These results are in agreement with many other authors (**Abd El-Aziz *et al.*, 2001; Kalender *et al.*, 2005; Yagmurcaa *et al.*, 2007)** who stated that one of the most prevailing hypothesis of hepatic damage from doxorubicin administration is the ability of the drug to produce reactive oxygen species (ROS) and suppress antioxidant defense mechanism. They also revealed that the increased lipid peroxidation play a critical role in liver injury.

Histopathological examination of liver sections of doxorubicin-administration rats supported the previous biochemical results. The liver exhibited fatty changes, vascular and hydropic degeneration, perivascular fibrosis, necrosis of some hepatocytes, haemrrhage, kupffer cell multiplication and bile ductule proliferation. These results are in concurrence with **Yagmurcaa *et al.* (2007)** who noticed degradation of hepatocytes, congestion, necrosis and proliferation of bile ductules in doxorubicin-treated rats.

The treatment of doxorubicin-administered animals with purslane shoot and seed ethanolic extracts successfully improved the elevated serum activities of ALT, AST, ALP and GGT and serum bilirubin concentration. The lowered serum total protein, albumin and globulin levels were potentially ameliorated in doxorubicin -administered rats treated with the tested extracts. These results are in agreement with previously published reports **(Singal *et al.*, 1987; Fadillioglu *et al.*, 2003; Yagmurca *et al.*, 2004 and Karaman *et al,* 2006)**. Moreover, **Tawfeq (2008)** reported that the purslane extract caused a significant reduction in the doxorubicin-induced toxicity in mice. **Omoniyi and Mathew (2006)** found that the aqueous extract of purslane potentially improved the functional status of liver as indicated by a decrease in serum activities of classical enzymes of liver function.

These ameliorations in biochemical serum parameters of liver functionin the present study are associated with the improvement in liver histological changes. The seed extract seemed to be more potent than shoot parts. The liver showed moderate vascular changes as a result of shoot extract and hyperemic central vein and kupffer cell multiplication as a result of treatment with seed extract.

The improvement of liver function and intergrity may be mediated *via* the antioxidant activity of purslane shoot and seed extracts. This is confirmed by the current study which revealed a significant decrease of lipid peroxidation and increase in CAT, SOD, peroxidase and glutathaione- S-transferase activities and glutathione levels.

Conclusion

The co-administration of purslane shoot and seed ethanolic extracts potentially prevented the deleterious effects of doxorubicin on liver. The effect of seed ethanolic extract seemed to be more potent than that of shoot ethanolic extracts. This improvement effect in liver injury may be mediated *via* enhancement of the antioxidant defense system. However, further clinical studies on human beings are required to assess the efficacy and safety of the purslane ethanolic extracts.

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Production of Pectinase by Fungi isolated from Degrading Fruits and Vegetable

*Adesina, Felicia C.1, Adefila, Olutola A.1, Adewale, Adeyefa1, O and Ummi Habiba O1, Agunbiade, Shadrach O. 2

1Dept of Microbiology, Faculty of information Technology and Applied Sciences Lead City University, Ibadan, Oyo State, Nigeria.
2Dept of Biochemistry, Faculty of information Technology and Applied Sciences Lead City University, Ibadan, Oyo State, Nigeria.
*adesinafelicia@yahoo.com, +2348123329944

ABSTRACT: Peelings of fruit and vegetable waste are usually thrown away as waste and they constitute environmental nuisance because they are found in heaps littering our environment and contributing to environmental pollution. This work was therefore aimed at using these fruit peelings as substrates in solid state fermentation for production of fungal pectinase. Pectinase producing fungal isolates from spoilt fruit peels and pomace were selectively obtained among other fungal isolates by culturing in successive enrichment medium. The obtained isolates were screened on agar plates containing 0.5% pectin as the sole carbon source. Two fungal isolates *Rhodotorulla* spp and *Mucor mucorales* were selected due to the large clearance zones they had on the plates and were used in solid state fermentation for the production of pectinase. Substrates used for the production of pectinase were dried and milled orange and pineapple peelings and water melon pomace while incubation was for 14 days. Assay was carried out every other day. Crude enzyme extract from both isolates were characterized. Highest pectinase activity of 82.95U/dry weight of substrate (dw) was recorded for *Rhodotorulla* spp. using orange peelings as substrate on the 8th day while the least was 16.12U/dw on water melon pomace as substrate on the 2nd day. Mucor had its highest pectinase activity of 46.05U/dw on orange peelings as substrate on the 12th day. Optimum temperature and pH for pectinase by *Rhodotorulla* spp was at 35˚C and 6.0 respectively while that of *Mucor mucorales* was 45˚C and 5.8 respectively. Pectinase by *Mucor mucorales* was relatively stable even at 65˚C. Michaelis Menten (K_m) constant value for pectinase by *Rhodotorulla* spp was 3.0 mg/ml while the maximum velocity (V_{max}) was 0.023364U/mg/min. K_m value of pectinase by *Mucor mucorales* was 15 mg/ml while the V_{max} was 0.043364U/mg/min. The molecular weights of pectinase by Rhodotorulla spp. were 35 and 45 kDa while that of *Mucor mucorales* was 60 kDa. Fruit waste therefore could be used as substrates for the production of microbial pectinases.

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Keywords: Pectinase, Waste, Fruit, Fungi.

INTRODUCTION

Pectinases hydrolyze pectin, the soluble complex polysaccharides that occur widely in plant cell walls. Pectinases are commercially used in many processes (Kashyap *et al*., 2001) and nearly 25% of the global enzyme sales are attributed to pectinase (Kaur *et al*., 2004).It degrades polygalacturonan by hydrolysis of the glycosidic bonds that link galacturonic acid residues.

Pectinase have been reported to be produced by a large number of bacteria and fungi such as *Bacillus* spp., *Clostridium* spp., *Pseudomonas* spp., *Aspergillus* spp., *Monilla laxa*, *Fusarium spp.*, *Verticillium* spp., *Penicillium* spp., *Sclerotinia libertiana*, *Coniothyrium diplodiella*, *Thermomyces lanuginosus*, *Polyporus squamosus*, nematodes, yeasts and protozoa (Jayani *et al*., 2005). The microbial world has shown to be very heterogeneous in its ability to synthesize different types of pectolytic enzymes with different mechanisms of action and biochemical properties (Favela-Torres *et* al., 2005; Gummadi & Panda, 2003). Pectic enzymes are produced by both prokaryotic microorganisms, which primarily synthesize alkaline pectinases, and by eukaryotic microorganisms, mostly fungi that synthesize acid pectinase (Kashyap *et al*., 2001, Hoondal *et al*., 2002; Jayani *et al*., 2005). Furthermore, the production of these enzymes has also been described in yeast (Alimardani-Theuil *et al*., 2011; Blanco *et al*., 1999).

Pectinases are used in the textile industry as they are capable of depolymerising the pectin breaking it into low molecular water soluble oligomers improving absorbency and whiteness of textile material and avoiding fiber damage (Sonia *et al*., 2009). Pectinase is used in juice clarification. There is use of xylano-pectinolytic enzymes in paper and pulp industry. Pectinases are effective in biobleaching of mixed hard wood and bamboo kraft pulp, as pretreatment of kraft

pulp with xylano-pectinolytic enzymes from same alkalo thermotolerant isolate produced pulp with superior quality facilitating adaptation of environment friendly technology in paper pulp industry (Amanjot *et al*., 2010). Pectinase also find application in the degumming of plant fibers, retting of plant fibers, pectinase from *Bacillus species* are used in waste water treatment. Pectinase are further used in coffee and tea fermentation by breaking pectin present in tea leaves, oil extraction by avoiding emulsification formation, improvement of chromaticity and stability of red wines (Botella *et al*., 2005), pectinase improve wine characteristics of colour and turbidity, biscouring of cotton. Pectinases possess biological applications in protoplast fusion technology and plant pathology (Ernesto *et al*., 2006).

It is known that the fruits and vegetables are highly perishable and a lot are wasted as a result of this. Also these fruits are not easily disposed off because a large number of them tend to spoil easily and they are found degrading in heaps in our environment. Instead of allowing them to constitute nuisance in the environment, the perished fruits and vegetables could be turned into wealth by utilizing them as substrates for certain enzyme production. Fungi and yeasts are common colonizers of degrading fruits and vegetables, therefore using both or either of them to produce microbial enzymes such as pectinase in solid state fermentation of degrading fruits and vegetables and their peelings will go a long way in bringing in wealth for the nation

Materials and Methods

Sterilization Process

All glass-wares were sterilized using hot-air oven (Gallenkampus Model NYC-101) at 180^{0}C for three hours. All media were sterilized by autoclaving in an autoclave (Model YM50) at a temperature of 121^{0}C and 15psi for 15minutes. Inoculating needles, cork borers and blades were sterilized by flaming until red-hot. All subculturing and inoculation was carried out in lamina flow chamber.

Enrichment for Isolation of Pectinase Producers

1gm of each sample was aseptically inoculated into each 100ml of 0.5% (w/v) pectin broth containing the following in g/l: $CuSO_4$. $5H_2O$(0.5), KCl(0.5), $MnSO_4$. $7H_2O$(0.01), $FeSO_4$ $7H_2O$(3.00), $NaNO_3$(1.00), $ZnSO_4$(0.50) and Pectin(5) in 1000ml 0.1M Phosphate buffer (pH6.2) flasks and covered back. The flasks were incubated on rotary shaker (Model) at 30ºC±2ºC for 7 days. After 7 days. 1ml of each previously enriched medium was inoculated into 100ml of another newly prepared 0.7% (w/v) pectin broth in 250 ml flask and covered with aluminum foil. Each flask was incubated on rotary shaker (Model) at 30ºC±2ºC for 7 days. This was done four times consecutively and increasing the concentration of pectin in the broth by 0.2g by each subsequent preparation isolation was done Nitinkumar and Bhushan (2010).

Plate Screening of Pectinase Producers

Pectin agar plates were prepared containing in g/l the following: $CuSO_4$. $5H_2O$(0.5), KCl(0.5), $MnSO_4$. $7H_2O$(0.01), $FeSO_4$ $7H_2O$(3.00), $NaNO_3$ (1.00), $ZnSO_4$ (0.50), 1% (w/v) pectin and agar (15g) in 1000ml phosphate buffer (pH6.2) IN 1000ml Erlenmeyer flask. The medium was sterilized by autoclaving and allowed to cool down to about 40ºC. The medium was then dispensed aseptically into sterile petri dishes and allowed to set in a lamina flow. 0.1ml of each of the last enrichment medium was inoculated on each pectin agar plate and incubated at 30ºC±2ºC for 5 days. After 5 days, the plates were each flooded with 1% acetyl trimethyl ammonium bromide (CTAB) to observe the zones of clearance which indicates production of pectinase by isolates Nitinkumar and Bhushan (2010).

Identification of Selected Isolates

Pure culture of selected isolates was obtained by subculturing the isolates on Potato Dextrose Agar plates consistently until pure culture of each isolate was obtained and later transferred to PDA slants. The culture slants were kept after wards in the refrigerator at 4ºC. Pure cultures were subcultured every 2 weeks to maintain isolates. A composite of cultural and morphological characteristics were used to identify the fungi. Cultural characteristics included morphology on culture plates such as mycelia growth, size, type, pigmentation (surface and reverse) and sporulation were used (www.dr.fungus.com).

Production of Pectinase by Selected Isolates

Collection and Preparation of Samples

Fruit peels (pineapple, water melon and orange) and spoilt vegetables were collected from Orita market, Ibadan, Oyo State, Nigeria. The samples were collected in clean, properly labeled, polythene bags and taken to the laboratory for further work. Each substrate was milled into about 10mm size and dried in the oven at 60ºC until the weight was constant. 5g of each substrate medium was weighed into each 100ml Erlenmeyer flasks and each was moistened with 10ml of a moistening medium consisting of $CuSO_4$, $5H_2O$ (0.5), $MnSO_4$. $7H_2O$(0.01), $FeSO_4$ $7H_2O$(3.00), $NaNO_3$ (1.00), $ZnSO_4$ (0.50) in 1000ml of 0.1M phosphate buffer (pH6.2) in a 1000ml Erlenmeyer flask, covered with aluminum foil and sterilized by autoclaving at a temperature of 121°C, 15psi for 15minutes. After sterilization, each flask was allowed to cool to room temperature. The pure culture of each isolate on slants was homogenized carefully in 10 ml of sterile distilled water and 2ml of the obtained homogenate was introduced into each flask containing the substrate. The flaks were incubated at 30±2°C for

14days and assay was done every other day throughout incubation period.

Assay for Crude Pectinase

Extraction of crude pectinase was done by simple contact method (Krishna *et al*., 1996). 0.5% (w/v) of pectin was prepared in phosphate buffer (pH 6.2) as the enzyme substrate. 1ml each of the crude enzyme extract was added to 1ml of the pectin solution in each Mac Cartney bottle and was left for 10 minutes at room temperature. 1ml of DNSA was added to the mixture thereafter reaction was stopped by adding 1ml of Rochelle's salt and boiled in water at 90°C for 5 minutes. Then the mixture was diluted by adding 2ml of phosphate buffer. The absorbance was measured Spectrophotometrically at 595 nm in a spectrophotometer (752W-UV-VIS grating spectrophotometer) (Miller, 1959). A standard graph was generated using standard glucose solution. One unit of Pectinase activity was defined as the amount of enzyme which liberated 1µm glucose per min.

Enzyme characterization

Effect of pH on pectinase activity

The effect of varied pH on the enzyme produced by each isolate was determined. Conical flasks containing buffer solution of varied pH (5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2 and 7.4) were prepared and 0.5%(w/v) of pectin was dissolved in each of them. Each bottle containing the enzyme substrate mixture was incubated at 30º ± 2ºC for 10 minutes. 1ml of 3, 5 dinitrosalicylic acid (DNSA) was later added to each mixture to terminate the reaction and later boiled for 5 minutes. The absorbance was read at 595nm using a spectrophotometer (752W UV-VIS Grating Spectrophotometer) (Miller, 1959).

Effect of temperature on pectinase activity

The effect of different temperature on the enzyme produced by each isolate was determined. I ml of crude pectinase was introduced into each bottle containing 1ml of 0.5% (w/v) pectin in Phosphate buffer (pH6.2). Each bottle containing the reaction mixture was incubated at varied temperatures of 35ºC, 40ºC, 45ºC and 50ºC. 1ml of 3, 5 dinitrosalicylic acid (DNSA) was added to each bottle containing enzyme-substrate mixture to terminate the reaction and later boiled for 5 minutes. The absorbance was read at 595nm using a spectrophotometer (752W UV-VIS Grating Spectrophotometer) (Miller, 1959).

Determination of Thermostability of Pectinase

Thermostability of the crude pectinase was determined by exposing the enzyme to temperature ranges of 45˚C – 70˚C at different durations of 0.5hour, 1 hour, 1.5 hours, 2 hours and 2.5 hours. After each exposure enzyme assay was carried out to determine the activity of the pectinases.

Effect of Different Concentration of Pectin on Enzyme Activity and Determination of K_m and V_{max} of Pectinase

The effect of different pectin concentration on the activities of the enzyme produced by the isolates was studied. Different concentrations (0.025%, 0.075%, 0.05%, 0.10%, 0.125% (w/v) pectin in phosphate buffer (pH6.2). 1ml of was introduced into each McCartney bottle containing each concentration of pectin and incubated at 30º ± 2ºC for (10 minutes. Thereafter, 1ml of 3,5 dinitrosalicylic acid (DNSA) was added to each reaction mixture to terminate the reaction and later boiled for 5 minutes. The absorbance was read at 595nm using a spectrophotometer (752W UV-VIS Grating Spectrophotometer) (Miller, 1959).

Determination of Molecular Weight of Crude Pectinase

Molecular weight of the crude pectinase was determined by the method of Laemli, (1970).

RESULTS

Four fungal isolates that were able to degrade pectin were obtained from the degrading fruit and vegetable waste. Two isolates were however selected for further work after the screening test because they had higher relative pectinase activity. These isolates were identified as *Rhodotorulla spp* (a yeast) and *Mucor mucorales.* Results of the screening test were presented on Table 1. From the results shown on Figs. 1 and 2, all the substrates supported the production of pectinase by the isolates but the amount of pectinase produced varied on each substrate and by each isolate. Pectinase production was highest by *Rhodotorulla spp* on the 8th day (82.95U/g dry weight of substrate (dw)) in Orange peelings as shown on Fig. 1 while maximum pectinase was produced by *Mucor mucorales* on the 12th day (46.05U/g dry weight of substrate (dw))) on the same substrate (Fig. 2). The least production of the enzyme by both isolates was on the 2nd day of incubation (Figures 1 and 2).

Optimum activity of the pectinase produced by *Rhodotorulla spp* was at 35°C (58.7U/ml) while the least activity of the enzyme was at 60°C (9.01U/ml). Highest activity of pectinase produced by *Mucor mucorales* was at 45°C (49.3U/ml) while the lowest was at 30°C (30.7U/ml). Comparing the activities of the pectinase by the isolates, pectinase by *Mucor mucorales* was able to withstand higher temperature than that produced by *Rhodotorulla spp* (Fig. 3).

Pectinase by *Rhodotorulla spp* and *Mucor mucorales* had optimum activities at pH values of 6.0 and 5.8 respectively and the least for each of them were pH values of 7.4 with activities of 25.6U/ml and 8.3U/ml respectively as shown on Figure 4.

Substrate concentration of 0.5% (w/v) prompted the highest activity of the pectinase produced by *Mucor mucorales* and *Rhodotorulla spp* respectively while the least was at concentrations of 1.0% (w/v) for *Mucor mucorales* and 0.75% (w/v) for *Rhodotorulla spp*. This is presented on Figure 5. The maximum velocity for pectinase by *Mucor mucorales* was 0.043 U/mg/min while that of *Rhodotorulla spp* was 0.023 U/mg/min Michaelis Menten constant for pectinase by *Mucor mucorales* was 15.0 mg/ml while it is 3.5 mg/ml for *Rhodotorulla spp* as obtained from the double reciprocal curve plotted for the enzymes (Figure 6).

The molecular weight profiles of the crude pectinases from the isolates were 90kDa *Rhodotorulla spp* and 35, 45 and 60 kDa for *Mucor mucorales* as shown on Plate 1.

Discussion

Fungal isolates used in this work for the production of pectinase were *Mucor mucorales* and *Rhodotorulla spp*. Fungal isolates have been successfully used for the production of pectinase by different researchers (Silva *et al*., 2002; Suresh and Viruthagiri, 2010; Banu *et al*., 2010), infact the most common source of microbial pectinase is from *Aspergillus niger* (Castilhoa *et al.,*1999). Agro industrial wastes have been considered as for solid state fermentation and production of microbial enzymes because they serve as cheaper substrates for microbes (Pandey *et al*., 2002). Orange bargaisse and other fruit waste have been used by other researchers as substrate for the production of microbial enzymes and in all cases have served as good sources of the substrate needed to induce the synthesis of such enzymes (Silva *et al*., 2002; Seyis and Aksoz, 2005). The composition of the different substrates used may have affected the amount of pectinase produced by each of the isolates. On the other hand, the consistency of the substrate particles may have interfered in their packing during the fermentation thereby affecting the gas and heat exchange within the system (Mitchell *et al*., 2000). Low pectinase production observed in both isolates on all the substrates on the 2nd and forth days may be because the microorganisms were trying to adapt to the new substrates as sources of carbon since they were subcultured from PDA slants whose carbon source is simpler than the new substrates. The relatively low production of pectinase by these isolates after an optimum production on the 8th day for *Rhodotorulla* spp and12th day for *Mucor mucorales* may be because fungal pectinase are subject to catabolite repression by high free sugar concentration affecting inducible and constitutive enzymes (Aguillar & Huitron, 1987 and Guevara *et al*., 1997).

Silva *et al*., (2002) found orange bargaisse to be the best substrate for polygalacturonase production by *Penicillium sp* which is in agreement with what was obtained in this work in which orange peelings supported best pectinase production by *Rhodotorulla* spp. In the production of alkaline pectinase by bacteria isolated from decomposing fruit materials, bacterial isolate from orange waste showed the maximum pectinase enzyme activity (Bhardwaj *et al* 2010). This may be because orange peels have very high pectin content and is more acidic than the other substrates making it a condusive condition for yeasts (Okafor, 2007). The optimum production of pectinase by the bacteria isolate was found to be at 72 hours in submerged fermentation (Bhardwaj *et al* 2010) which is in variance with what was obtained in this work.

Every enzyme has its optimal activity and stability up to a certain temperature and get denatured at higher temperature (Lehninger *et al*., 1992). Optimum temperature of pectinase produced by *Mucor mucorales* was 45˚C while that of *Rhodotorulla* spp was 35˚C, while this results agrees with that of some researchers it is in various with that of others. In screening of pectinase producing bacteria and their efficiency in biopulping of paper mulberry bark (Poonpairoj *et al*., 2001), pectinase activity from bacterial strains N05 and N10 had the optimum activity at pH 10 and temperature 35°C. Blanco *et al*., (1999) reported that some yeasts (*Tephrosia candida* and *Kluyveromyces fragilis*) produced pectinase with maximal activities at temperatures up to 60°C.

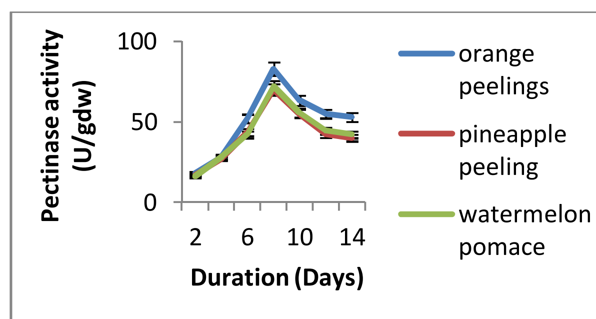

Figure1:Time course for the production of pectinase by *Rhodotorulla spp*

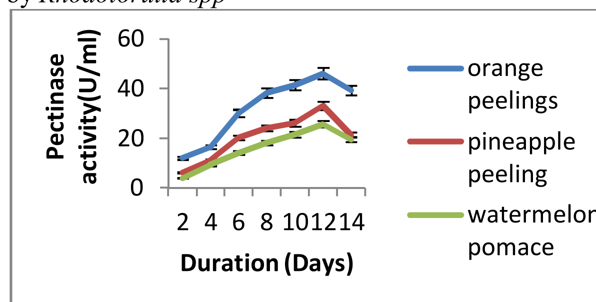

Figure2:Time course for the production of pectinase b y *Mucor mucorales*

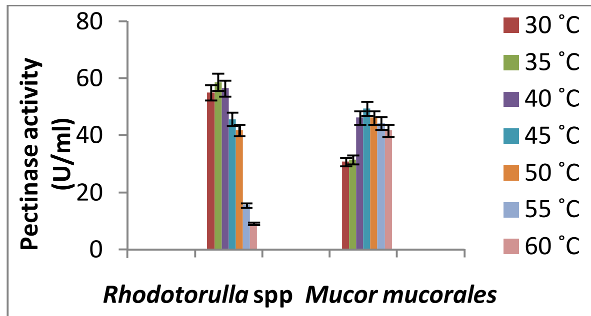

Figure3: Effect of temperature on pectinase by the fungi

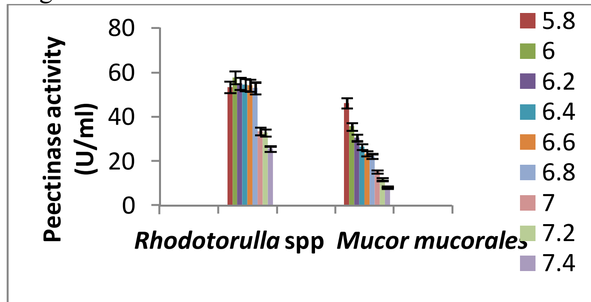

Figure 4: Effect of different pH on pectinase by the fungi

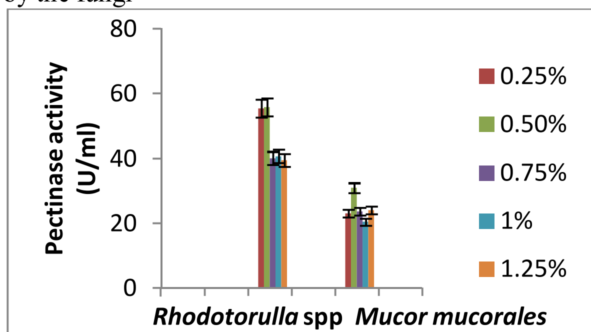

Figure 5: Effect of different substrate concentration on pectinase by the fungi

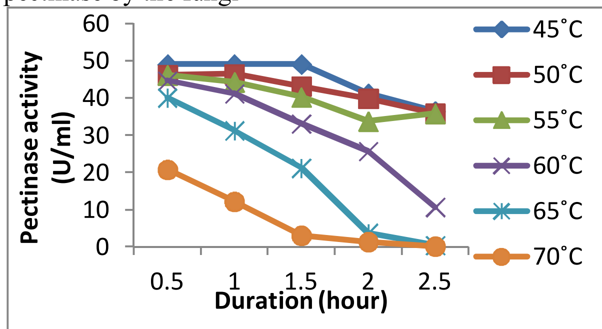

Figure 8: Thermostability of pectinase synthesized by *Mucor mucorales*

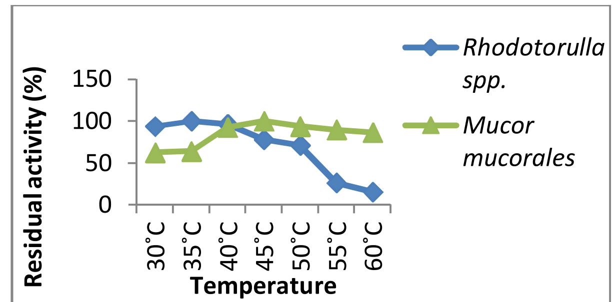

Figure 6: Residual pectinase activity of isolates at varied temperature

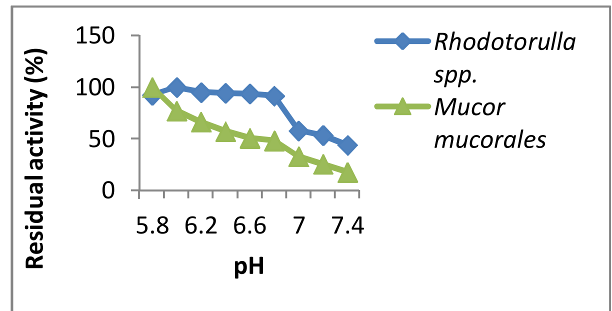

Figure 7: Residual pectinase activity of isolates at different pH

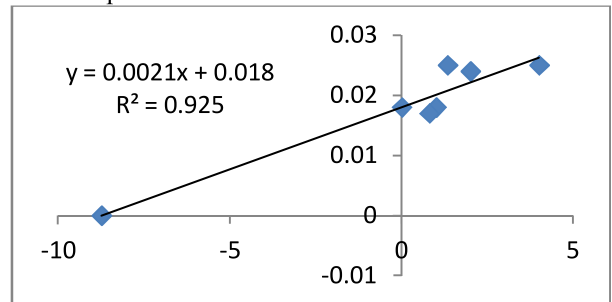

Figure 8: Lineweaver-Burk graph showing reciprocal of K_m and V_{max} values of pectinase by *Rhodotorulla* spp.

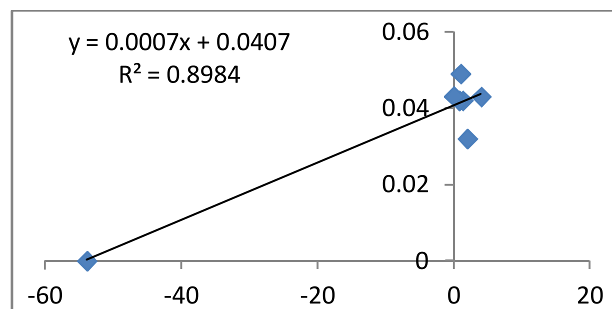

Figure 9: Lineweaver-Burk graph showing reciprocal of K_m and V_{max} values of pectinase by *Mucor mucorales*.

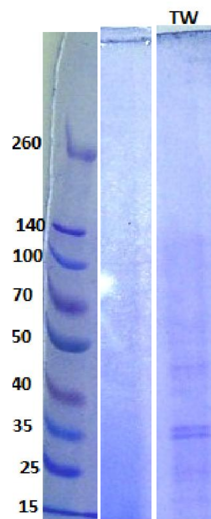

Figure 10: Molecular weight of pectinases produced by *Rhodotorulla spp* and *Mucor mucorales*
KEY:
First Lane: Molecular weight Marker
Second Lane: Pectinase by *Rhodotorulla* spp.
Third Lane: Pectinase by *Mucor mucorales*

Also Kluskens *et al*., (2005) reported the optimal temperature for a polygalaturonase from *Streptomyces* sp. QG- at 60°C and the hyperthermophilic bacterium *Thermotoga maritima* was at 80°C. One of the pectinase characterized in this work had its maximum activity at 35°C which is within the mesophilic range since the isolate is generally a mesophilic fungus (Alexopoulus *et al*., 1980).

Marcia *et al.,* (1999) recorded a maximum activity of the pectinase produced by *Penicillium* spp. to be at pH 6.0, Pereira *et al*., 2002 also reported that the optimum pH for polygalaturonase production by *Penicillium sp* was found to be at pH 6 these results are within the range of the pectinase produced by *Rhodotorulla* spp which had maximum activity at pH 6.0. However Banu *et al*., 2010 obtained a result that is a little deviated from the result obtained in this work recording maximum activity at pH6.5 for *Penicillium* spp they worked with. Much work has not been done on production of pectinase by *Mucor* spp. and *Rhodotorulla* spp. Maximal activity of pectinase synthesized by *Mucor mucorales* used in this work was at pH 5.8, Alexopoulus *et al*., (1980) reported that fungi prefer acidic pH range for growth and so the enzymes synthesized by them maintain activity within this range too. This result is however close to what was obtained in this study and. Fungal pectinase with high affinity for alkali pH had however been reported by Denis *et al.,* (2005) in *P. viridicatum* with maximal activity at pH 8.0 and able to maintain 80% of its activity at pH 9.

Reda *et al*., 2008, reported the maximum production of pectinase from *Bacillus sp* at pectin concentration of 1% while Pereira *et al*., 2002, reported the optimum pectinase production from *Penicillium* sp obtained from soil at pectin concentration of 1.5%. Both results were in variance with results obtained in this work.

Conclusion

The exploration of microbial biodiversity in decaying plant matter has allowed, especially in recent years, to identify and characterize new pectin-enzyme-producing microorganisms. Also, it has been technically possible on the one hand to select wild strains and constitutive mutants that produce a single enzyme, and on the other hand, the heterologous expression in bacteria and yeast of numerous genes which encode pectin enzymes, obtaining producing strains of interest.

The result from this *research* work shows that pectinase was produced by *Rhodotorulla spp* and *Mucor spp* used in this work. *Mucor spp* produced pectinase at an optimum temperature of 45 ºC which is a desirable property for microbial pectinase that is targeted for industrial use. This is because most of the industrial processes in which these pectinases are used are carried out under these environmental conditions. Thus, the pectinase obtained from this work has a potential for industrial use. It therefore obvious from the results obtained in this work that vegetable and fruit waste can be used as substrate for solid state production of microbial pectinase which can be a source of income for Nigeria if more work is done on the economics of the production process by optimizing the production conditions.

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The Socioeconomic Impact of Adopted Agroforestry Practices on the Livelihoods of Rural Small Scale Farmers in Northern Rwanda

Isaac Emukule Ekise [1], Alphonse Nahayo[1], Jennifer Rono[1] and Jean Berchmans Twahirwa[2].

[1] Department of Forestry and Nature Conservation, Higher Institute of Agriculture and Animal Husbandry, P.O. Box 210, Musanze, Rwanda
[2] Department of Rural Development and Agribusiness, Higher Institute of Agriculture and Animal Husbandry P.O. Box 210, Musanze, Rwanda
iekise@yahoo.com

Abstract: Understanding the impact of agroforestry practices on rural farming households' livelihoods is the best way of seeking strategies to improve the farming systems and hence improve the welfare of households. In this regard, a survey on the impact of agroforestry practices was conducted in Jenda Sector in 2012 with the aim of obtaining information of the contribution of these new techniques using a sample size of 116 households selected using random sampling method. SPSS and Excel programs were used to process and analyze data. The results of the survey showed that woodlots (59%), windbreaks (3%), fruit trees (3%), boundary tree planting (62%), live fences (31%), intercropping or Taungya (5%) and homegardens (28%) are the most commonly preferred agroforestry practices among households in the sector. The most preferred tree species are *Erythrina abysssinica* (100%), *Alnus acuminata* (94%), *Grevillea robusta* (77%) and *Iboza liparia* (72%). The households affirmed that agroforestry practices increased soil fertility (65%), increased farm income (59%), conserved soil and water (76%), reduced chances of crop failure through diversification (42%), saved time used to collect fodder and fuelwood from forests (46%) and maintaining and improving surrounding environment (39%). Honey (4%), timber (8.9%), fodder (45%), firewood (74%), stakes (82%), fruits (24%), charcoal (17%), building poles (46%), and medicines (32%) are some of the agroforestry products produced on their farms. Agroforestry practices on the farms accounted for 40% of total annual income. The estimated multiple linear regression model showed that the socioeconomic factors influencing income generation positively were age, sex, experience in farming, household size, education, health status and land size with an R^2 value of 50%. The variation in income due to the stochastic error term was accounted for by 50%. The study also showed that lack of capital (1.12 mean rank), lack of planting materials (1.71 mean rank), lack of labour (3.06 mean rank), and lack of technical advice (mean rank 3.19) were the major constraints hampering the full agroforestry impact. In conclusion, this study has established that agroforestry practices (techniques) are indeed transforming the rural livelihoods of households and these practices should be upscaled in other sectors of the country.

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Keywords: Agroforestry practices, transforming , livelihoods, rural small scale farmers.

1. Introduction

Considering high population growth rates, increasing poverty levels and scarcity of land, the need for technologies that would boost food production including crops and animals, forest and wood products as well as sustaining the use of land cannot be over emphasized (Young, 2004). International concern is to find alternative farming systems that are ecologically and economically sustainable as well as culturally acceptable to farmers.

Agroforestry, which is a collective name for all land-use systems and practices where woody perennial plants are deliberately grown on the same land management unit as agricultural crops and/or animals, either in spatial mixture or in temporal sequence (ICRAF, 2003) has been suggested by several development experts as a new solution to rural development needs (Rocheleau *et al*, 1989).

An agroforestry practice denotes as a distinctive arrangement of components in space and time (Nair, 1993). Examples of agroforestry practices are tree home gardens, woodlot, windbreaks/shelterbelts, boundary planting, live fences, alley cropping, improved fallow, Taungya, plantation crop combinations, silvopastoral practices, Agroforestry for fuelwood production, intercropping under scattered or regularly planted trees, Agroforestry for reclamation of problem soils, Buffer – zone agroforestry, apiforestry and acquaforestry.

Agroforestry practice leads to the production of some economic products such as food, fodder, fuelwood, medicinal substances, gums and resins, tannins, essential oils, fibers and waxes (Rehm and

Espig, 1991). Oram (1993) reported that agrofoestry provides a wider range of products, more secure subsistence or more cash income from wood products to enable the farmer to buy food. Nair (1993) indicated that the combination of several types of products which are both subsistence and income generating, helps farmers to meet their basic needs and minimizes the risk of the production system's total failure.

Some important service roles of agroforestry are: soil conservation, either erosion control (presence of a permanent soil cover, barrier effect against run-off), soil fertility maintenance (incorporation of organic matter into the soil, nutrient pumping from the deep layers of the soil through the tree's roots, these nutrients then improve the crops through litter and mulch, nitrogen fixation) or soil physical properties maintenance (Young, 1989).

Also, agroforestry practices, through different products, help farmers to pay schools fees for the children, provide house building materials, and fodder for the animals, honey and stakes among others. In addition, the agroforestry practices contribute to income generation for the farmers as well as they are able to pay health insurance, increase social relationship between the households and reduce the poverty in the households. Agroforestry therefore helps to mitigate deforestation, combat land depletion, and as a result, can contribute to the alleviation of rural poverty (ICRAF, 2001).

The contribution of agroforestry and forest products is particularly important to rural communities in terms of food and nutritional requirements, medicines, fodder for livestock, gums, fibre, construction materials and related domestic requirements. In addition many agroforestry products like honey, wax and bamboo generate income to rural communities once they are marketed, hence of improving the well-being or livelihood of rural populations (FAO, 2009).

This study has determined the contribution of agroforestry practices to the livelihoods of rural farmers in Jenda Sector. The results from the present study provide information on commonly used agroforestry products, their contribution to income generation and social uses in the study area. The results also can be used for decision making on agroforestry use in order to improve the well-being of the rural people while promoting the sustainable use of agroforestry practices without compromising our environment.

The combination of several types of products in agroforestry, which are both subsistence and income generating helps farmers to meet their basic needs and minimizes the risk of the production system's total failure. Agroforestry can help mitigate deforestation because it addresses in general, the issues of tree planting, can combat land depletion because of its potential for soil conservation and as a result contribute to the alleviation of rural poverty (ICRAF, 2003).

Given the immense agricultural and environmental potential of agroforestry it is no wonder that it is being promoted for adoption among farmers in most developing countries especially in Africa where productivity is low and more marginal lands are increasingly being brought under cultivation. Also, the agroforestry practices, through different products, help the farmers to get the schools fees for the children, house building materials, and fodder for the animals, honey and stakes. In addition, the agroforestry practices contribute to income generation for the farmers as well as they are able to pay health insurance, increase social relationship between the households and reduce the poverty in the households.

In Rwanda, there is a national concern to combat environmental degradation and those emanating from poor agricultural practices (deforestation, soil erosion) have received a lot of attention. However, technology transfer and adoption has not been very easy in the country as a result of several existing barriers, which have not yet been fully overcome. Some of the barriers that militate against agroforestry adoption include illiteracy, inadequate credit facilities, non-availability of farm inputs and socio-cultural factors (MINAGRI, 2007).

In Nyabihu District, as elsewhere in the country the integration of trees in farm management unit was considered as the strategies adopted for enhancing the diversification of the productions (Nyabihu, 2011). Trees are the principal source of energy for cooking, materials for construction, domestic utensils mostly in rural areas, with a wide range of other product and services including fruit, medicine, livestock feeding, fencing, etc.

The understanding of the importance of agroforestry by the farmers in Nyabihu District in general and in Jenda Sector in particularly would be the best way of improving that technique ,hence result in improving the households' welfare. In that context, a research study on the socio-economic impact of agroforestry practices was conducted in Jenda Sector with the aim of getting the real information of the contribution of these new techniques introduced in agricultural farming on the livelihood of households in this study area.

2. Material and Methods

2.1. Description of the study site

Jenda Sector is one of the twelve Sectors of Nyabihu District in Western province. It is bordered

at the East by Mukamira Sector, to the North by Democratic Republic of Congo, to the West by Bigogwe Sector, to the South by Karago Sector. Its surface area is 36.6 km^2 for 39,365 inhabitants. The number of households is 5415. Jenda Sector is subdivided into seven cells, such as Bukinanyana, Gasizi, Kabatezi, Kareba, Nyirakigugu and Rega and it consists 40 villages *(Imidugudu*), (Jenda Sector, 2012).

Jenda Sector experiences four seasons well distributed during the whole year as follows:
Short dry season: December till February
Large wet season: March till May
Large dry season: June till August
Short wet season: September to November
The average temperature is 14.9º centigrade and the average annual rainfall is 1337.2 mm.

The altitude of Jenda Sector is between 2000 m and 2500 m. It means that there are many mountains and hills because it is located in the North of Rwanda where there are chains of mountains (Jenda Sector, 2012).

The soil is volcano soil that has fertility. The reaction of soil is neutral; it is a better soil when it is exploited in the best conditions. The soil of Jenda Sector is very permeable with low depth on mountains. This type of soil is vulnerable to many erosion phenomena in the area with abrupt slope; in The general the soil of Jenda is very fertile (Jenda Sector, 2012).

This Sector has one health center, six primary schools, and three secondary schools, 76.4% of households have access to health care and 87% of households use the clean water (Jenda, 2012). In

Jenda Sector there are the following household categories: Vulnerable (in abject poverty), very poor, poor, the resourceful poor, the food rich and the money rich (Jenda Sector, 2012).

The economy of Jenda is based on agriculture and livestock farming. The main crops grown in Jenda Sector are food crops (potato, maize, sorghum, wheat, beans, marketing gardening and fruits) and cash crops (tea and pyrethrum). The system of keeping is still traditional. The animals kept are .cows, goats, sheep, pig, hens, rabbits, and bee keeping and the exotic races are still at low level (Jenda Sector, 2012). This Sector is crossed by road and electric line from Musanze District to Rubavu District. This allows transport of products and supplying different centers with electric power. Some people of Jenda Sector use biomass energy. The crops like potatoes are sold in different areas of Jenda Sector where the farmers have set up the commercial site. The prices are fixed by farmer and his/her customer. There are also some roads facilitate the forwarding of these crops (Jenda Sector, 2012).

2.2. Sampling procedures in the study

Determination of sample size at sector level

A sample is a portion of the population selected to achieve the objectives of the study. This study adopts purposive sampling procedure where a sample of n private households is selected by using the formula by Kothari (2004) as given by Dagnelie (2006) below:

$$n = \frac{z^2 \times p \times q \times N}{d^2(N-1) + z^2 \times p \times q}$$

Where**:**
n= sample size,
N= size of population (number of households),
Z= coefficient normal distribution,
q= probability of failure,
d= margin error,
p= probability of success.

In Kothari (2004) the margin of error varies between 5 % and 10 %. The study used the margin error of 9 %, the confidence level of 95 %, probability of success p=0.5, failure probability is q=0.5, and tabulated $Z_{0.25}$=1.96. The sample was selected proportionally from 5415 households of the study Sector.

Dermination of sample size at sector level

For determining the sample size at Sector level the proportional allocation formula is used:

$$ni = \frac{Ni \times n}{N}$$

Where:
ni= the sample size proportion to be determined;
Ni= the population proportion in the stratum;
n= the sample size;
N= the total population.

Using proportionate sampling the number of households interviewed in each cell is depicted in Table 1 below.

Table 1: Number of Households and sample size in each cell

Cell	Number of households/cell	The sample size proportion determined
Bukinanyana	1023	22
Gasizi	876	19
Kabatezi	938	20
Kareba	891	19
Nyirakigugu	773	16
Rega	914	20
Total	**5415**	**116**

2.3. Data collection

Data was collected through household interviews for households selected in each cell. A structured questionnaire was the principal instrument in this exercise. First, the questionnaire was pretested using 10 households, adjusted and then used finally to collect data. The questionnaire was translated into the local language to facilitate communication with household heads who usually are only proficient using the local language called "Kinyarwanda".

3. Results

3.1. Types of Agroforestry Practices adopted by households in Jenda Sector

Figure 1 shows the different Agroforestry practices adopted by farmers in Jenda Sector.

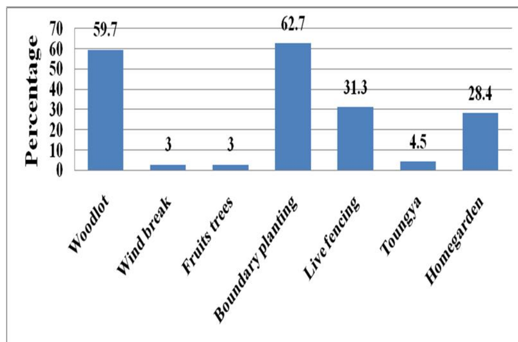

Figure 1: Agroforestry practices in Jenda

Agroforestry practices adopted by farmers in the study area include Boundary planting, (62.7 %), woodlot (59.7%), life fencing (31.4%), home gardens (28.4 %), taungya (4.5%), windbreaks (3 %) and fruits trees in intercropping practice (3 %). From that, it is clear that in Jenda Sector, the farmers do and adopt agroforestry practices in their farms with socio-economic and environmental considerations. The boundary planting is one of the agroforestry practices which is preferred by the farmers in Jenda Sector.

Types of agroforestry species planted in the study area

The tree crops in the land use system included *Persea americana, Alnus accuminata, Leucaena diversifolia, Calliandra sp, Erythrina abyssinica, Iboza riparia, Markhamia lutea, Cyphomandra betacea*, etc.

Figure 2 presents the proportions of farmers growing various species in the study area.

In figure 2, the most grown species in Jenda Sector are *Erythrina abyssinica, Alnus accuminata, Grevillea rubusta* and *Iboza riparia* corresponding to 100 %, 94 %, 77.6 % and 71.6 % respectively. These species have been shown to be more adaptable and productive in the highlands area like Busogo area. In addition, the fruit trees grown are *Persea americana* and *Cyphomandra betacea* with 7.5 % and 6 %. The *Erythrina abyssinica* specie is very dominant in Jenda Sector due to its considerable role in traditional believes.

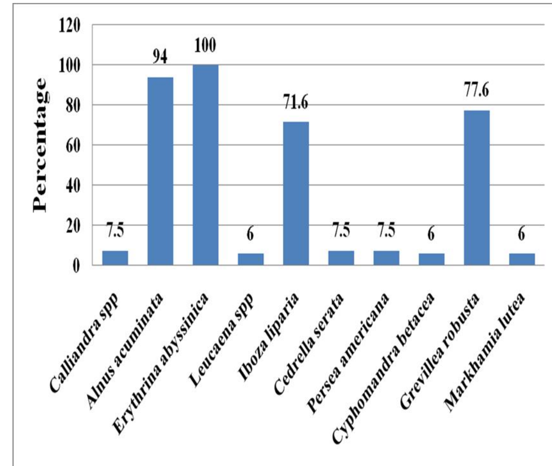

Figure 2: Types of Agroforestry species adopted in Jenda Sector

3.2. The Roles of Agroforestry practices in Jenda Sector

Service roles

The service functions of agroforestry practices known by the farmers of Jenda Sector are presented on the figure 21 bellow.

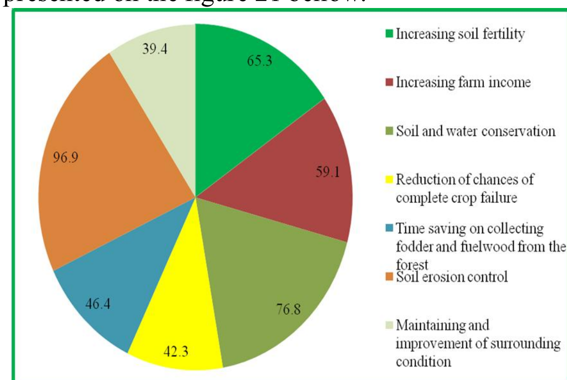

Figure 3: Service roles of agroforestry practices in Jenda Sector

The results presented on figure 21 show that the farmers of Jenda Sector 96.9% of them agree that the agroforestry practices play a vital service role in soil erosion control and 76.8 % of the farmers know that the agroforestry practices contribute in soil and water conservation; while the 65.3 %,59.1 %,46.4 %, 42.3 % and 39.4 % of farmers are aware of increased soil fertility, increased farm income, saved time for collecting fodder and fuel wood from the forest, reduced chances of complete crop failure and

maintained and improved surrounding condition as the service roles of agroforestry practices in Jenda Sector respectively. In terms of improving surrounding condition, agroforestry like forestry purifies air i.e. it reduces green house gases, creates microclimate, protects livings things against sun light and provides shade.

Productive roles

A part from the service roles of agroforestry practices there are productive roles that are played by those practices in the study area. The most productive roles of agroforestry practices found in Jenda Sector are mentioned on the figure 22.

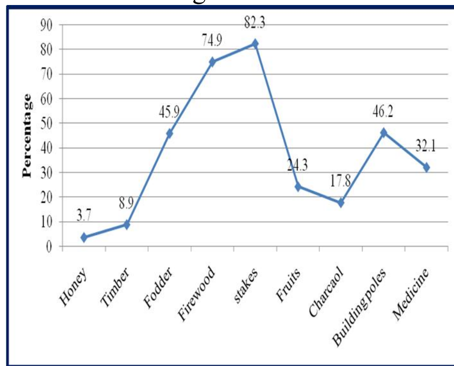

Figure 4: Productive roles of agroforestry practices in Jenda Sector

In figure 4, the products gained from agroforestry practices which are found in Jenda sector are stakes (82.3 %), firewood (74.9 %), building poles (46.2 %), fodder for animals (45.9%), medicines (32.1 %), fruits (24.3 %), charcoal (17.8 %), timber (8.9 %) and honey (3.7 %).

Social roles of Agroforestry practices

The social uses of agroforestry products in Jenda Sector are shown in figure 5 presented below.

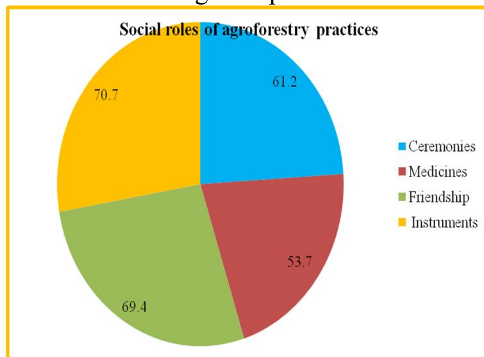

Figure 5: Social use of agroforestry products

As indicated in figure 5, the social uses of agroforestry products are varied. Instruments are ranked highly by 70.7 % of households, maintaining friendship with the neighbors is ranked second by 69.4 % for example when one farmer gives his/her neighbor or friend a bundle of firewood or stakes, the society or community is made cohesive and use in ceremonies (like marriage, funerals and birth celebration) is affirmed by 61.2 % of households while provision of medicines is ranked 4th by 53.7 % of households.

3.3. The socio-economic factors affecting the income levels of the farmers

Table 2 below summarizes the influence of socioeconomic factors on household income.

Table 2: Coefficient of regression model on income

Model	Unstandardized Coefficients Betas	p-value
(Constant)	-144045	0.189
Age (X_1)	25689.7 (β1)	0.350
Sex (X_2)	36815.4 (β2)	0.446
Experience in farming (X_3)	789.3(β3)	0.988
The size of the family (X_4)	49106.3(β4)	0.005
Education (X_5)	146417.8(β5)	0.000
Health status (X_6)	10392.6(β6)	0.768
The land size (X_7)	101338.6(β7)	0.000

Source: Survey data

The regression model equation is:

$Y = -144045 + 25689.7X_1 + 36815.4X_2 + 789.3X_3 - 49106.3 X_4 + 146417.8 X_5 + 10392.6 X_6 10138.6 X7 + \varepsilon_{i.}$

The size of the family, education and the land size are statistically significant at 5% level of significance because the p-value (0.005, 0.000, 0.000 respectively) are less than an α-value (significance level) of 0.05 while others factors are not statistically significant because the p-values (0.446, 0.350, 0.998, 0.800, 0.768 respectively) are greater than an α value of 0.05.

3.4. Constraints affecting adoption of Agroforestry in the study area

The results obtained on the constraints affecting the adoption of agroforestry practices in the study area are presented in the figure 6.

According to the results indicated in Figure 6, 86.1 % of the surveyed farmers had the constraints of lacking capital, 75.8 % of them had the constraints of lacking seedlings, and 17.2 % are still lacking technical advice while 13.6 % presented the constraints of lacking labour or manpower. This is because some farmers were old and could not use larger portion of their land for agroforestry since it was labour-intensive. The statistical analysis of the results obtained on the relationship between farmers constraints and the adoptions of agroforestry in Jenda Sector are presented in table 7.

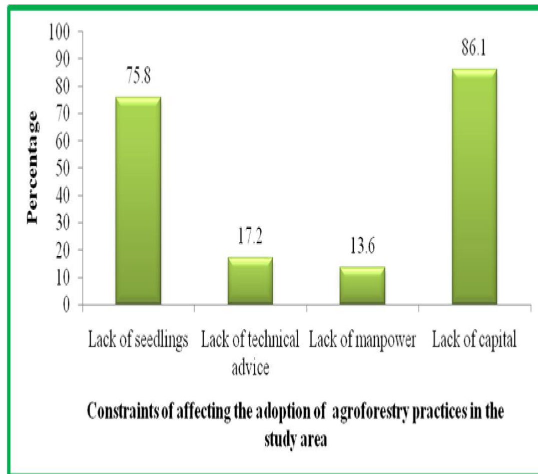

Figure 6: Constraints affecting adoption of agroforestry in the study area

Table 3: Statistical analysis on the farmers 'constraints in adoption of agroforestrypractices

Farmers 'constraints	Mean Rank
Lack of seedlings as a constraints for agroforestry practice	1.71
Lack of technical advice as a constraints for agroforestry practice	3.19
Lack of labor as a constraints for agroforestry practices	3.06
Lack of capital as a constraint for agroforestry practices	1.12

According to Friedman Test as indicated in Table 3, the lack of capital and seedlings had the lowest mean ranks, this allows us to affirm that lack of capital and seedlings are viewed as the most critical constraints affecting farmers adoption of agroforestry practices in Jenda Sector. Therefore the hypothesis is accepted. In general, the constraints have the different negative effect on adoption of agroforestry practices as shown by the analysis where p-value (0.000) < α (0.05).

3.5. Solutions suggested by farmers to alleviate the constraints

After identifying different constraints that affect the adoption of agroforestry practices it was very necessary to find out the possible suggestions in order to overcome those constraints. The results obtained on them are presented in Figure 7 below.

Referring to the results obtained on the suggestions of farmers in order to overcome the mentioned constraints, 91.8% suggest the availability of financial capital, either by loans, credit or subsidies for enhancing the agroforestry practices, (82.3 %) request the availability of different agroforestry species and75.9 % of the farmers prefer the availability sufficient nurseries establishment for getting planting materials.

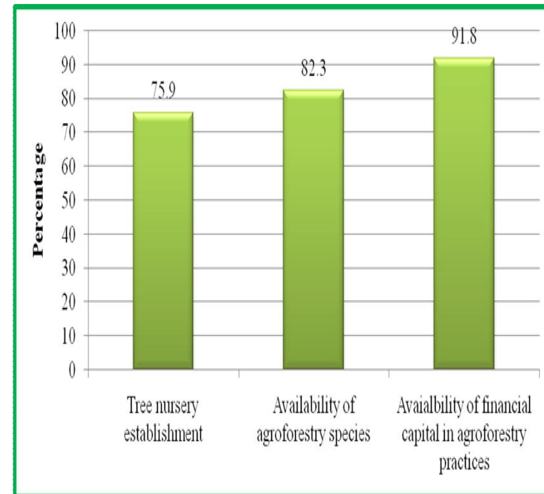

Figure 7: Suggested solutions to mentioned constraints

4. Discussions

The age range 40-59 years constitute the majority of respondents (62%), which shows that younger farmers are more likely to adopt a new technology because they had more schooling than the older generation and could reap the benefits of tree crops in their life time. According to ICRAF (2003), younger farmers are more likely to adopt a new technology, since they have had more schooling than the older generation or perhaps have been exposed to new ideas as migrant labors.

Many of the agroforestry practices like woodlot inter cropping, planting of windbreaks, as well as fruit trees on cropland in the study area involved strenuous activities, which are done manually. The fact that women constitute about one-quarter of agroforestry practitioners shows that women are also into agroforestry. However, it can be said that women are mostly interested in planting and cultivating food crops to meet household consumption needs rather than tree crops.

Eckman (1992) deduced from his studies that individuals within a household may have different rights depending on gender. He found that in some African Countries, for example, women plant and tend fire-wood or fruit trees but do not have right to harvest fruits or wood; these may be sold or appropriated by male members. Leach and Mearns (1988) have emphasized division between men and women in access to natural resources and their management and use as common in African land management system. They concluded that forestry

development initiatives must therefore not just "consider" women but aim at giving them equality with men in control over resources, in decision making over resource production and use, and empowerment to evolve self – directed problem solving strategies.

They all invariably point out that women are commonly collectors of water, fuel wood, foodstuffs and medicinal herbs and that they are directly affected by environmental degradation. In their decision making, women place higher value on taking care of the environment than the male family members do.

From the results in figure 5, it can be deduced that since the factors that affect the adoption of agroforestry practices may not be very different from those of general adoption of agricultural practices, the rich experience of farmers can be used to improve agroforestry using indigenous technical knowledge to bring about the desire results. According to ICRAF (2003), the very experienced farmers in farming are more likely to adopt a new technology since they are capable to understand the innovations through different comparisons.

The high level of literacy rate would result in increase of technical efficiency and decreased conservationism among farmers. This would also contribute to the acceptance of agroforestry innovations (Sarfo Mensah, 1994). According to Tripp (1993), education is an important socio-economic variable that may make a farmer more receptive to advice from an extension agency or more able to deal with technical recommendations that require a certain level of numeracy or literacy.

The reasonably high household sizes probably indicate that farmers were youthful and highly reproductive. The large family size of the bulk of farmers (63 %) could provide labour which is an incentive to agroforestry adoption. According to the National Institute of Statistics of Rwanda (2007-2008), the mean household size was 4.6 persons in general and 4.5 persons in rural areas and 4.8 persons in urban areas. However, the consequences of large family size are increased pressure on the ecosystem, land fragmentation and tree ownership problems.

Generally, the married people are sensitive to innovations since they have many opportunities to participate in training and formation about agroforestry. However, the widow, divorced and single farmers are still resistant to the new information dissemination as they are alone in households.

In Jenda Sector, as the highest number of the farmers is married, it is clear that the adoption of agroforestry practices will be done adequately and in rational manner; as well as they have been explained clearly by the agroforestry extensionists the importance and functions of agroforestry in improvement of their livelihoods. That has been confirmed by Sarfo-Mensah (1994) in his study carried out in Ghana. Also, he has concluded that the married farmers are able to adopt agroforestry technologies than other categories of farmers.

According to the National Institute of Statistics of Rwanda (2007-2008), the main sources of income of Rwanda are agriculture and allied activities.

The inherent land can be pledged for money or used as collateral in securing loans from financial institutions like banks. It is very easy to plant perennial crops (tree crops) on inherent land since the land belongs to you. Many also said the use of land for woodlot, intercropping and *Persea americana* intercrop which were all agroforestry practices would help them raise their income levels through the sale of tree products. This may encourage many from using their land for agroforestry purposes. This shows that even if the interviewed farmers obtained their land by inheritance, they use other methods (purchase and renting) in order to increase their farm land. According to the results of MINITERE (2007), the average landholdings in Rwanda are very small. This pushes some farmers to purchase and renting land for increasing the size of their land farm in order to diversify their activities included forest plantations.

The small farm sizes constitute an obstacle to farm improvement. According to the results of MINITERE (2007), the average landholdings in Rwanda are very small, less than 25 % cultivate the land of more than 0.5 ha, 50 % cultivate less than 0.5 ha, and more than 25 % cultivating less than 0.2 ha).

For example, it will be difficult for smallholder farmers to expand their farms. This finding supports Benneh (1976) who argued that miniature farm sizes and the manner, in which they are fragmented and scattered, constitute an obstacle to farm improvement since they do not enable farmers to take advantage of economies of scale of production.

In Jenda Sector, 100% of farmers use their lands for food production, 47.8% for tree production and only 4.5% for fodder production. This confirms that the majority of farmers in the study area are agriculturally dependent. Most farmers had the desire to grow food crops in order to provide food for household consumption. They also wanted to increase income by incorporating tree crops. Referring to MINAGRI (2006), the land use consolidation will help to implement the crop intensification program not only food crops, but also, cash and fodder crops.

As it is presented on figure 15, the rearing domestic animals in Jenda Sector contribute in increasing household income (97%), getting farm yard manure (94 %), getting money through purchase (91 %), improvement of social relationship with neighbours (74.6%), milk drinking (49.3%) and getting family consumption (13.4%). According to MINICOFIN (2006), apart from agriculture in generating income to the farmers, the animal rearing is also the considerable source of income in Rwanda, about 24 % of national income.

Agroforestry practices undertaken by farmers in the study area include Boundary planting (62.7%), woodlot (59.7%), life fencing (31.4%), home gardens (28.4%), taungya (4.5%), windbreaks (3%) and fruits trees in intercropping practice (3%). From that, it is clear that in Jenda Sector, the farmers do and adopt agroforestry practices in their farms with socio-economic and environmental considerations. This was supported by ICRAF (2003) while conducting its study on the socio-economic and environmental of tree farming in Kenya, where they have found that most farmers cultivated and grown trees for protecting their lands, income generation, fodder production and medicinal products issues.

Majority of the farmers (67.16 %) indicated that they have been supported by the NGOs as extension agents while 64.7 % were supported by Government institutions in order to promote agroforestry practices in their fields. The non-governmental organizations, which complemented the efforts of government extension services, were European Union and CARE.

As the Government institutions, there were MINAGRI, REMA, PAREF and NAFA. Farmers received extension education on improved cultural practices. This is an incentive to agroforestry adoption and its subsequent impact on the livelihood of farmers in the study area. This agrees with Adams (1982) who concluded that techniques or innovations normally provide the means of achieving sustained increases in farm productivity and income and that it is the extension workers job to encourage farmers to adopt innovations of proven value.

The results are in agreement with Wollenberg (1998), in many countries, a wide range of forest products is commonly used in traditional ceremonies, funeral, and inauguration ceremonies of the chiefs, initiations, birth celebrations and conflict resolution.

However, 6 % of the farmers had their source of finance through money lenders while 4.5 % of them had the source of financing farming activities by family members' support.

The size of the family, education and the land size are statistically significant at 5% level of significant because the p-value (0.005, 0.000, 0.000 respectively) are less than an α-value (significance level) of 0.05 while others factors are not statistically significant because the p-values (0.446, 0.350, 0.998, 0.800, 0.768 respectively) are greater than an α value of 0.05.

According to the results obtained on the figure 24, the 86.1 % of the surveyed farmers had the constraints of lacking capital, 75.8 % of them had the constraints of lacking seedlings, and 17.2 % are still lacking technical advice while 13.6 % were presented the constraints of lacking labour/manpower. This is because some farmers were old and could not use larger portion of their land for agroforestry since it was labour-intensive. According to Sarfo-Mensah (1994), lack of seedlings and capital were the main farmers'c onstraints to adopt agroforestry technologies than other constraints.

According to Friedman Test as indicated in the table 4, lack of capital and seedlings had the lowest mean rank, this allow us to conclude that lack of capital and seedlings are viewed as the most critical constraints affecting farmers in the adoption of agroforestry practices in Jenda Sector.

In general, the constraints have the different negative effect on adoption of agroforestry practices as shown by the analysis where p-value (0.000) < α (0.05). This has been confirmed by Sarfo-Mensah (1994) in his study in Ghana. It has concluded that lack of seedlings and capitals were the main farmers 'constraints to adopt agroforestry technologies than other constraints. According to the results of statistical analysis on the agriculture and formal/informal job are the sources which generate high income to the farmers. This is affirmed by the mean of income generated by different activities per year. The results of this study show that the agriculture occupies the first place with an average of 678909.09 Rwf per year followed by formal/informal job with a mean of 205283.13 Rwf per year but also the agroforestry practices contribute to the total household's income with an annual average of 43860.87Rwf.

From the Friedman Test depicted in Table 7, the lack of capital and seedlings had the lowest mean ranks, this allow us to conclude that the lack of capital and seedlings are viewed as the most critical constraints affecting farmers adoption of agroforestry practices in Jenda Sector. Therefore the hypothesis is accepted. In general, the constraints have the different negative effect on adoption of agroforestry practices as shown by the analysis where p-value (0.000) < α (0.05).

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Corresponding Author:
Isaac Emukule Ekise
Department of Forestry and Nature Conservation
Busogo Campus, Higher Institute of Agriculture & Animal Husbandry, P.O. Box 210, Musanze, Rwanda
E-mail: iekise@yahoo.com

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Responses of Wheat – Rice Cropping System to Cyanobacteria Inoculation and Different Soil Conditioners Sources under Saline Soil

Wafaa, M. T. Eletr , F. M. Ghazal, A. A. Mahmoud and Gehan, H. Yossef

Soils, Water and Environ. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt
Dr.wafaaeletr @yahoo.com - fekryghazal@ymail.com- ezgeales@hotmail.com

Abstract: A Field experiment was conducted in the clayey soil of the farm at Sahl El-Hossynia Agric. Res. Station in EL-Sharkia - Governorate, Egypt. The institute farm is located at 31° 8' 12.461" N latitude and 31° 52' 15.496" E longitude. wheat crop (*Triticum aestivum* L) was planted during winter season (2010-2011) and rice crop (*Oryzae sativa*) was planted during summer season (2011) to study the effectiveness of cyanobacteria inoculation combined with different sources of soil conditioners to improve soil chemical properties, soil biological activity and reflected to productivity of both wheat and rice crop system; total content of mineral nutrients of both tested plants were taken in consideration.

Results indicated that, in general, applying cyanobacteria inoculation in combined with some soil conditioners decreased slightly pH and EC values, while organic matter (OM) and Saturation Percent (SP) were increased as compared to control treatment. Also, applying cyanobacteria in combined with fulvic acid (FA) and /or humic substances (HS) significantly superior for decrease EC, SAR and ESP values in soil at both studied seasons. Moreover, cyanobacteria inoculation combined with compgypsum increases organic matter (OM) content in soil after two cultivated seasons.

In addition, positive significant responses existed for available N, P and K as well as soil biological activity (total count bacteria, CO_2 evolution, dehydrogenase activity and nitrogenase activity) in the studied soils under cultivation with both wheat and rice as a result of applied gypsum combination with cyanobacteria inoculation as compared to control treatment.

On the other hand, wheat and rice yields (straw and grain) along with total content of macronutrients (N, P and K) increased significantly in response to cyanobacteria inoculation in combination with gypsum as compared to other treatments and\or control treatment.

In conclusion, the application of cyanobacteria inoculation combined with humate organic acids helpful to improve the soil properties of saline soils. Also, the cyanobacteria inoculation combined with gypsum improved available and uptake macronutrients reflected that on the yield components.

[Wafaa, M. T. Eletr , F. M. Ghazal, A. A. Mahmoud and Gehan, H. Yossef. **Responses of Wheat – Rice Cropping System to Cyanobacteria Inoculation and Different Soil Conditioners Sources under Saline Soil.** *Nat Sci*2013;11(10):118-129]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 18

Key words: Cyanobacteria inoculation, soil conditioners, gypsum, compost, humic acids, fulvic acid, polyvinyl acetate, wheat (*Triticum aestivum* L.), rice (*Oryzae sativa*).

1. Introduction

Wheat and rice are important cereal crops in Egypt. They are also major cash crops for the farmers and handsome amount of foreign exchange is earned through export of rice. Thus, their role in strengthening the economy of the country may not be neglected.

The main problem at Sahl El-Hossynia soil is related to high salinity conditions. Soil degradation caused by salinizations and sodication were of universal concern. Saline (EC > 4 dSm^{-1}), or salt affected soil is a major environmental issue, as it limits plant growth and development, causing productivity losses **(Qadir et al., 2008).** Salt affected soils are characterized by excessively high levels of water- soluble salts, including sodium chloride (NaCl), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$), among others. In the salinity case, NaCl is a major salt contaminant in the soil. It has a small molecule size and when oxidized by water, producing sodium ions (Na^+) and chloride ions (Cl^-), which are easily absorbed by the root cells of higher plants and transferred to the whole plant using xylem uploading channels, also cause ionic and osmotic stresses at the cellular level of higher plants, especially in susceptible species **(Rodriguez-Navarro and Rubio, 2006).** There are many procedures that can be used to improve salt affected soils, such as, water leaching, chemical remediation including gypsum ($CaSO_4.H_2O$), calcite ($CaCO_3$), calcium chloride ($CaCl_2$) and phytoremediation including organic matter such as farmyard manure, green manure, organic amendment, compost and their components, **(Feizi et al., 2010).**

In Egypt, improving salt affected soils is considered as an important part in the agricultural security program. Management of the salt affected soils requires a combination of agronomic practices depending on chemical amendments, water quality and local conditions including climate as well as crop economic policy.

Gypsum is commonly used as amendment for the reclamation of saline – sodic and sodic soils and reducing the harmful effects of high sodium irrigation water because of its solubility, low – cost, availability and ease of handling **(Abdel-Fattah, 2012).** The relative effectiveness of gypsum and sulfuric acid has received the most attention because they are widely used as reclamation amendments. In addition, **Khan et al. (2010)** found a positive significant improvement in saline-sodic soil properties, i.e., EC, SAR and pH in response to gypsum applied in ridges, farmyard manure and agricultural practices that resulted in an increase in wheat grain yield by 42 % over control. Besides,**Cha-um et al. (2011)** evaluated the efficiency of the same treatment on remediation of saline soil and found that rice recorded of 79.6 % spikelet fertility in response to gypsum and FYM against 46.4 % for the same soil without the use of gypsum and FYM. Also, **Abdel-Fattah (2012)** revealed pronounced decreases in EC, pH, SAR and ESP in a saline-sodic soil due to the application of gypsum and two types of compost either they applied solely or in combination, compared with control. They added that combined treatments were more efficient.

The significance of organic matter has been proved through its effect on improving the physical conditions of soils for crop growth besides its role as fertilizers. Compost is one from of organic matter producer, which can be used to improve the soil physical, chemical and biological properties of salt affected soils and it can be converted as ideal manure with high contents of macro and micronutrients. The application of organic manures, as compost or humic substances, increased the available N, P & K and organic carbon content in the soil and moreover, the reduction of soil bulk density and pH **(Dhanushkodi and Subrahmaniyan, 2012).**

Biofertilizers are non-bulky, less expensive, ecofriendly agricultural in puts, which could play a significant role in improving plant nutrients supplies as complementary and supplementary factors. Cyanobacteria play an important role in maintenance and building up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer **(Song et al., 2005).** The acts of cyanobacteria include: (1) Increase in soil pores and production of adhesive substances. (2) Excretion of growth – promoting substances such as hormones (auxin, gibberellins), vitamins and amino acids **(Rodriguez et al., 2006).** (3) Increase in water holding capacity through their jelly structure. (4) Increase in soil biomass after their death and decomposition. (5) Decrease in soil salinity and preventing weeds growth **(Saadatnia and Riahi, 2009).** (6) Increase in soil phosphate by excretion of organic acids. Furthermore, **Palaniappan et al. (2010)** pointed out that the cyanobacteria are being used as biofertilizer for plants, as food for human consumption and for the extraction of various products such as vitamins and drug compounds.

The present study aims to evaluate the efficiency of different soil conditioners sources in combination with cyanobacteria inoculation on wheat - rice yields grown in saline soil in a wheat-rice cropping system.

2. Materials and Methods

Field experiments were conducted in clay soil at Sahl El-Hossynia Agric. Res. Station Farm in EL-Sharkia Governorate; Egypt to study the effect of cyanobacterial inoculation in combination with different soil amendments on saline soil. The farm is located at 31° 8' 12.461" N latitude and 31° 52' 15.496" E longitude. Some physical and chemical characteristics of the studied soil are presented in Table (1).

The experiments were carried out during two successive seasons; on both wheat (SaKha, 93) in winter season (2010 - 2011) and rice (SaKha, 104) in summer season (2011). The experimental design was a randomized complete block design with three replications.

The experiment included nine treatments as follows:

1. (T1) 100 % mineral fertilizer (N, P &K) (recommended doses).
2. (T2) Cyanobacteria (SBCI) only.
3. (T3) SBCI + Humic acids 3% (v/v).
4. (T4) SBCI + Fulvic acid 3% (v/v).
5. (T5) SBCI + Humic substances 3%(v/v).
6. (T5). SBCI + sulphur (16 Kg fed^{-1}.)
7. (T6) SBCI + gypsum ((4 ton fed^{-1}).
8. (T8) SBCI + compgypsum (2 :1) at rate of 6 ton fed^{-1}
9. (T9) SBCI + polyvinyl actete (0.20% w/v).

Some properties of humic acids, fulvic acid and compost are presented in Tables (2 a) and (2 b).

Dried flakes from the soil based cyanobacteria inoculum (SBCI) were inoculated to wheat plants 10 days after sowing at the rate of 6 kg fed^{-1}, while rice received SBCI inoculum 30 days after sowing at the rate of 3 kg SBCI fed^{-1}. Cyanobacteria inoculum (SBCI) is composed a mixture of *Anabaena*

fertilissima, Nostoc linckia, Nostoc commune and *Nostoc muscorum.* The cyanobacterial inoculum was prepared as described by **Vennkataraman (1972).**

Table (1): Some characteristics of the experimental soil

Soil characteristics	Values
Particle size distribution %	
Coarse Sand	5.40
Fine Sand	4.20
Silt	40.40
Clay	50.00
Texture class	Clay
Chemical properties	
pH (suspension 1:2.5)	8.09
EC dS m^{-1} (saturated paste extract)	10.90
Organic matter %	0.62
Soluble cations and anions (meq L^{-1})	
Ca^{++}	43.30
Mg^{++}	39.70
Na^{+}	79.80
K^{+}	1.70
CO_3^{--}	- - -
HCO_3^{-}	5.78
CL^{-}	87.80
SO_4^{--}	70.92
SAR	12.40
ESP	14.60
Available macro& micronutrients (mg kg^{-1})	
N	196.00
P	7.00
K	133.00
Fe	9.00
Mn	1.88
Zn	1.82
Cu	5.40

Table (2 a): Some characteristics of humic and fulvic acids

Determination	Humic acids	Fulvic acid
EC dS m^{-1}	61.0	59.00
pH	5.00	2.00
Available nutrients (mg L^{-1})		
Fe	0.44	0.33
Mn	0.058	0.048
Zn	0.94	0.64
Cu	0.03	0.09

Table (2 b): Some characteristics of compost applied in the Experiment

Analysis	Values
Moisture %	**12.00**
pH (1 :10)	**8.02**
EC dS m^{-1}	**3.14**
OM %	**24.50**
C :N	**29.6 :1**
Total N %	**0.48**
NH_4 – N mg Kg^{-1}	**55**
NO_3 – N mg Kg^{-1}	**155**
Total P %	**0.38**
Total K %	**0.60**

All treatments applied before cultivation except for cyanobacterial inoculation and received mineral fertilizers at the recommended doses for both wheat and rice crops. Superphosphate (15 % P_2O_5) at a rate of 200 Kg fed^{-1} added basically before sowing during soil preparation. Nitrogen added at rates of 340 Kg fed^{-1} and100 Kg fed^{-1} for both wheat and rice, respectively, in three split equal doses after 15, 30 and 60 days from sowing in the form of ammonium sulfate (20 % N). While, potassium added at the form potassium sulfate (48 % K_2O) at a rate of 50 Kg fed^{-1} in two equal doses at sowing and 30 days from sowing for both wheat and rice.

At harvest, surface soil samples collected and subjected to the analysis of some soil chemical properties as described by **Cottenie et al. (1982)**.

Straw and grains of both wheat and rice crops collected from each plot, oven dried at 70°C for 48 h, and the weighed up to a constant dry weight, ground and prepared for digestion according to **Page et al. (1982)**. The digests were then exposed to the estimation of N, P, K and Na (**Cottenie et al., 1982).**

Sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) carried out according to **Abdel – Fattah (2012).** Sodium adsorption ratio (SAR) estimated by using the following equation, where ionic concentration of the saturation extracts is expressed in meq L^{-1}.

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

Exchangeable sodium percentage (ESP) was estimated by using the following equation

$$ESP = \frac{100\ (-0.0126 + 0.01475\ SAR)}{1 + (-0.0126 + 0.01475\ SAR)}$$

Obtained results were subjected to statistical analysis according to **Snedecor and Cochran (1980)** and the treatments were compared by using the least significant difference (L.S.D. at 0.05 level of probability).

As well as, at harvest, a part of the collected soil samples was devoted for the determination of the soil biological activity in terms of nitrogenase activity (N_2-ase) **(Hardy et al., 1973)**, dehydrogenase activity (DHA) **(Casida et al., 1964)**, carbon dioxide evolution **(Pramer and Schmidt, 1964)** and total count bacteria **(Allen, 1959).**

3. Results and Discussion

Soil chemical properties

Data in Table (3) show the changes of some soil chemical properties in response to the application of different soil conditioner materials along with the inoculation with cyanobacteria.

Saturation percent (SP) and organic matter (OM):

Results revealed that the saturation percent (SP) has slightly insignificant increase due to the applied treatments compared to control. However the exception was for the treatments of comp-gypsum and PVA in combination with cyanobacteria inoculation at winter and summer seasons (wheat and rice crops), which recorded insignificant decreases for the saturation percent.

With regard to organic matter (OM), results showed that all applied treatments increased significantly the OM compared to control treatment. This trend was true for both seasons. Application of comp-gypsum in combination with cyanobacteria (T8) recorded high significant increases in OM content of soil being 0.48% and 0.80% in winter (wheat) and summer (rice) seasons against 0.26% and 0.65% for control treatment, respectively. Cyanobacteria play an important role in maintenance and building up the soil fertility. The acts of these cyanobacteria include: (1) Excretion of growth – promoting substances such as hormones, vitamins, amino acids as organic matter **(Rodriguez et al., 2006),** (2) increase in soil biomass after their death and decomposition **(Saadatnia and Riahi, 2009)**. Also, under salt stress condition, application of cyanobacteria to the soil lead to increase the soil organic matter, which is consequently, increased the soil biological activity by increasing the soil CO_2 evolution leading to increase the soil fertility **(Singh et al., 2008).**

Table (3): Effect of cyanobacteria inoculation in combination with some soil conditioners on some chemical properties in soil under saline condition

A:- Wheat

Treatments		pH	O.M (%)	EC (dSm^{-1})	SP	Anions (meq L^{-1})				Cations (meq L^{-1})			
						CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
NPK (Control)		7.61	0.26	9.63	79.7	-	13.3	47.3	38.2	18.8	24.6	55.0	0.42
Cynobacteria only		7.57	0.30	7.23	80.0	-	9.58	30.7	45.0	16.6	22.8	44.4	0.45
Cynobacteria	Humic acids(HA)	7.61	0.47	6.47	80.0	-	10.8	28.0	38.1	19.2	22.4	34.1	1.22
	Fulvic acid(FA)	7.54	0.42	5.38	81.2	-	9.17	11.7	33.3	15.0	11.5	26.3	1.29
	Humic substances (HS)	7.52	0.47	5.37	84.7	-	12.5	27.0	14.2	18.3	10.9	23.3	1.18
	Sulphur (S)	7.55	0.47	6.32	82.2	-	12.5	22.7	39.2	23.3	17.6	32.3	1.13
	Gypsum	7.60	0.32	8.30	80.5	-	10.0	30.7	58.9	28.2	21.6	49.4	0.39
	Compgypsum	7.57	0.48	7.80	79.8	-	10.8	13.3	40.4	25.8	32.3	33.9	0.53
	PVA	7.55	0.46	7.33	78.7	-	10.0	16.0	48.6	20.2	19.2	34.8	0.42
LSD. 5 %		NS	0.11	4.03	NS	-	2.19	11.5	27.0	9.99	13.4	16.9	0.44

B:- Rice

Treatments		pH	OM %	EC (dSm^{-1})	SP	Anions (meq L^{-1})				Cations (meq L^{-1})			
						CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
NPK (Control)		7.73	0.65	8.57	80.0	-	8.00	38.9	42.2	24.4	27.7	41.1	1.20
Cynobacteria only		7.72	0.69	6.66	85.5	-	8.66	29.7	29.0	17.8	21.7	26.8	1.05
Cynobacteria	Humic acids(HA)	7.80	0.71	7.35	82.0	-	8.67	33.8	39.8	22.2	25.2	33.8	1.00
	Fulvic acid(FA)	7.76	0.78	6.37	83.0	-	8.66	25.6	32.2	23.3	15.4	26.8	1.05
	Humic substances (HS)	7.74	0.67	6.21	81.0	-	8.69	26.9	35.8	23.3	19.4	27.8	1.00
	Sulphur (S)	7.77	0.68	7.51	84.0	-	5.78	32.4	45.4	28.9	18.5	35.0	1.16
	Gypsum	7.73	0.69	8.32	82.0	-	8.67	40.5	41.1	28.9	23.3	37.2	1.11
	Compgypsum	7.77	0.80	6.93	78.5	-	8.67	31.1	38.8	24.4	20.6	32.5	1.03
	PVA	7.79	0.66	6.68	75.0		8.68	28.4	38.3	22.2	22.0	30.0	1.10
LSD. 5 %		NS	NS	0.86	NS	-	1.82	3.99	3.33	0.02	2.89	4.31	0.02

Soil electric conductivity (EC) and soil reaction (pH).

Results revealed that the values of pH and EC in soil have insignificant and significant decreases, respectively, as affected by the studied treatments compared to control. Application of fulvic acid (FA) and humic substances (HS) in combination with cyanobacteria led to significant superior decreases of pH and EC values in winter and summer seasons. However, an exception being obtained for pH at summer season. On the other hand, the application of gypsum in combined with cyanobacteria (T6) was the least affected treatment than other treatments. In this concern, the applied cyanobacteria treatments

reduced EC, according to **Molnar and Ordog (2005)** who noted that some plant growth promoting regulators (PGPRs) are found to be released by cyanobacteria; these PGPRs represent the defense systems that encounter the salt stress leading to decrease the soil EC degree. Also, the cyanobacteria have the ability to excrete extracellular a number of compounds, like polysaccharides, peptides, lipids, organic acids leading to decrease the soil pH **(El-Ayouty et al., 2004).** Organic matter, including FA, FYM and HS as well as gypsum may function as salt-ion chelating agents, which detoxify the toxic ions, especially Na^+ and Cl^-, as indicated by low EC in soil treated with both organic matter and gypsum **(Zahid and Niazi, 2006).** Recently, **Khan et al. (2010)** found that the application of gypsum improved the soil chemical properties by reducing the EC and pH parameters that might be due to substitution of exchangeable Na by Ca that produced more soluble salts (NaCl or Na_2SO_4) and was leached by the irrigation water **(Lebron et al., 1994).** Concerning the effect of organic matter as compost, FA, HA and HS on the decreasing the soil pH, their effect illustrated by the indirect effect in decreasing sodium and the direct effect of organic acids, which formed either during decomposition of compost or by the application of HA and FA **(Abdel – Fattah, 2012).**

Soluble cations and anions.

Regarding the effect of different sources of soil conditioners in combination with cyanobacteria on soluble cations and anions contents in soil at both tested seasons, results indicate that the same trend observed in pH and EC was true for both soil cations and anions in response to the applied treatments.

Soil sodicity.

To complete the picture, the calculation of the sodium absorption ratio (SAR) and exchangeable sodium percentage (ESP) are as expressive of the salinity. Soil sodicity in terms of SAR of the soil paste extract and ESP of the soil are presented in Fig. (1), both SAR and ESP decreased considerably by the application of all treatments compared to control. The SAR at applied of different sources of soil conditioners ranged from 11.8 (100% NPK) to 6.09 (cyanobacteria + HA) for the first season (wheat crop) and ranged from 8.06 (100% NPK) to 6.02 (cyanobacteria + HA) for the second season (rice crop) compared with the initial values of 12.4, thus exhibiting a decrease of between 4.84 to 50.9 % and 34.7 to 51.5 % for the first and second seasons, respectively. The ESP gave the same trend as that of the SAR. The ESP values showed decreases ranged between 4.79 to 51.6 % for the first season and 34.4 to 51.4 % for the second season. The application of humic substances (HS) in combination with cyanobacteria inoculation showed greater decrease in SAR and ESP than other treatments. The data agree with results reported by **Khan et al. (2010)** and **Abdel - Fattah (2012)** who showed that the application of organic amendments either singly or in combination decreased in SAR and ESP compared to control, This behavior can be attributed to the decrease in soil salinity resulted from the organic amendments that may function as salt – ion chelating agents, which detoxify the toxic ions, especially Na^+ and Cl^-, as indicated by low EC in soil treated with amendments.

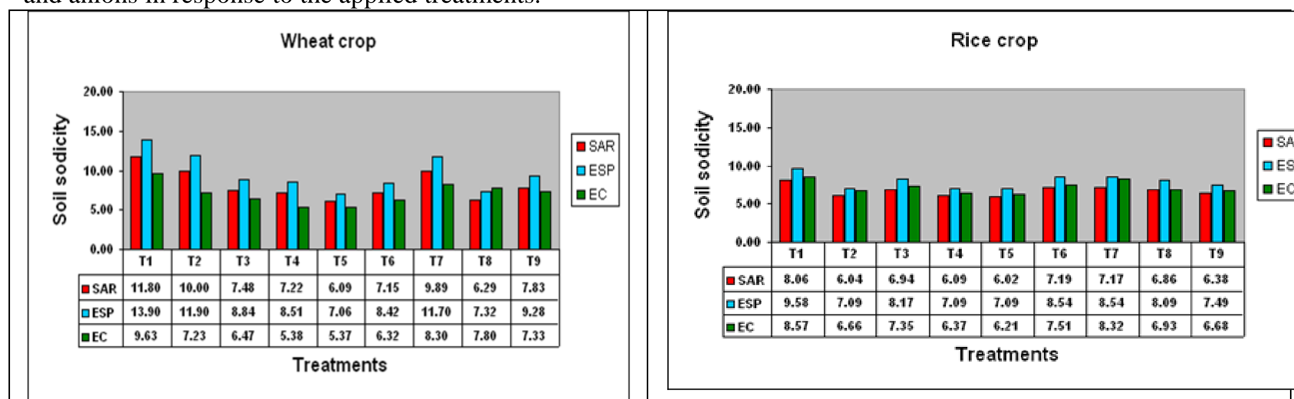

Fig. (1): Effect of inoculation with cyanobacteria and different soil conditioners sources on SAR and ESP in soil under saline condition after wheat- rice harvested.

Finally, the EC values was plotted against ESP and SAR as well as plotted between for the last at two seasons, these parameters are showed in Fig (2). ESP (r^2 =0.587) and SAR (r^2 = 0.598) in the soil cultivated with wheat were positively related to the increase EC values.

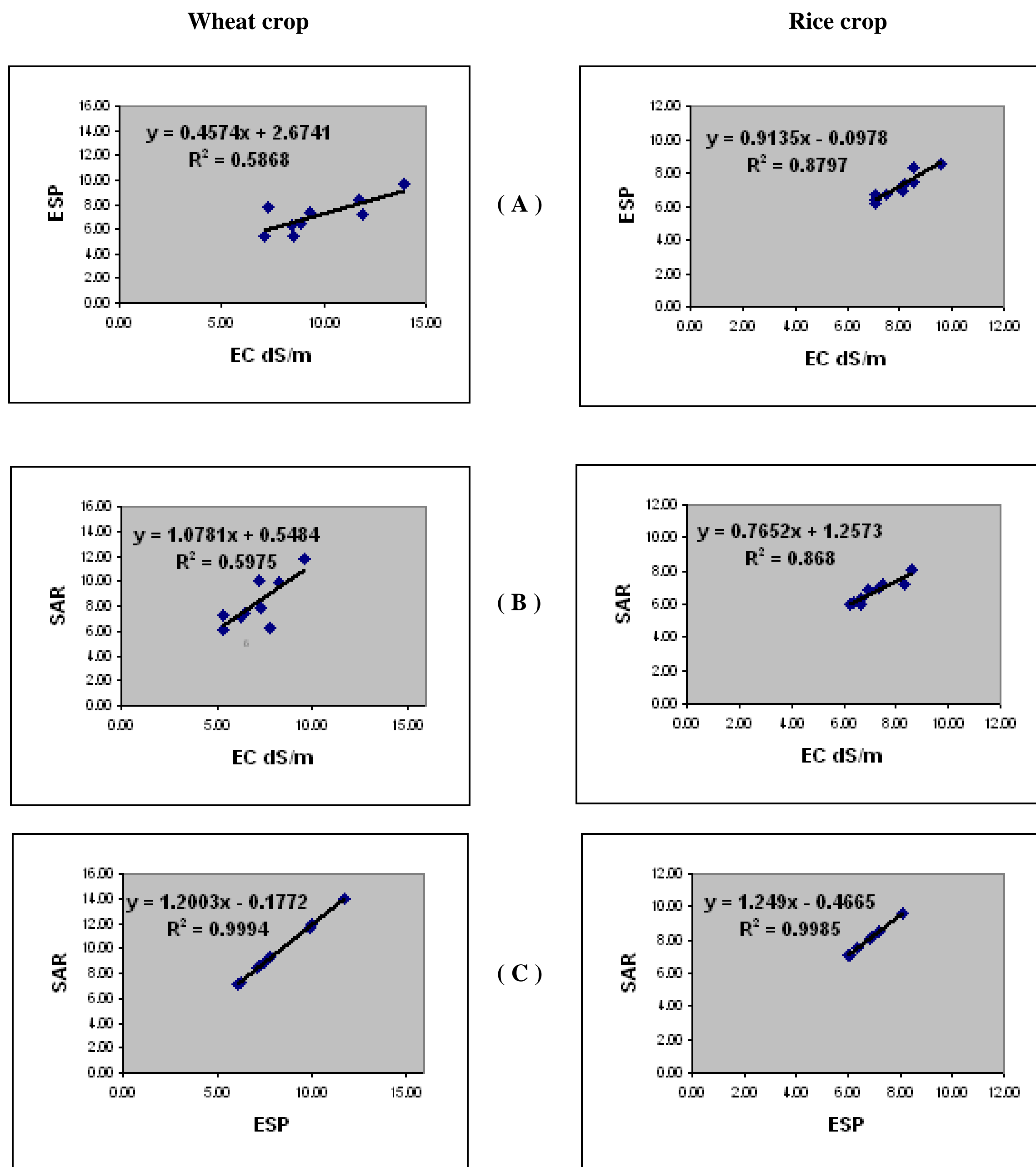

Fig. (2): Relationship between ESP and EC (A), SAR and EC (B) and SAR and ESP (C) in soil under saline condition after wheat- rice harvested.

The same trend was observed for ESP (r^2 = 0.879) and SAR (r^2 = 0.868) in the soil of rice. Also, the relationship between SAR and ESP was positively (r^2 = 0.99). This indicated the positive effect among the all studied parameters (EC, SAR and ESP). As illustrated above, the relation between EC, ESP and ASR is more obvious in the case of rice than in wheat. These may be due to the leaching effect.

Nutrients availability in soil after harvesting of both wheat and rice cropping system.

The data representing availability of soil macronutrients (N, P and K) after wheat and rice harvesting are shown in Table (4). Statistical analysis showed that all applied treatments increased significantly the soil macronutrients availability (N and K) compared to the control treatment. This trend was true for both crops. On the other hand, the applied treatments had not significantly increased the

soil P availability. However, the use of gypsum in combination with cyanobacteria inoculation gave the highest soil available N, P & K values after harvesting of both wheat and rice.

Table (4): Effect of cyanobacteria inoculation in combination with some soil conditioners on available macronutrients in soil under saline condition after wheat and rice harvested

Treatments		Available of macronutrients mg kg^{-1} soil					
		N	P	K	N	P	K
		A:- Wheat			B:- Rice		
NPK (Control)		238	12.6	245	137	5.39	242
Cynobacteria only		334	22.2	315	201	9.43	313
Cynobacteria	Humic acids(HA)	299	19.2	281	191	7.70	329
	Fulvic acid(FA)	288	17.8	279	173	6.72	329
	Humic substances(HS)	325	18.5	319	201	8.02	320
	Sulphur (S)	345	22.1	337	205	9.97	335
	Gypsum	364	28.5	345	210	10.7	342
	Compgypsum	327	17.2	332	196	6.97	332
	PVA	266	16.8	262	173	5.87	305
LSD. 5 %		48.6	8.92	43.3	33.1	3.97	36.5

In this respect, **Dhanushkodi and Subrahmaniyan (2012)** pointed out that the application of compost as soil conditioners increased available N in soil compared to control and reported that available N increased due to mineralization of native N by soil organisms. The addition of organic soil conditioners well decomposed and humified organic matter adds mineralized nitrogen and humic substances, this reduces the loss of N from leaching and volatilization. Also organic acids produced during decomposition of organic matter reduced the activity of polyvalent cations through chelation and reduced the P fixation and increase the availability of P. Furthermore, soil conditioners induced the availability of K because soil conditioners itself adds an appreciable quantity of K to the soil and also due to rapid decomposition and mineralization which release higher amount of NH_4^+ ion leading to increase the availability of K in soil.

Obtained results also showed that the highest significant values of soil available N, P and K were due to for the application of gypsum combined with cyanobacteria as compared to other tested treatments. Increases in the values of soil available macronutrients at winter season (wheat), as compared to control, recorded 52.9%, 126% and 40.8% for N, P and K, respectively. The corresponding increases in N, P and K at summer season (rice) recorded 53.3%, 98.5% and 41.3%, respectively. In spite of that, application of polyvinyl acetate (PVA) combined with cyanobacteria was generally non-significantly inferior. These results agree with those reported by **Dhanuskkodi and subrahmaniyan (2012)** who mentioned that the gypsum is the most economical amendment to reclaim the sodic soils. This due to that, the gypsum application sustained the available nutrients status of soil that perhaps due to the reclamation effect on soil. However, lowering pH and EC values of the soil by the use of gypsum is due to downward movement of Na owing to its replenishment by calcium as a result of solubilization of gypsum. Also sodium can be leached from the soil as Na_2SO_4. The availability of all nutrients in soil remarkably improved by the application of gypsum, which creates more favorable environment in soil and maintain elements in a more available form due to reclamation effect. On the other hand, **Singh et al. (2008)** reported that the cyanobacteria added to the soil, under salt stress condition, led to increase the soil biological activity, which is consequently increased the soil fertility that in turn is reflected positively on the availability the macro and micro-nutrients in soil. Recently, **Sahu et al. (2012)** reported that cyanobacteria play an important role to build-up soil fertility that consequently increases the yield. Biofertilizer are essential components of organic farming and play vital role in maintaining long term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micro nutrients, convert insoluble phosphorus in the soil into forms available to plants, thereby increases their efficiency and availability. The blue-green algae (cyanobacteria) are capable of fixing the atmospheric nitrogen and convert it into an available form of ammonium required for plant growth.

Soil biological activity.

Data in Table (5) indicate the effect of inoculation with cyanobacteria combined with different soil conditioners on the soil biological activity in terms of total count bacteria, CO_2 evolution, dehydrogenase activity and nitrogenase

activity after wheat and rice harvesting under wheat – rice cropping system. Results revealed that all the treatments that received cyanobacteria inoculation increased the soil biological activity compared to the control treatment (NPK only). However, the use of gypsum along with cyanobacteria gave the highest values of total count bacteria, CO_2 evolution, dehydrogenase activity and nitrogenase activity in both wheat and rice soils. The corresponding values were 18 x 105 cfu g dry soil^{-1}, 141.46 mg CO_2 100 g dry soil^{-1} day^{-1}, 295.11 mg TPF g dry soil^{-1} day^{-1}and 1936.79 mmole C_2 H_2 g dry soil^{-1} day^{-1} (wheat soil) and 21 x 105 cfu g dry soil^{-1}, 171.56 mg CO_2 100 g dry soil^{-1} day^{-1}, 415.65 mg TPF g dry soil^{-1} day^{-1} and 2250.12 mmole C_2 H_2 g dry soil^{-1} day^{-1} (rice soil) for count bacteria, CO_2 evolution, dehydrogenase activity and nitrogenase activity, respectively. Also, it was noticed that the values of the soil biological activity terms in rice soil were higher than those recorded in wheat soil. Generally, the combination of the humic substances and/or gypsum with cyanobacteria enhanced relatively the soil biological activity in both wheat and rice soils after harvesting under wheat-rice cropping system. In this concern, **Zulpa et al. (2008)** studied the effect of cyanobacteria products of *Tolypothrix tenuis* and *Nostoc muscorum* on the microbiological activity and the nutrient content of the soil. The biomass and extracellular products of both strains increased the soil microbial activity. *N. muscorum* and *T. tenuis* biomasses increased the soil oxidizable C (15 & 14%), total N (10 & 12%) and available P (22 & 32%), respectively. *T. tenuis* extracellular products increased by 28% oxidizable carbon and N. muscorum extracellular products increased by 15% the available phosphorus. These are caused the soil biological activity to be increased also because they are a continuously renewable carbon source. **Caire et al. (2000)** established that cyanobacteria can increase the soil enzymatic activity. **Aref and EL- Kassas (2006)** found that cyanobacteria inoculation to maize field enhanced significantly any of total count bacteria, cyanobacteria count, CO_2 evolution, dehydrogenase and nitrogenase activities compared to the control treatment received no inoculation. They explained that bio-fertilization with cyanobacteria led to increase microorganisms' community and in turn soil biological activity in soil through increasing the organic matter and microbial activity. **Saruhan et al. (2011)** revealed that humic compounds added to soil increased the soil fertility through increasing the soil microbial population including beneficial microorganisms. They explained that humic substances are major components of organic matter, often constituting 60 to 70% of the total organic matter, thus they may enhance the plant nutrients uptake through stimulation of microbiological activity. **Ulkan (2008)** postulated that addition of humic acids to soil in wheat cultivation stimulated the soil microbiological activity that led to increase the soil fertility.

Table (5): Effect of cyanobacteria inoculation in combination with some soil conditioners on soil biological activity after wheat and rice harvesting under saline condition

Treatments		Total count bacteria x 10^{5} *cfu g dry soil^{-1}		CO_2 Evolution (mg CO_2 100 g dry soil^{-1} day^{-1})		**DHA activity mg TPF g dry soil^{-1} day^{-1}		***N-ase activity (mmole C_2H_2 g dry soil^{-1} day^{-1})	
		Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice
NPK (Control)		11	14	112.15	131.16	220.12	315.12	950.12	1215.12
Cynobacteria only		13	16	125.12	145.48	256.23	335.85	1200.13	1624.65
Cynobacteria	Humic acids(HA)	16	19	137.13	161.75	284.25	390.13	1239.25	1834.85
	Fulvic acid(FA)	15	17	131.14	156.18	270.18	370.23	1226.45	1756.23
	Humic Substances. (HS)	14	16	128.12	152.13	262.25	358.78	1218.95	1678.94
	Sulphur (S)	13	15	124.96	142.13	251.76	328.58	1189.14	1465.84
	Gypsum	18	21	141.46	171.56	295.11	415.65	1936.79	2250.12
	Compgypsum	17	19	138.65	160.98	280.65	395.15	1715.43	2178.95
	PVA	12	12	121.50	136.18	230.12	320.14	985.68	1153.64

**cfu = Colony formed uint^{-1}. ** DHA = Dehydrogenase activity. ***N-ase = Nitrogenase activity.*

Yield and its components at harvest stage.

Statistical analyses of data in Table (6) show that the straw, grains and 1000-grain weight of both wheat and rice yields had significantly influenced by different applied treatments, compared to the control treatment.

The highest values of yield components were recorded by the treatment received gypsum in the presence of cyanobacteria. On the other hand, the

lowest values for both yield components were recorded by the treatments received industrial conditioner (PVA) combined with cyanobacteria inoculation. Relative percentage in yield components of wheat plants, as compared to control, recorded 38.8%, 134% and 15.6% for straw, grains and weight of 1000-grain, respectively; the corresponding increases in components of rice plants recorded 23.8%, 52.6% and 50.0%, respectively. These results agree with those reported by **Khan et al. (2010)** who showed that the increase in wheat yield on the ridges supplemented with gypsum may be due to ameliorative effect of gypsum that lowers the SAR and EC for soils. Also, **Dhanushkodi and Subrahmaniyan (2012)** pointed out that the application of gypsum improve soil physico-chemical environment in the root zone and lowering the pH and ESP leading to increase the rice yield. The supply of nutrients through gypsum provides conductive physical environments leading to better aeration, root activity and nutrient absorption and the consequent complementary effect that resulted in higher grain yield. With respect to effect of cyanobacteria, results confirmed by the findings of **Youssef et al. (2011)** who found that the application of humic acids enriched with cyanobacteria led to increase significantly yield components of barley and faba bean crops under salt stress condition. Also, **Paudel et al. (2012)** found that the blue - green algae (cyanobacteria) inoculum applied with low doses of NPK, gave significant increase in all parameters of rice yield over control.

Furthermore, cyanobacteria is characterized by their cytokinins, gibberellins and auxins content that enhance the plant growth and moreover, these materials is proved to overcome the adverse effect of salinity in saline soil **(Strik and Staden, 2003).**

Table (6): Effect of cyanobacteria inoculation in combination with some soil conditioners on the yields of wheat and rice under saline condition

A: - Wheat

Treatments		Weight of straw Kg fed^{-1}.	Relative percentage	Weight of grains kg fed^{-1}.	Relative percentage	Weight of 1000 - grain (g)	Relative percentage
NPK (Control)		896	-	1996	-	57.6	-
Cynobacteria only		1069	19.3	3363	68.5	63.5	10.2
Cynobacteria	Humic acids (HA)	1184	32.1	4083	104	63.6	10.4
	Fulvic acid (FA)	1148	28.1	3147	57.7	61.2	6.25
	Humic substances (HS)	1118	24.8	2980	49.3	63.6	10.4
	Sulphur (S)	1090	21.7	3308	65.7	65.6	13.9
	Gypsum	1244	38.8	4673	134	66.6	15.6
	Compgypsum	1016	13.4	2661	33.3	65.8	14.2
	PVA	921	2.79	2428	21.6	60.4	4.86
LSD. 5 %		161	-	1215	-	5.43	-

B:- Rice

Treatments		Weight of straw Kg fed^{-1}.	Relative percentage	Weight of grains Kg fed^{-1}.	Relative percentage	Weight of 1000 grain (g)	Relative percentage
NPK (Control)		564	-	487	-	10.0	-
Cynobacteria only		636	12.8	575	18.1	10.6	6.00
Cynobacteria	Humic acids (HA)	675	19.7	646	32.6	10.6	6.00
	Fulvic acid (FA)	639	13.3	703	44.4	15.0	50.0
	Humic substances(HS)	664	17.7	651	33.7	13.3	33.0
	Sulphur (S)	671	18.9	587	20.5	15.0	50.0
	Gypsum	698	23.8	743	52.6	15.0	50.0
	Compgypsum	653	15.7	632	29.8	13.3	33.0
	PVA	567	18.3	560	14.9	10.9	9.00
LSD. 5 %		116	-	123	-	2.62	-

Total contents of macronutrients in plants.

With respect to total content of macronutrients for wheat and rice plants, generally, results revealed that the application of different treatments gave significant positive influences on total content of macronutrients in straw and grains for wheat and rice plants as compared to control (Table 7). Application of gypsum a combined with cyanobacteria inoculation increased significantly the total content of macronutrients for both straw and grains of wheat

and rice crops. Also, the behavior of the total macronutrients content followed the same trend of those recorded by yield components. In spite of that application of PVA combined with cyanobacteria decreased this parameter.

Table (7): Effect of cyanobacteria inoculation in combination with some soil conditioners on nutrients total content in both wheat and rice under saline condition

A: - Wheat

Treatments		**N Kg fed^{-1}.**		**P Kg fed^{-1}.**		**K Kg fed^{-1}.**		**Na Kg fed^{-1}.**	
		Straw	Grains	Straw	Grains	Straw	Grains	Straw	Grains
NPK (Control)		13.6	12.4	4.10	9.28	7.74	4.40	1.94	1.23
Cynobacteria only		21.5	17.8	7.10	16.5	22.1	6.03	4.22	3.60
Cynobacteria	Humic acids (HA)	28.6	19.8	8.26	15.0	18.9	7.38	6.91	5.64
	Fulvic acid (FA)	22.0	19.1	6.43	20.2	20.9	5.68	3.63	2.74
	Humic substances(HS)	19.1	18.9	7.52	17.1	17.4	6.10	5.21	4.15
	Sulphur (S)	23.2	18.6	7.43	17.5	15.1	6.97	6.91	4.98
	Gypsum	31.5	21.2	12.1	27.1	25.7	8.56	7.53	6.59
	Compgypsum	17.3	16.6	5.32	16.5	17.9	5.65	3.14	3.07
	PVA	14.5	14.1	5.25	10.0	12.2	4.70	2.29	1.95
LSD. 5 %		8.57	3.52	3.12	11.4	17.1	1.85	2.51	2.16

B:- Rice

Treatments		**N Kg fed^{-1}.**		**P Kg fed^{-1}.**		**K Kg fed^{-1}.**		**Na Kg fed^{-1}.**	
		Straw	Grains	**Straw**	Grains	**Straw**	**Grains**	**Straw**	**Grains**
NPK (Control)		8.72	12.3	0.96	0.74	6.07	1.13	1.45	0.32
Cynobacteria only		10.3	14.0	0.68	1.29	7.08	1.59	1.92	0.67
Cynobacteria	Humic acids (HA)	10.3	14.8	0.90	1.24	6.38	1.61	1.68	0.73
	Fulvic acid (FA)	10.6	16.1	0.63	1.13	7.13	1.94	1.67	0.69
	Humic substances(HS)	10.2	15.1	1.12	1.25	7.64	1.67	1.92	0.63
	Sulphur (S)	9.71	14.1	1.07	1.14	7.01	1.59	1.63	0.55
	Gypsum	11.6	17.6	1.36	1.59	7.89	2.13	2.37	0.82
	Compgypsum	10.1	14.9	0.86	1.11	6.68	1.65	1.48	0.55
	PVA	8.98	12.3	0.69	0.64	6.34	1.44	1.29	0.47
LSD. 5 %		2.36	3.09	0.44	0.28	2.23	0.35	0.54	0.23

The application of algal extracts significantly increased the contents of the total chlorophyll and antioxidant phenomenon. As well as algal extracts exhibited strong positive correlation with the increase of wheat fresh weight, grain weight and yield and yield components. They explained that algal spray application significantly increased the plant nutrients content and had a positive effect on plant growth, oxidation behavior and activity of antioxidant enzymes in plants affected by salt stress. Furthermore, both cyanobacteria and *Azolla* extracts are characterized by their cytokines, gibberellins and auxins content that enhance the plant growth and furthermore these materials is proved to overcome the adverse effect of salinity in saline soil **(Aref et al., 2009).** Recently, mentioned that the application of gypsum creates more favorable environment in soil and maintain elements in more available form due to declamatory effect, which is consequently increased the soil fertility that in turn is reflected positively on the uptake of nutrients by plants. In addition, biofertilizers play a significant role in improving plant nutrients supplies as complementary and supplementary factors. They help in increasing the biologically fixed atmospheric nitrogen, also increases the availability of native and applied P and other crop nutrients. **(Dhanushkodi and Subrahmanlyan, 2012).**

The total contents of N, P, K and Na ranged from 13.6 to 31.5 Kg fed^{-1}, 4.10 to 12.1 Kg fed^{-1}, 7.74 to 25.7 Kg fed^{-1} and 1.94 to 7.53 Kg fed^{-1} for straw of wheat plants as well as 12.4 to 21.2 Kg fed^{-1}, 9.28 to

27.1 Kg fed^{-1}, 4.4 to 8.56 Kg fed^{-1} and 1.83 to 6. Kg fed^{-1} for grains against 8.72 to 11.6 Kg fed^{-1}, 0.96 to 1.36 Kg fed^{-1}., 6.07 to 7.89 Kg fed^{-1} and 1.45 to 2.37 Kg fed^{-1}. for straw of rice as well as 12.3 to 17.6 Kg fed^{-1}, 0.74 to 1.59 Kg fed^{-1}, 1.13 to 2.13 Kg fed^{-1} and 0.32 to 0.82 Kg fed^{-1} for grains, respectively.

Moreover, results showed that the total content of sodium in straw for both wheat and rice was progressively increased compared to grains. This may indicate that the accumulation of salts in plants resulted from high salts in soil, which reflected on nutrient uptake by plants especially for sodium. Also, sodium uptake in both straw and grains decreased while K uptake increased in treatment of gypsum a combined with cyanobacteria may be possible reason of higher yield in condition of salinity soil. These findings were observed by **Cha-um et al. (2011)** who found that the Na^+ and Cl^- are absorbed rapidly by the root system of rice and accumulate in the whole plant, which will then display symptoms of toxicity in both vegetative and reproductive stages. In the present study, Na+ in Jasmine rice grown in saline soil treated with gypsum and FYM was very low, whereas K+ was enriched when compared to the control. The properties of organic matter **(Tejada et al., 2006)** and gypsum **(Chaudhry, 2001)** in treating saline soil have been reported for the purposes of saline soil remediation **(Makoi and Verplancke, 2010)** for the cultivation of crops such as rice, wheat **(Qadir et al. 2001)** ; organic matter, including FYM and green manure and gypsum may function as salt-ion chelating agents which detoxify the toxic ions, especially Na+ and Cl-, as indicted by low EC in soil treated with both FYM and gypsum (**Hanay et al., 2004 and Zahid and Niazi, 2006).**

Conclusion

In conclusion, the application of cyanobacteria inoculation combined with organic acids (fulvic acid and humic substances) is helpful to improve the soil properties of saline soils (EC, SAR and ESP). Also, the cyanobacteria inoculation combined with compogypsum and gypsum improved organic matter content and the available nutrients in soil. As well as, the improve of macronutrients uptake (N, P and K) in wheat and rice cropping system, which in is reflected on the yield and its components.

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Chemical Constituents and *In Vitro* Antimicrobial Activities of Five Botanicals Used Traditionally for the Treatment of Neonatal Jaundice in Ibadan, Nigeria

Gbadamosi I. T. and Obogo S. F.

Department of Botany, University of Ibadan, Nigeria.
gita4me2004@yahoo.com

Abstract: Neonatal jaundice is a main cause of morbidity and mortality among neonates in Nigeria and ethnobotanical investigation revealed the use of *Alstonia boonei* (De wild.), *Cocos nucifera* (L.), *Gossypium barbadense* (L.), *Tetrapleura tetraptera* (Schumach. & Thonn.) and *Vernonia amygdalina* (Del.) for the management of neonatal jaundice in Ibadan, Nigeria. This study analysed the five plants for their chemical components and antimicrobial activities to justify their therapeutic values in the management of jaundice. The powdered plant samples were screened for mineral and phytochemical components using standard protocols. The ethanol extracts (50 mg/ml) of samples were tested against five clinical pathogenic organisms at 10^{-1} – 10^{-6} cfu/ml inoculum concentrations using agar-well diffusion method. Data were analysed statistically. *G. barbadense* (0.26 mg/100g) had the highest Na content. *A. boonei* had the highest Zn and Fe contents, 6.40 and 5.13 mg/100g respectively. *A. boonei* contained the highest saponins (0.79%) and tannins (0.05%). At 1 x 10^{-4} cfu/ml, *G. barbadense* was most active on *K. pneumoniae* (29.90 mm); *T. tetraptera* was most active against *C. albicans* (21.90 mm) and *T. tetraptera* and *V. amygdalina* gave the same activity (14.90 mm) against *E. coli.* At 1 x 10^{-6}cfu/ml, only *T. tetraptera* showed 100% antimicrobial activity by being active against all test organisms. The mineral and phytochemical components of the plants could be responsible for their antimicrobial properties. The extracts, compounds as well as oils from the test plants especially *A. boonei* could be useful in combating infections and metabolic disorder associated with neonatal jaundice. Toxicity studies of the plant samples would ascertain their safety in treatments.
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Keywords: Botanicals; jaundice; neonates; mineral analysis; phytochemical screening; antimicrobial assay.

1. Introduction

Hyperbilirubinemia is the medical term for a high bilirubin level in the bloodstream and the cause of jaundice (Coleman, 2011). Jaundice itself is not a disease, but rather a sign of one of the many possible underlying pathological processes that occur at some point along the normal physiological pathway of bilirubin metabolism. Hyperbilirubinemia is often seen in liver diseases such as hepatitis or liver cancer. It may also indicate obstruction of the biliary tract, for example by gallstones or pancreatic cancer, or less commonly congenital in origin. Jaundice can also be associated with severe illnesses such as haemolytic disease, metabolic and endocrine disorders, enzymatic deficiencies of the liver, and infections (Bilgen *et al*, 2006).

Neonatal jaundice is the most common form of jaundice and it occurs in new-born babies of less than a month old. Factors influencing the occurrence of neonatal jaundice are body physiology, premature birth, effect of breastfeeding, breast-milk constituents, heredity (enzyme deficiency), blood or rhesus group incompatibility (between mother and baby), and infections (Kotal *et al*, 1996; Bilgen *et al*, 2006; Kumral *et al*, 2009; American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004).

Clinical symptoms of neonatal jaundice include irritability, jitteriness, increased high-pitched crying, lethargy and poor feeding; back arching; apnea and seizures (Nelson, 2013). Phototherapy is the most effective way of breaking down a neonate's bilirubin (Leung *et al*, 1992) and increased feedings also help move bilirubin through the neonate's metabolic system. Exchange transfusions are also useful in the treatment of neonatal jaundice. (American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004).

Neonatal jaundice associated with high morbidity and mortality is a common pediatric problem in West Africa (Sofoluwe and Gans, 1960). The condition is the commonest cause of neonatal admission to Children Emergency Room in Lagos University Teaching Hospital (LUTH), Nigeria (Ransome-Kuti, 1972). In a study on the incidence and causes of neonatal jaundice in Nigerian babies, Effiong *et al*. (1975) observed that G6PD (enzyme glucose-6-phosphate dehydrogenase) deficiency and ABO incompatibility were the major aetiological factors in babies with total bilirubin of 15mg/100ml.

Ahmed *et al*. (1995) reported septicaemia (50%) and G6PD deficiency (40%) as the major aetiological factors of neonatal jaundice in 587 neonates born in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. Neonatal jaundice accounted for 35% of all Neonate Intensive Care Unit (NICU) admissions at Federal Medical Centre, Abakaliki, Southeast, Nigeria. Septicaemia (32.5%) and prematurity (17.5%) were the leading aetiological factors in neonates (Onyearugha *et al*., 2011).

In view of non-drug orthodox methods of treatment of neonatal jaundice and the prevalence of neonatal jaundice in Nigeria, a phytodrug therapy would be desirable. Medicinal plants with reported therapeutic effects in the management of neonatal jaundice are *Artemisia capillaris*, *Scutellaria baicalensis*, *Rheum officinale*, *Glycyrrhiza glabra* and *Coptis chinesis* (Fok, 2001); *Boswellia serrata* (Patwardhan *et al*., 2010); *Alstonia boonei* (Adotey *et al*., 2012; *Moronkola and Kunle*, 2012) and *Gossypium barbadense* (Todou and Konsa, 2011). Rhei rhizome, ginseng, grapeseed, *Dioscorea villosa*, *Pseudolarix kaempferi*, *Hypericum perforatum* and soy have also been reported to be effective in the management of neonatal jaundice (Lazar, 2004).

In Nigeria, the stem bark of *A. boonei* is used widely to treat malaria, typhoid fever, gonorrhoea, yaws, asthma, dysentery, and as a galactagogue. *C. nucifera* roots have antipyretic and diuretic properties. Milk of young coconut is useful as laxative, antidiarrhoeic and counteracts the effects of poison. The oil is used to treat diseased skin and teeth, and mixed with other medicines to make embrocations. *G. barbadense* is widely used for the treatment of conjunctivitis, convulsions, jaundice, gastrointestinal disorders, and sexually transmitted infections such as gonorrhea, rheumatism and wounds. *T. tetraptera* is used traditionally for the treatment of skin infections. *V. amygdalina* has antibiotic value in traditional medicines, it is also used to treat diabetes, gastrointestinal disorders and as a worm expeller (www.prota4u.org).

This study presents scientific information on the therapeutic potentials of *A. boonei*, *C. nucifera*, *G. barbadense*, *T. tetraptera* and *V. amygdalina* in the management of neonatal jaundice.

2. Material and Methods

Plant materials

Twenty herb-sellers of a local herbal market (Bode) in Ibadan, Nigeria were interviewed on traditional knowledge of management and treatment of neonatal jaundice. *Alstonia boonei, Cocos nucifera, Gossypium barbadense, Tetrapleura tetraptera* and *Vernonia amygdalina* were frequently mentioned in the twenty recipes collected from the herb-sellers. Herbal recipes were documented. The local name, parts of plant used, method of preparation and mode of administration were also recorded (Sofowora, 1982). The plant materials were purchased from a local herbal market. The samples were identified at species level in the University of Ibadan Herbarium (UIH). The plant samples were washed, dried (27°C) and powdered. The powdered samples were stored (4°C) in air-tight bottles for further use.

Mineral analysis of powdered plant samples

The methods of Walsh (1971) and AOAC (2005) were used for the mineral analysis of samples. After wet digestion, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) were analysed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bausch scientific AAS). Phosphorus was determined using Vanadomolybdate (Yellow method) (AOAC, 2005). Percentage transmittance was determined at 400 nm using Spectronic 20 (Bausch and Lomb) Colorimeter.

Phytochemical screening of plant samples

Quantitative phytochemical analysis of powdered plant samples was carried out using AOAC (2005) methods.

Antimicrobial assay of ethanol plant extracts: The extracts (50 mg/ml) of samples prepared by cold extraction method in 80% ethanol, concentrated and used for the antimicrobial bioassay. The test organisms: *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were clinical isolates obtained from University College Hospital (UCH), Ibadan. The organisms were grown in nutrient broth for 18 h at 37°C. Six different inoculum concentrations ($1 \times 10^{-1} – 1 \times 10^{-6}$ cfu/ml) of each isolate were prepared in sterile distilled water from the broth cultures via serial dilution. 1ml of the inoculum was thoroughly mixed with 19 ml of sterile nutrient agar and poured into sterile Petri dish. The agar was left to solidify. Two wells of 4 mm in diameter were punctured in each agar plate and 60 µl of each extract was filled into the wells with the aid of a sterile micropipette. 80 % ethanol was used instead of extract in the control experiment. Also plates containing the test organisms in agar without extract were used as control. All experiments were done aseptically and each experiment was replicated three times. The plates were incubated at 37°C for 24 - 48 h. Readings were taken after 24 h and 48 h. The diameter of inhibition was measured in millimetres (mm).

Data analysis

Where applicable, data were statistically analysed using One-way Analysis of Variance (ANOVA) and expressed as mean ± SD. The Duncan

Multiple Range Test (DMRT) was used to test means for significance ($P < 0.05$).

3. Results and Discussion

The profile of the plant samples used in the management of neonatal jaundice is presented in Table 1. The plants belong to five different families: Apocynaceae, Arecaceae, Malvaceae, Fabaceae and Asteraceae. Table 2 shows the traditional methods of preparation of various botanicals as regimen for neonatal jaundice. The herbs are used singly in recipes and decoction is the most common method of preparation, the herbs are also prepared as soap, oil and syrup for administration. The folk methods of treatment take into consideration the fragility of the baby's body and system hence the mild methods of preparation and administration of herbal remedies.

The various mineral constituents of plant samples are presented in Table 3. *G. barbadense* (0.26mg/100g) gave the highest Na content and *C. nucifera* (0.04mg/100g) the least. K content was also highest in *G. barbadense* (0.48 mg/100g) and least in *T. tetraptera* (0.10 mg/100g). Calcium content was highest in *G. barbadense* (0.08 mg/100g) and least in *T. tetraptera* (0.01 mg/100g). *A. boonei* gave the highest P content of 0.41 mg/100g and *C. nucifera* (0.11 mg/100g) the least. Zinc (Zn) was highest in *A. boonei* (6.40mg/100g) and least in *C. nucifera* (3.25mg/100g). The iron (Fe) content of *A. boonei* (5.13mg/100g) was the highest and *T. tetraptera* (1.15 mg/100g) recorded the least. Overall, *G. barbadense* was highest in sodium, potassium and calcium. *A. boonei* also recorded highest values for phosphorus, zinc and iron. The results of the mineral constituents of test plants are in line with the reports of previous authors. Afolabi *et al*. (2007) reported the magnitude of presence of macroelements (ppm) in *A. boonei* bark in the order P>K>Na>Ca>Mg and only Fe (3.2 ppm) was detected as micronutrient. Although there is scarcity of information on the mineral constituents of coconut pod, the literature is replete with information on coconut water mineral components (Richter *et al*., 2005; Uphade *et al*., 2008). *G. barbadense* leaves contain copper (14.6 ppm) and zinc (1.6 ppm) (Apena *et al*., 2004). Essien *et al*. (1994) reported that the fruit shell, fruit pulp and seed of *T. tetraptera* contained varying amounts of nutrients such as protein, lipids and minerals. *V. amygdalina* contains calcium, iron, potassium, phosphorous, manganese, copper and cobalt in significant quantities (Eleyinmi *et al*., 2008). The various plant minerals have key roles to play in human health. Potassium regulates heartbeat, maintains fluid balance and helps muscle contraction. Calcium is important for developing and maintaining healthy bones and teeth. It assists in blood clotting, muscle contraction and nerve transmission. Phosphorus works in combination with calcium to develop and maintain strong bones and teeth. It also enhances the use of other nutrients. Magnesium helps nerves and muscles function. It activates over 100 enzymes in the body. Zinc is an essential part of more than 200 enzymes involved in digestion, metabolism and reproduction and wound healing. Copper helps in normal red-blood cell formation and connective tissue formation. It acts as a catalyst to store and release iron to help form hemoglobin. It also contributes to central nervous system function. Iron is essential for red blood cell formation and function and is very important for brain function (Schauss, 2013).

The five medicinal plants contained secondary metabolites in varied concentrations (Fig. 1). The % alkaloids was highest in *A. boonei* (0.47%), followed by *V. amygdalina* (0.19%), and the least was for *C. nucifera* and *G. barbadense* (0.16%). The % saponins was highest in *A. boonei* (0.79%), followed by *G. barbadense* (0.58%) and *C. nucifera* (0.26%) had the least. *A. boonei* and *V. amygdalina* had the highest tannins content of 0.05%. The % phenols was highest in *V. amygdalina* (0.08%). The highest glycosides content was observed in *T. tetraptera* (0.36%). Generally, *A. boonei* had the highest values of alkaloids, saponins and tannins.

At 1 x 10^{-4}cfu/ml inoculum concentration, the ethanol extract (50 mg/ml) of *A. boonei* was active on *E. coli* and *K. pneumoniae* with 11.90mm and 14.90mm zones of inhibition respectively (Fig. 2). *C. nucifera* extract was active on 2 out of 5 test organisms; it inhibited the growth of *S. aureus* (11.90mm) and *P. aeruginosa* (13.40mm). *G. barbadense* extract showed antimicrobial activities on 4 out of 5 isolates being most active on *K. pneumoniae* (29.90mm) and least active on *E. coli* (11.90mm). *T. tetraptera* fruit extract was active on three bacterial pathogens and *C. albicans*. The extract was most active against *C. albicans* (21.90mm) and least active on *S. aureus* (11.90mm). *V. amygdalina* extract had antimicrobial properties being most active on *K. pneumoniae* (23.40mm) and least active on *P. aeruginosa* (14.40mm).

Fig. 3 shows the antimicrobial effects of various plant extracts against organisms at 1 x 10^{-6} cfu/ml inoculum concentration. *E. coli* was susceptible to *C. nucifera* extract with 24.90 mm diameter of inhibition. The growth *K. pneumoniae* was inhibited by *T. tetraptera* extract with 44.90 mm zone of inhibition. Overall, at 1 x 10^{-6}cfu/ml inoculum concentration, only *T. tetraptera* showed 100% antimicrobial activity by being active against all organisms. In line with the results of the antimicrobial activities of the test plants are previous reports on their values as antibiotic plants. The antimicrobial activity of extracts and active compounds of *V. amygdalina* has been reported by previous authors

(Alabi *et al*., 2005; Erasto *et al*., 2006). Gbadamosi and Oyedele (2012) reported the antimicrobial activities of *A. boonei* and *T. tetraptera* in skin infections. The hot percolated ethanolic extract of *C. nucifera* was active against Gram + and Gram – bacterial isolates (Singla *et al*., 2011). *G. barbadense* has antibacterial and wound healing properties (Ikobi *et al*., 2012).

As neonatal jaundice is an indication that the baby's liver is limited in its ability to process bilirubin, the mineral and antioxidant constituents of the five plants may be therapeutically useful. According to White and Foster (2000) detoxification process of the liver initially requires magnesium, iron, molybdenum and essential fatty acids, then later extra vitamins A, C, E (antioxidants), zinc, copper, folic acid and some amino acids. *V. amygdalina* has antibiotic, antimicrobial, anticancer, antioxidant, antidiabetic, hepato-protective, nephro-protective, oxytocic and serum lipid modulation properties (Ijeh and Ejike, 2011). Also *T. tetraptera* has shown antimalarial and antioxidant properties (Lekana-Douki *et al*., 2011; Badu *et al*., 2012). Gbadamosi *et al*. (2011) reported the nutritional and phytochemical properties of *A. boonei* bark as an antimalaria herb. *C. nucifera* is rich in phosphorus and has antibacterial, antifungal, antiviral and antioxidant properties. The phytochemical components such as alkaloids, saponins and tannins present in the medicinal plants may have singular or synergistic effects in improving the ability of the neonate's liver to reduce high level of bilirubin. Since infection is a risk factor for neonatal jaundice (Bilgen *et al*., 2006) the antimicrobial activities of the medicinal plants will be useful in treating the health problem.

Table 1. Profile of test plants used in neonatal jaundice in Ibadan, Nigeria

Family	**Scientific name**	**Common name**	**Local name**	**Part used**
Apocynaceae	*Alstonia boonei*	Stool wood	Ahun	Bark
Arecaceae	*Cocos nucifera*	Coconut	Agbon	Pod (husk)
Malvaceae	*Gossypium barbadense*	Cotton	Owu	Leaf
Fabaceae	*Tetrapleura tetraptera*	-	Aidan	Pod (Fruit)
Asteraceae	*Vernonia amygdalina*	Bitter leaf	Ewuro	Leaf

Table 2. Herbal remedies for the management of neonatal jaundice in Ibadan, Nigeria

S/N	Herb	Herbal preparation and dosage	Preparation method
1.	*Alstonia boonei*	The bark (200g) of *A. boonei* is boiled in 1L of water for 15 mins. The water extract is used to bath the baby twice daily. Half teaspoonful of the extract is given to the baby orally twice daily after food.	Decoction
2.	*Cocos nucifera*	The pod (200g) of *C. nucifera* is cooked in 1L of water for 15mins and used to bath the baby. Powdered pod is soak in hot palm oil and menthol crystals are added to the oil. The oil is rubbed on the baby after bath.	Decoction and oil
3.	*Gossypium barbadense*	*G. barbadense* leaves (200g) are boiled in water (1L) for 15mins. One teaspoonful of the extract is given to the baby three times daily after food.	Decoction
4.	*Tetrapleura tetraptera*	*T. tetraptera* pod (200g) is cooked in water (1L) for 15 mins. The preparation is used to bath the baby twice daily. Half teaspoonful of the extract is taken by the baby twice daily after food. The powdered pod is mixed with local soap and used for bathing the baby twice daily.	Decoction and soap
5.	*Vernonia amygdalina*	Fresh leaves of *V. amygdalina* are collected and washed thoroughly. The leaf juice is extracted by pounding. The juice is added to pure honey (1:1) and mixed thoroughly. The baby takes one teaspoonful of the mixture three times daily. Paste is also prepared from the fresh leaves and mixed with local soap. The soap is used for bathing daily.	Syrup and soap

Table 3. Mineral components (mg/100g) of powdered plant samples

Botanical	Na	K	Ca	P	Mg	Zn	Cu	Fe
A boonei	*$0.12^c \pm 0.00$	$0.31^c \pm 0.00$	$0.06^c \pm 0.00$	$0.41^a \pm 0.00$	$0.49^a \pm 0.00$	*$6.40^a \pm 0.28$	$9.05^a \pm 0.35$	$5.13^a \pm 0.14$
C. nucifera	$0.04^e \pm 0.00$	$0.08^e \pm 0.00$	$0.03^d \pm 0.00$	$0.11^e \pm 0.00$	$0.23^e \pm 0.00$	$3.25^e \pm 0.21$	$4.35^c \pm 0.21$	$2.14^d \pm 0.21$
G. barbadense	$0.26^a \pm 0.00$	$0.48^a \pm 0.00$	$0.08^a \pm 0.00$	$0.39^b \pm 0.00$	$0.47^b \pm 0.00$	$5.63^b \pm 0.28$	$8.50^a \pm 0.28$	$3.88^b \pm 0.14$
T. tetraptera	$0.10^d \pm 0.00$	$0.12^d \pm 0.00$	$0.01^e \pm 0.00$	$0.26^d \pm 0.00$	$0.31^d \pm 0.00$	$3.66^d \pm 0.21$	$4.80^c \pm 0.14$	$1.15^e \pm 0.21$
V. amygdalina	$0.17^b \pm 0.00$	$0.34^b \pm 0.00$	$0.06^b \pm 0.00$	$0.29^c \pm 0.00$	$0.40^c \pm 0.00$	$4.28^c \pm 0.49$	$6.35^b \pm 0.21$	$3.15^c \pm 0.21$

Legend: *Value = Mean ± standard deviation. Values within a column followed by the same superscript are not significantly different at P = 0.05. Na - Sodium; K - Potassium; Ca - Calcium; P - Phosphorus; Mg- magnesium; Zn - Zinc; Cu - copper; Fe - Iron.

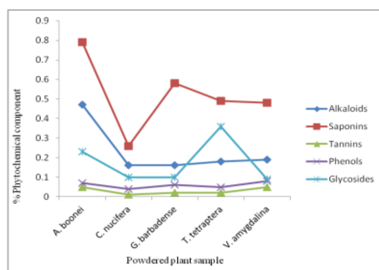

Figure1. Phytochemical components of powdered plant samples

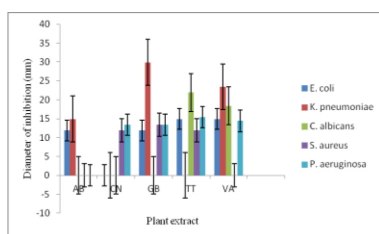

Figure 2. *In vitro* antimicrobial activities of ethanol extracts of test plants at 1 x 10^{-4} cfu/ml inoculum concentration

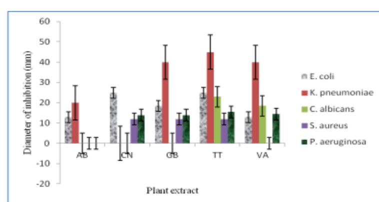

Figure 3. *In vitro* antimicrobial activities of ethanol extracts of test plants at 1 x 10^{-6} cfu/ml inoculum concentration

Conclusion

The test plants contained minerals and secondary metabolites that could be responsible for the observed antimicrobial activity of the plants. *A. boonei* is a valuable plant in the management of neonatal jaundice because of its high magnesium, copper, zinc and iron contents. Mg and Zn play significant roles in metabolism, copper and iron are essential in red blood formation and function. Enhanced metabolism flushes out bilurubin out of the baby's system. A decoction of *A. boonei* is good for the mother as galactogogue as well as for the baby for the treatment of jaundice especially in breast feeding and infections associated neonatal jaundice. Of importance is the significant antimicrobial activities of *G. barbadense* and *V. amygdalina* in infections associated neonatal jaundice. The isolation and purification of active components of *A. boonei*, *G. barbadense* and *V. amygdalina* could improve pharmaceutical treatments for neonatal jaundice. Toxicity studies of the three plants will certify their safety in treatments.

Corresponding Author:
Dr. Idayat T. Gbadamosi
Department of Botany
University of Ibadan, Nigeria
E-mail: gita4me2004@yahoo.com

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Effect of Water Deficit on Growth of Some Mango (*Mangifera indica* L.) Rootstocks

Abdel-Razik, A. M.

Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.
dr.ahmedyoussef57@yahoo.com

Abstract: The present study aimed to investigate the effect of water deficit on the growth of mango rootstock seedlings. The experimented seedlings were obtained by seed propagation of two mango cultivars, namely: Sabre and Zebda. One year old mango rootstock seedlings (*Mangifera indica* L.) were grown in pots in a green house, where they subjected to four water levels, 100% (control), 80%, 60% and 40% available water. The results indicated that, Sabre stock seedlings gave the highest values of shoot growth, leaves number, leaf area, root/shoot ratio, succulence grade, T.S.S%, leaf proline and leaf nutrient content compared with that of Zebda rootstock seedlings.
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Key words: Mango, Water deficit, Osmotic potential, Proline, Leaf nutrient content.

1.Introduction

Mango is one of the most important fruit crop in Egypt, which meet a great demand in local market and in export. Mango trees in Egypt depend on irrigation to get their need of water in most cultivated area. Hence, the amount of given irrigation water depends on many factors such as: type of soil, climate condition in the cultivated area, methods and systems of irrigation, characteristics of the plant rootstock and others. On the other hand, irrigation is necessary to insure stable yield with high quality. However, insufficient irrigation amount might cause plant water deficit which, might lead to permanent wilting of shoot and fruit growth if unrelieved and plant dehydration leading to plant death.

According to Ashley, (1993), the exposure of rootstock seedling to some moisture stress at nursery may cause some degree of "hardening" against current and later drought periods. However, the degree of hardening will vary among varieties and species of used rootstocks.

It is remarkable that few studies have been carried out in Egypt to determine the optimal water requirements of different mango stock seedlings. The previous done work in the last ten years on the water requirements of the mango stock seedlings, brought about by seed propagation, indicated that, irrigation deficit affected growth and leaf content of mango (Romero *et al,* 2004; Cifre *et al*. 2005, Tognetti *et al*. 2005, Luvahu *et al.* 2007).Therefore, the present study aims to emphasize on the two mango stock cultivars grown under different water deficit and their effect on vegetative growth, leaf water deficit succulence grade, osmotic potential, plant proline content and leaf nutrient content.

2. Material and Methods

The experimentation was carried out during seasons of 2012 and 2013 in the green house of the research farm of Horticulture Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. To study the suitable irrigation requirement for two seedling cultivars that can be used as mango rootstocks. Therefore, uniform and healthy one year old seedlings of mango stocks cultivars, Sabre (from south Africa) and Zebda(local rootstock) were chosen as plant material for this study. The irrigation treatment consisted of three replicates, each replicate consisted of three stocks.Mango stock seedlings of each cultivar were similar in growth on the base of seedling height (cm), planted in 15th February 2012 and 2013, into 30 cm porous clay pots, filled with 7 kg sandy soil. On 1st March, the irrigation treatments started as follow:

Treatment 1: Each pot received 100% available water (control) during whole period of experiment.

Treatment 2: Each pot received 80% available water during whole period of experiment.

Treatment 3: Each pot received 60% available water during whole period of experiment.

Treatment 4: Each pot received 40% available water during whole period of experiment.

The irrigation treatments were carried out by weighing the pots every three days and adding the depleted amount of water to attain the specific percentage of available water in the treatment.

The measurements:

1. The vegetative growth:

1.1. Shoot length in cm: the vegetative growth was expressed in one main shoot per seedling that was left to grow. The increase in shoot length was adjusted through calculating the difference between

shoot length in cm at the beginning of the spring flush and the cessation of growth. The vegetative growth was collected from the seeding and dried under 70 °C for 48 hours to determine the dry weight.

1.2. Leaves number per seeding was recorded at cessation of growth.

Leaf area was determined according to Ahmed and Morsy (1999) as follows:

Leaf area = 0.7 ×Leaf blade length (cm) × blade length (cm) – 1.06 = ----- cm^2

The fourth distal leaf was used

2. The root and shoot dry weight :

The root system and shoot of seedling was separated at cessation of growth and washed with tap water, then dried under70 °C for 48 h, Root/shoot ratio was determined.

3. Physical and Biochemical characteristics of the seedling rootstock:

3.1. The physical parameters measured were:

3.1.1. Water saturation deficit:

Leaf disks (1 cm^2) were taken from adult leaves (the fourth distal adult leaf) at cessation of growth and weighed, then put in distilled water for 45 min. Leaf disks, thereafter were dried at 70°C for 24 hrs. The saturated disks were used to adjust the W.S.D. and R.W.C.

$$\text{W.S.D.} = \frac{\text{Sat. wt. - fresh wt.}}{\text{Sat. wt. - dry wt.}} \times 100$$

2-1-2. Succulence grade =

$$\frac{\text{Leaf water content in (g)}}{\text{Leaf surface (cm}^2\text{)}} \quad (\text{g / cm}^2)$$

2-2- Biochemical characteristics:

2-2-1 Osmotic potential of leaf sap was determined at growth cessation according to Hifny and Abdel-all (1981) who found that TSS % in leaf cell sap showed an identical trend to that of osmotic potential values, 5 g of fresh leaf blade was mixed with 25 ml distilled water with electrical mixer where TSS % was measured in the filtrate using hand refractometer.

2-2-2Proline content of leaf:

Proline content was colorimetrically estimated at 520 μm from leaf extract according to method of Bates *et al*. (1973).

2-2-2 Leaf nutrients (N,P and K) content:

Total nitrogen was determined by a micro-kjldahl (Jackson,1967) at the end of growth season.

Phosphorus was determined by using flam photometer according to Murphy and Riely (1962).

Potassium was determined by using atomic Absorption spectrophotometer according to Brandifeld and Spincer (1965).

Statistical Analysis :

Analysis of variance (ANOVA) was performed using two way ANOVA from SAS software (1989).

3.Results and Discussion

1. Effect of different water deficit on seedling growth:

1.1. Growth increase:

Data in Table (1) showed an increase in shoot length (cm) during the growth period under different water stress, in the two studied mango stock cultivars. The results showed that the control treatment (100% available water) had increased the growth of most seedling shoots, which recorded the maximum height, while the lowest plant height was resulted from40% available water treatment. Differencesin growth increase (plant height) among the two studied stock cultivars were insignificant at 100% available water levels, on the other hand, the differences of shoot growth of both stock seedlings was significant under the effect of low available water, (40%,60%or80% available water). Sabre seedlings induced the highest growth increase at 80%, 60% and 40% available water

The treatment of 40% available water showed the least increase in seedling growth when compared with those resulted under higher available water percentages. The present result was in agreement with those of others who found that the little increase in shoot growth that caused by water deficit had resulted from the direct adaptation under high stresses of water deficient.

(Tahir*et al*. 2003 and Perez *et al*. 2007) who suggested that Abscisic acid might play the main hormonal role regarding drought tolerance. An increase in ABA- leaves content may depress growth of plants which suffering from high water stress (Loveys and Kriedemann, 1973) Moreover, re-watering the wilted vine plants decreased ABA content of soft wood to 50%. The ABA could regulate water transpiration, water uptake and the morphogenetical adaptation to high water stress. It might also increase the permeability of root-tissues to water and decreased the ion transport in root-xylem.

1-2 Number of leaves and leaf surface area (cm^2):

Data in Table (1) indicated that a significant increase was observed in number of leaves in plants under 100% available water compared to those under 60% and 40% of available water. Water deficits decrease leaf growth by slowing rates of cell division and expansion due to turgor loss and

increased synthesis of abscisic acid (Tezara *et al.*2002).Water deficit also causes low leaf initiation (Boyer,1976). Reducing the number of leaves could be a phenomenon by the plants to minimize the surface transpiration (McCree, 1985). In mango, water deficit causes a reduction in leaf development (Luvaha *et al.* 2005, Abdel-Razik and Abd-Raboh, 2007). Sabre stock seedling induced higher leaves number and leaf surface area at all water levels.

1-3 Root and shoot growth:

As can be seen in (Table 1) root (d.wt) and shoot growth (d.wt) expressed as dry weights in the two rootstocks cultivars was proportional to the percentage of water availability in the two seasons (2012 and 2013). 100% available water caused the maximum significant root and shoot dry weight, followed descendingly by that of 80% and 60% available water, while the least obtained root and shoot growth was induced by 40% available water in the two studied cultivars and in the two studied seasons. The data indicated that the decrease in water availability caused a marked reduction in dry weight of roots and shoots. Differences among the two studied cultivars in roots and shoots at all water levels were significant.

Increasing the available water caused a marked increase in root and shoot dry weights. This is apparently due to the role of water in the early plant growth processes such as cell division and cell enlargement in the mango seedlings.

There is differential sensitivity of roots and shoots to water deficit. Root growth being less sensitive to water deficit and this caused the increase in root to shoot ratio (George, 2007). The reduction of dry weights of root and shoot under water stress were apparently caused by increased water deficit to the plants which may have also impacted negatively on nutrient uptake (Luvaha, 2005).

These results are in harmony with those of others, who found that a decrease in soil moisture induced a high decrease in dry weight of plant roots (Tahir *et al.* 2003). They added that the mango rootstock selected for drought resistance showed a high respiration intensity, a minimum water deficit and large number of absorbing rootlets particularly after drought periods.

1-4Root/ shoot ratio

The root / shoot ratio expressed as dry weight was increased with increasing water deficit (table 1). The values of Root/shoot ratios for the sevier stressed seedlings (40% of available water) were significantly higher than those of control treatment (100% of available water).According to Luvahu *et al.* (2007) the ratios of R/S were measured in seedling that was subjected to high water deficient 40% available water. They added that, Abscisic acid (ABA) accumulated in the hypocotyl region in water deficit plants can inhibits growth. This however has no effect on root growth.

Comparing the values of root/shoot ratios of the two studied seedling under the effect of percentages of available water 100 %, 80%, 60% and 40%, the results in table (1) indicated that Zebda stock at seeding elucidated higher Root/shoot ratios. As while the differences was insignificant between the two stocks under 40% available water.

Table (1):**Effect of different water deficit on the vegetative growth of mango seedlings cessation of growth.**

Variety	Treatment	Growth increase		Leaves number				Leaf surface area			
		2012	2013	2012		2013		2012		2013	
				Beg	End	Beg	End	Beg	End	Beg	End
Sabre	100%	18.81	19.18	29	46	26.33	40	59	85.91	57.32	79.62
	80%	14.86	16.70	25	32	26	31	58.57	84.59	56.16	82.31
	60%	8.90	10.02	24	27	26	29.33	56.18	81.35	55.43	76.08
	40%	6.45	6.59	24.33	27	27.66	29	50.69	57.52	56.16	58.23
Zebda	100%	23.83	25.05	25.66	34.33	28.33	37.33	55.95	73.63	56.20	75.75
	80%	13.39	14.63	26.66	30.33	24.33	29.66	58.09	74.92	56.25	72.69
	60%	6.31	7.00	24.33	28.00	25.00	28.33	52.96	70.72	54.17	70.97
	40%	4.03	5.28	26.00	26.33	25.33	26.33	53.23	55.05	52.34	55.11
LSD 0.05											
Cultivar		2.76	3.42	3.46	3.58	1.72	1.82	8.19	12.77	6.57	7.42
Treatment		3.91	4.83	4.9	5.06	2.43	2.58	11.58	18.06	9.29	10.5
C X T		5.53	6.84	6.93	7.16	3.44	3.65	16.38	25.54	13.14	14.85

Table (1) Continue :

variety	Treatment	Root dry weight 2012	Root dry weight 2013	Shoot dry weight 2012	Shoot dry weight 2013	Root / Shoot ratio 2012	Root / Shoot ratio 2013
Sabre	100%	40.89	44.49	66.03	69.0	61.92	64.48
	80%	33.064	35.53	48.12	46.6	68.71	70.24
	60%	35.0	36.91	36.02	37.52	97.17	98.37
	40%	25.4	26.3	24.02	24.9	105.74	105.62
Zebda	100%	31.57	33.01	48.1	49.04	65.63	67.31
	80%	29.03	30.79	34.9	35.19	83.18	87.49
	60%	29.66	30.23	29.1	29.03	101.92	104.13
	40%	22.6	26.16	20.96	24.2	107.82	108.09
LSD 0.05							
Cultivar		4.22	4.35	3.07	5.18	8.23	9.002
Treatment		5.97	6.16	4.35	7.33	11.64	12.73
C X T		8.44	8.71	6.15	10.37	16.47	18.005

2- Physical and chemical characteristics of the vegetative growth:

2-1- Physical parameters:

2-1-1: Water saturation deficit (W.S.D.):

Water saturation deficit was increased by decreasing the percentage of available water. Fig (1) showed that water saturation deficit of mango seedling leaf was increased by decreasing the available water in the two mango studied stock cultivars. The lowest values of water saturation deficit were noticed at 100% available water (the control), while the highest values were noticed at 40% available water in the two studied stocks cultivars. At the levels of 100, 60 and 40% available water, Zebda showed higher value of saturation deficit more than Sabre stock cultivars, while at 80% available water Sabre stock cultivar showed the highest value. Differences among stocks were mostly insignificant.

This finding was in agreement with that obtained by Tahir *et al*. (2003) on mango, who found that water saturation deficit was decreased by increasing soil moisture and added that the rootstock selected for drought resistance showed a minimum water saturation deficit values.

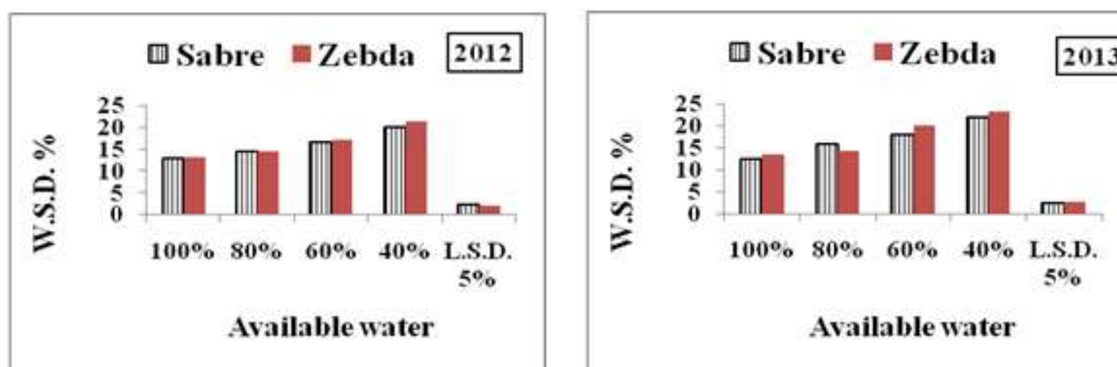

Fig. (1): Effect of different water deficit on leaf water saturation deficit.

2-1-2Succulence grade of leaves:

Data in fig (2) indicated that, succulence grade under different available water treatment was increased as the available water increased. The maximum values of leaves succulence grade were obtained at the control treatment (100% available water). On the other hand, the lowest values were obtained at 40% available water. This may be due to the increased water uptake by increasing available water percentage, which increases the relative water content and succulence grade of leaves. Sabre seedlings showed higher succulence grade of leaves at 60 and 40% available water levels than stock cultivar Zebda in the two seasons. Differences among stocks were significant at the previous two water levels at the first season. These results were in harmony with those obtained by Abdel-Razik and Abd-Raboh (2007), who found that, succulence grade in some mango cultivars was correlated with soil moisture level.

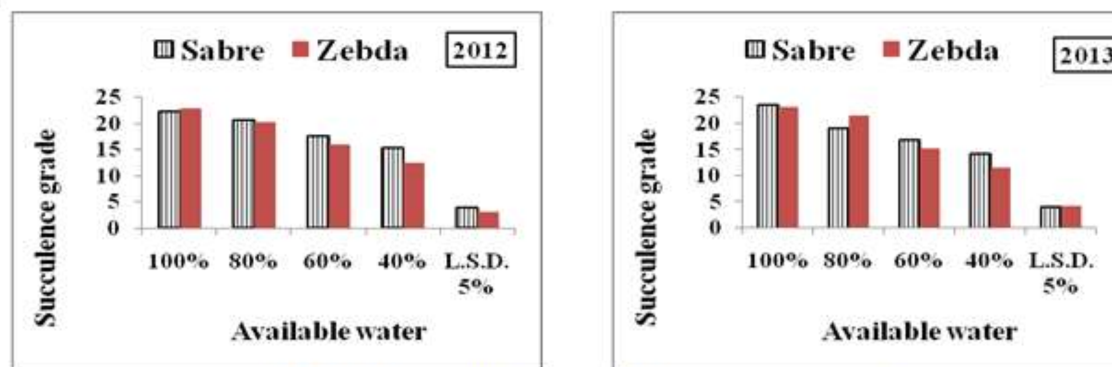

Fig. (2): Effect of different water deficit on leaf succulence grade.

2-2 Biochemical characteristics:

2-2-1 Osmotic potential as TSS % of leaf sap.

The results in fig (3) indicated that total soluble solids percentages of leaf filtrate in both cultivars had increased by decreasing the available water, so that the highest values were at 40% of available water, while the lowest values at 100% available water. It is worthy to mention that the increase in TSS % values in mango leaf filtrate might increase the resistance of leaves to drought. It was true for the two stocks in both seasons. Sabre seedings showed higher TSS % values of leaf cell.sap at all the levels of avaible water than Zebda rootstock, Hifny *et al* (2013) stated that values of leaf osmotic potential might follow the similar trend of TSS% during the active period of shoot growth.

The results were in harmony with those obtained by Mehanna *et al*. (2012) who found that leaf water potential of olive cvs. increased by increasing rate of water stress.

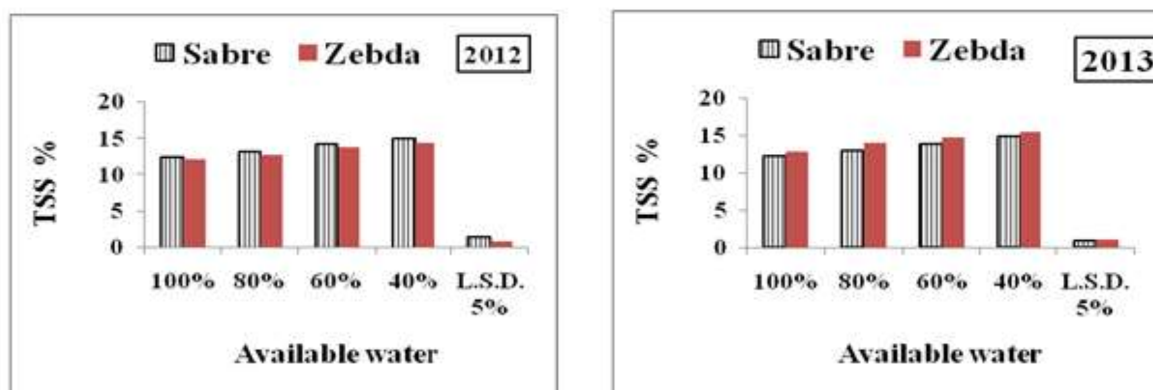

Fig. (3): Effect of different water deficit on TSS % in leaf sap.

2-2-2 Proline content% (f. wt.):

Data in fig.(4) indicated that, when water decreased, high value of proline was induced, such that the lowest value of proline was induced at 100% available water, while the highest value was induced at 40% available water. Differences among stock cultivars concerning proline content (%) in mango leaves were insignificant in the two seasons. Sabre seedlings produced the higher proline level in their leaves at all levels of available water in the two seasons. High proline content indicates that the seedling leaves suffer from drought more than that of low proline content. In other words, production of high proline content in the leaves indicates that such plant is less tolerant to drought than that of low proline content. It could be concluded that Sabre rootstock cultivar sensitive to drought than Zebda rootstock.

This results were in agreement with those obtained by Abdel-Razik and Abd-Raboh (2007), who found that mango plant produced a high values of proline as a result of high water stress.

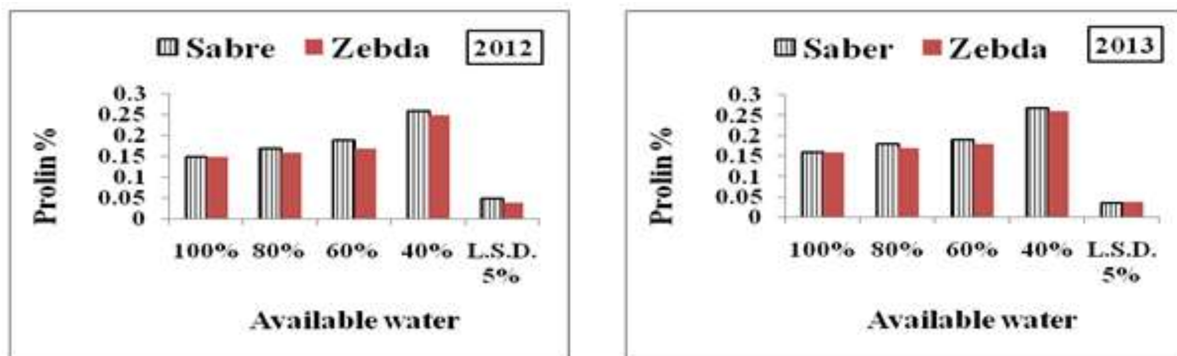

Fig. (4): Effect of different water deficit on proline content in leaf sap.

***2-2-3 Leaf nutrient (N,P,K) content:**

Nitrogen content% (d.wt.):

Data in Table(2) showed that the highest values of leaf nitrogen content was induced at 100% available water, while the lowest nitrogen value was induced at 40% available water. This was true for two mango stocks.

Sabre stock possessed higher values of leaf nitrogen content at the 80%,60% and 40% available water levels than that of Zebda. Differences between the two stock kind regarding N content were not significant. These results were in harmony with those obtained by Tahir *et al*. (2003), who found that leaf nitrogen content was decreased by decreasing the available water percentage. They added that, under soil water stress nitrogen solubility was decreased and the plant did not receive the necessary nitrogen amount.

Phosphorus content% (d.wt.):

As for phosphorus Table (2) indicate that the highest value of leaf phosphorus content under the effect of 100% available water and the lowest values were noticed at 40 % available water for the two studied stock cultivars.

Sabre stock leaves contained the higher values of leaf phosphorus than Zebda stock, but differences among the two studied stocks was insignificant at all availble water pecent. The results were in harmony with those obtained by luvaha,(2005),who stated that water deficit negatively affect on nutrient uptake and transport.

Potassium content% (d.wt.):

Table (2) showed that leaf potassium content in the two mango studied stocks were increased by increasing water availability. The highest values of potassium content were found at 100% available water followed descendingly by 80%,60% and 40% available water for the two studied stock cultivars. Sabre seedling leaves insignificantly showed higher potassium content in all water deficit treatments than those in Zebda seedling stock cultivar.

Potassium has long been associated with the water economy of plants. Its availability in soil is decreased by lack of soil moisture. A good supply of the plant with potassium ions might avoid the effect of drought. Thus lack of moisture in the soil increases the plant need for potassium (Walter, 1968).

The present results ascertain those obtained by Abdel-Razik and Abd-Rabboh (2007), who found that the increase in available water causes the increase in leaf potassium content.

Table (2) Effect of different water deficit on leaf nutrient content.

Variety	Treatment	N% (d.wt)		P% (d.wt)		K% (d.wt)	
		2012	2013	2012	2013	2012	2013
Sabre	100%	1.82	1.84	0.091	0.087	1.27	1.19
	80%	1.78	1.81	0.087	0.084	1.16	1.09
	60%	1.66	1.70	0.082	0.083	1.09	1.03
	40%	1.47	1.51	0.078	0.081	1.07	0.99
Zebda	100%	1.85	1.86	0.086	0.091	1.22	1.24
	80%	1.76	1.75	0.085	0.087	1.14	1.07
	60%	1.57	1.6	0.082	0.081	1.05	1.01
	40%	1.42	1.45	0.079	0.081	0.97	0.99
LSD 0.05							
Cultivar		0.125	0.115	0.007	0.009	0.072	0.077
Treatment		0.177	**0.163**	0.010	0.013	0.102	0.109
C X T		**0.250**	**0**.231	4.89	1.84	0.144	0.154

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Effect of Some Growth Regulators on Yield and Fruit Quality of Manzanillo Olive Trees

Abdrabboh, G.A

Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Nasr city, Cairo, Egypt.
Gabdrabboh65@yahoo.com

Abstract: The present work is an attempt to elucidate the effect of some growth regulators on fruit drop, yield and quality of olive cv. Manzanillo. Thirteen years old olive trees were foliar sprayed with both GA_3 and NAA individually and additively at 50 and 75 ppm. Maximum fruit drop % was recorded by using NAA at 75 ppm. Spraying trees with 75 ppm of GA_3 and NAA at either 50 or 75 ppm decreased fruit drop% in comparison to those sprayed with NAA only. Spraying trees with GA_3 at 75 ppm caused maximum fruit yield/ tree in comparison to those of other treatments including control. Maximum fruit weight, volume, length, diameter as well as fruit shape index were obtained when trees were treated with GA_3 at 75 ppm. GA_3 and NAA either individually or additively increased TSS % as well as TSS/Acid ratio of fruit juice and decreased total acidity than control. Maximum oil content (% of dr. wt.) was recorded when trees were sprayed with 75 ppm of GA_3 in comparison with other treatments including control. Accordingly, it is preferable to spray Manzanillo olive trees with GA_3 and NAA individually or additively 10 days after fruit set to improve tree yield and fruit quality.

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Key Words: GA_3, NAA, Manzanillo olive, Fruit drop, yield, Fruit quality

1.Introduction

Olive tree (*Olea europaea,* L.) is an evergreen tree belongs to Oleaceae family has a high economic value to Egypt and to many countries in Mediterranean sea region since they use it for pickling, oil extraction or for both purposes (**Payvandi *et al.,* 2001**). Olive is successfully cultivated in the irrigated semi arid areas in Egypt. Recently, olive growers complained the low productivity of olive especially in the new reclaimed areas such as Sinai region, on both sides of the desert roads and also in the northwestern coast. The low fruit productivity of the olive trees may be due to that olive has a very marked alternate bearing phenomenon in all production regions through the world. Also, fruit quality of Manzanillo olive cultivar decreased linearly with an increase in yield (**Krueger *et al.,* 2004**).

Major losses in profit are occurred in "On" season since the fruit being too small for table olive usage **(Lavee, 2006)**, while in an "Off" season, the increase in fruit size dose not make up for the loss in yield. Spraying olive trees with GA_3 increased the annual vegetative growth of long and short fruit bearing branches thus it increased fruit yield of the following year and appeared to stabilize fruit production over two years period and reduce the degree of alternate bearing compared with control (**Boulouha *et al.,* 1993**). **Rotundo and Gioffre (1984)** reported that GA_3 treatments increased the weight, length, width of olive fruits and flesh weight in comparison to control. Also, fruit thinning using thinning materials such as NAA at different concentrations improved fruit quality and reduced alternate bearing in various crops **(Link, 2000).** Fruit thinning approximately two weeks after full bloom (FB) increased vegetative growth, flower bud differentiation, fruit size and cumulative yield of consecutive years in various table and oil olive cultivars **(Dag *et al.,* 2009)**. Application of plant growth regulators such as Gibberellic acid and Naphthalene acetic acid individually or in combinations on olive trees may improve cropping potential and fruit quality. Therefore, this study aimed to explore the effect of application GA_3 and NAA on controlling fruit drop and improving yield and fruit quality of Manzanillo olive trees under semi arid conditions in Egypt.

2. Materials and Methods

Olive tree (*Olea europaea,* L. CV. Manzanillo) trees of similar vigor, age (13 years old) and size were selected for foliar spray treatments during the two successive seasons of 2010 and 2011 at a private farm in Wadi El-Faregh, Behira Governorate, Egypt. Trees were grown in sandy soil, 5x6 m apart and irrigated through drip irrigation system. The orchard received normal cultural practices according to the farm plan.

1. The treatments:

GA_3 and NAA were applied individually or in combinations, 10 days after fruit set as foliar spray on the trees as follows:

1.1 T1= Control
1.2 T2= GA_3 at 50 ppm
1.3 T3 = GA_3 at 75 ppm

1.4 T4 = GA_3 at 75 ppm+NAA at 50 ppm
1.5 T5 = GA_3 at 75 ppm+NAA at 75 ppm
1.6 T6 = NAA at 75 ppm.

Each treatment had five replicates with one tree per a replicate. A complete randomized block design was adopted in this experiment. Each tree was received 10 L of the applied solution plus 5cm per liter of tween 20 to avoid the surface tension. The trees of control treatment were sprayed with tap water.

2.The measurements:

2.1. Fruit drop percentage:

Four main branches in each replicate were tagged and the number of fruits per 1m length of fruiting shoots was recorded twice, 10 days after fruit set and at harvest date. Consequently, the fruit drop % was recorded according to the following equation:

Fruit drop% =

$$\frac{\text{No. of fruits at fruit set - No of retained fruits at harvest}}{\text{No. of fruits at fruit set}} \times 100$$

2.2. The yield: At harvesting time (last week of October) of each season, yield of each tree was recorded in Kg/tree **(Sibbett *et al.*, 1986)**. A random sample of 30 fruits was collected from each replicate at harvest to determine the following characteristics:

2.3. Fruit physical characteristics: Fruit weight (g), Fruit volume (cm^3), Fruit length (cm), Fruit diameter (cm), and Fruit shape index (L/D ratio) were recorded.

2.4. Fruit chemical characteristics: Fruit samples were taken (30 fruits per a replicate at harvest to determine the chemical characteristics such as TSS %, Total acidity % and Fruit oil content (% dry weight).

Oil determination: Oil extraction and determination (% dr.wt) was determined according to **A.O.A.C (2000).**

Statistical analysis: The obtained data were subjected to analysis of variance (ANOVA) according to **Snedecor and Cochran (1968)** using Mstat program. Least significant differences (L.S.D) were used to compare between means of treatments according to **Waller and Duncan (1969)** at probability of 5%.

3. Results and Discussion

3. Effect of growth regulators on Manzanillo olive trees:

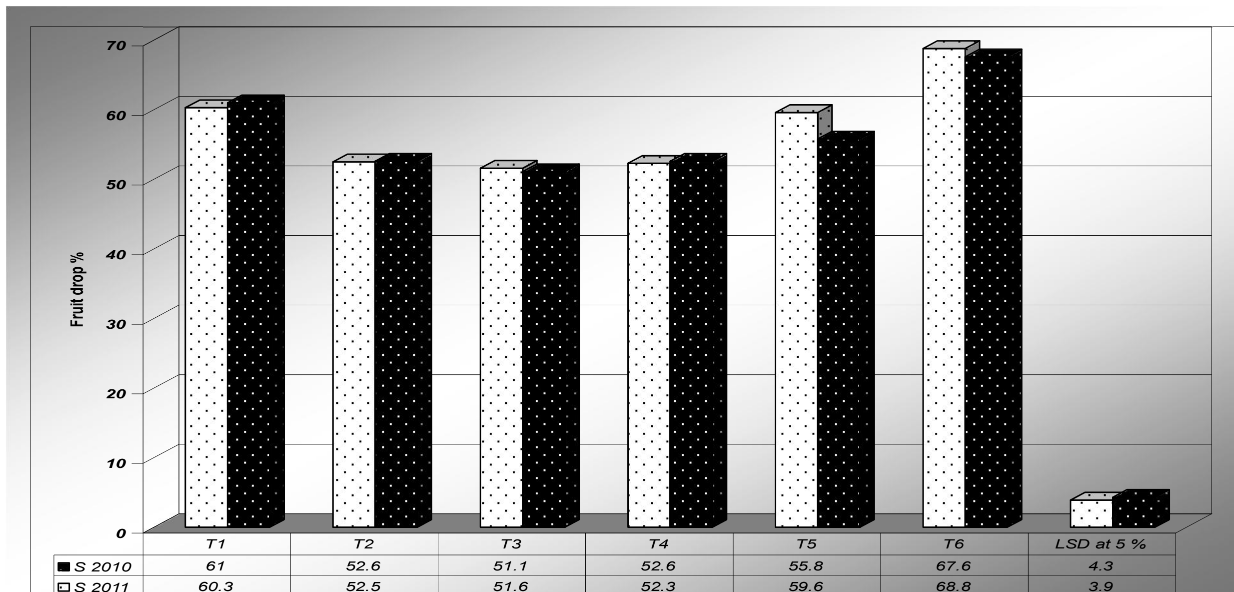
3.1. Fruit drop percentage:

Fig.(1) indicated that spraying Manzanillo olive trees with GA_3 generally reduced fruit drop % in the two seasons in comparison with other treatments including control. The results showed that fruit drop percentage was reduced by increasing GA_3 concentration since the fruits attained the lowest drop percentage when the trees treated with GA_3 at 75 ppm as foliar spray. The present result is in agreement with that obtained by **Daood (2002)** who cleared that spraying Picual olive trees with 25, 50 or 100 ppm of GA_3, 10 days after fruit set significantly increased the retained fruit percentage and reduced the total fruit drop percentage in comparison with the control. The results also are in agreement with those of **Abdrabboh (2009)** who cleared that fruit drop % of Picual olive trees was decreased by spraying the trees with GA_3 at 30 or 60 ppm in comparison to that of control. Gibberellins are still used commercially on various fruit tree species to reduce fruit drop% especially in fruit species which their harvest agree to be during May and June **(Crous, 2012)**. The results in Fig.(1) also indicated that spraying Manzanillo olive trees with NAA either at 50 0r 75 ppm, 10 days after fruit set showed a significant increase in fruit drop percentage in comparison with other treatments and control. The maximum value of drop percentage was achieved at 75 ppm of NAA. The last result is in harmony with those of **Crous, (2012)** working on Manzanillo olive trees who found that fruit drop percentage of olive was increased by increasing NAA concentration. Therefore, NAA is applied for fruit thinning in many fruit species especially olive trees during the "On" year to regulate annual bearing of trees and to improve fruit quality (**Lavee, 2006 and Dag *et al.*, 2009**). The results in Fig. (1) Indicated also that the additive treatment combined GA_3 at 75 ppm plus NAA either at 50 or 75 ppm decreased drop percentage of Manzanillo cultivar when compared with individually applied NAA. However, the additive treatment which contained NAA at 50 ppm slightly decreased the fruit drop percentage compared with that which contained NAA at 75 ppm only in the first season. **El-Shewy (1999)** supported the present results since he reported that adding GA_3 at 75 mg / L plus 50 mg/L of NAA reduced fruit shedding of Guava either during June or at pre-harvest drop. It can be concluded that spraying olive trees with GA_3 at 50 or 75 ppm caused a significant decrease in fruit drop percentage in comparison to control. On the other hand, spraying trees with NAA 50 or 75 ppm either additively or in combinations with GA_3, increased fruit drop percentage in the two seasons in comparison with other treatments including control.

3.2. Effect of growth regulators on the yield (kg/tree):

Results in Fig. (2) showed that yield of Manzanillo olive trees significantly increased by increasing GA_3 concentration from 50 ppm to be 75 ppm. The increase in yield was significant in comparison with that of control. The maximum increase in yield per tree was obtained by spraying

GA_3 solution at 75 ppm. The results are in agreement with that obtained by **Ramezani and Shekafandeh (2009)** working on Shengeh olive cultivar who found that spraying olive trees with GA_3 at different concentrations 10 days after fruit set significantly increased yield of trees. They also reported that this increase in yield may be attributed to the ability of GA_3 in reducing fruit drop and consequently increasing fruit yield / tree. **Ramezani and Shekafandeh (2009)** also reported that increasing yield of olive due to GA_3 sprays may be attributed to its effect on increasing levels of IAA. The results in Fig.(2) elucidated also that spraying the trees of Manzanillo olive cultivar with75 ppm of NAA individually exhibited insignificantly decrease in the yield (kg/ tree) in the two seasons in comparison with control. These results may be acceptable with regard to the thinning effect of NAA especially at high concentration. The results are in harmony with that obtained by **Crous (2012)** working on Manzanillo olive trees as he reported that spraying trees with NAA at high concentrations decreased tree yield in comparison to control due to its thinning effect on trees. Data of the present results also showed that treating Manzanillo olive trees with a combination of GA_3 at 75 ppm and NAA at either 50 or 75 ppm caused slight increase in fruit yield / tree in comparison with NAA alone in first season while insignificant increase was noticed in the second one. Slight insignificant difference was recorded between NAA at 50 ppm and 75 ppm combined with 75 ppm of GA_3.These results are in a harmony with those of **El-Shewy (1999)** who reported that spraying guava trees with combination of NAA and GA_3 led to an increase in fruit yield/ tree in comparison with control. Spraying the Manzanillo olive trees with 75 ppm NAA individually results in significant decreases of olive yield/ tree.



	T1	T2	T3	T4	T5	T6	LSD at 5 %
S 2010	61	52.6	51.1	52.6	55.8	67.6	4.3
S 2011	60.3	52.5	51.6	52.3	59.6	68.8	3.9

Fig.(1): Effect of GA_3 and NAA (ppm) on fruit drop % of Manzanillo olive trees in 2010 and 2011 seasons.

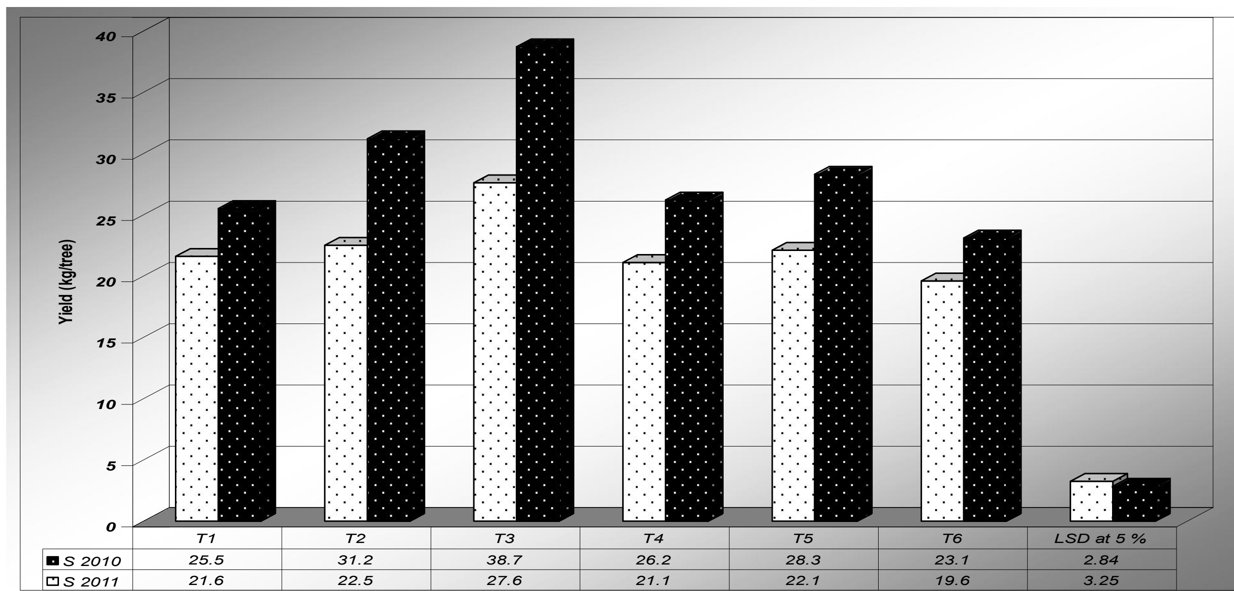


Fig.(2): Effect of GA_3 and NAA (ppm) on yield (kg/tree) of Manzanillo olive trees in 2010 and 2011 seasons.

3.3.Effect of growth regulators on fruit quality

3.3.1.Fruit physical properties:

Fruit weight, volume, length and diameter.

Figs. (3, 4, 5, 6 and 7) clearly showed that spraying Manzanillo olive trees with GA_3,10 days after fruit set at 50 or 75 ppm significantly increased fruit weight, fruit volume, fruit length and fruit diameter in comparison to those of control treatment. The results also cleared that GA_3 at the two tested concentrations gave insignificant increase in fruit shape index (length/ diameter ratio) and produced nearly round fruit due to higher growth rate of diameter than that of the control treatment especially in the second season. The results are in agreement with that of (**Abdrabboh, 2009**) working on Picual olive cultivar and with those of **(Ramezani and Shekafandeh, 2009) working** on Shengeh olive cultivar who reported that spraying olive trees with GA_3 ranged from 25 to 100 ppm increased the physical fruit parameters than control. The present results may be attributed to stimulative influence of this bioregulator on cell extension and/or cell division. This result had been also supported by **Eman *et al.* (2007)** who reported that the role of GA_3 in improving fruit quality such as fruit weight and fruit size may be due to its role in increasing cell elongation**.** Fruit size increase in response to exogenously applied GA_3 according to the view of others can be associated with an increase an increase in cell size of the mesocarp **(Zhang *et al.,* 2007).** The role of GA_3 in increasing the fruit mesocarp could be interpreted through its action on sink demand by enhancement of phloem unloading or/and the metabolism of carbon assimilates in fruit **(Ramezani and Shekafandeh, 2009)**. The results in Figs. (3, 4, 5, 6 and 7) also cleared that spraying Manzanillo olive trees 10 days after fruit set with NAA either individually or in combination with GA_3 slightly increased fruit weight, fruit volume, fruit length and fruit diameter than those of control treatment. These results are in harmony with that obtained by **Lavee (2006) and Crous (2012)** who reported that NAA positively influenced fruit quality, i. e., fruit size, flesh/pit ratio and oil content and improved return bloom. As a matter of fact, NAA induces thinning out of the fruits such that the retained fruits at harvest should be little in number. The share of each fruit as a sink from the assimilates should be greater than control and the fruits becomes bigger.

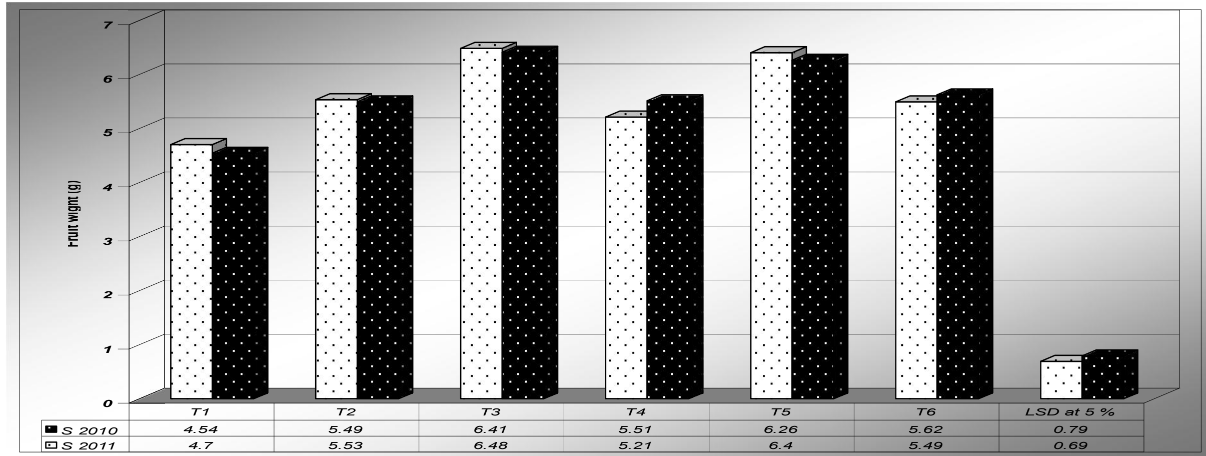


Fig.(3): Effect of GA_3 and NAA (ppm) on fruit weight (g) of Manzanillo olive trees in 2010 and 2011 seasons.

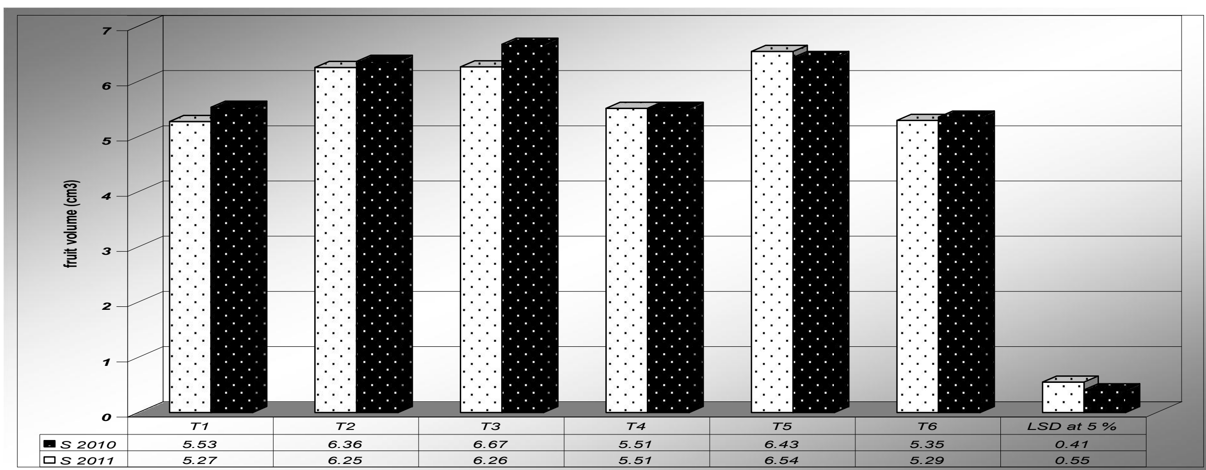


Fig.(4): Effect of GA_3 and NAA (ppm) on fruit volume (cm3) of Manzanillo olive trees in 2010 and 2011 seasons.

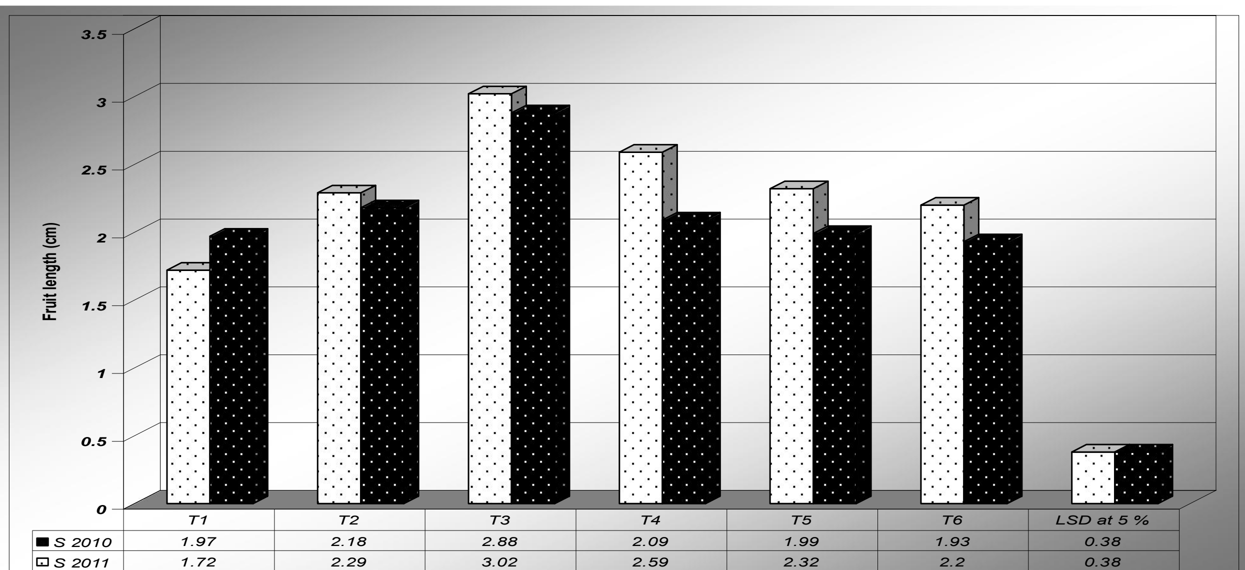


Fig.(5): Effect of GA_3 and NAA (ppm) on fruit length (cm) of Manzanillo olive trees in 2010 and 2011 seasons.

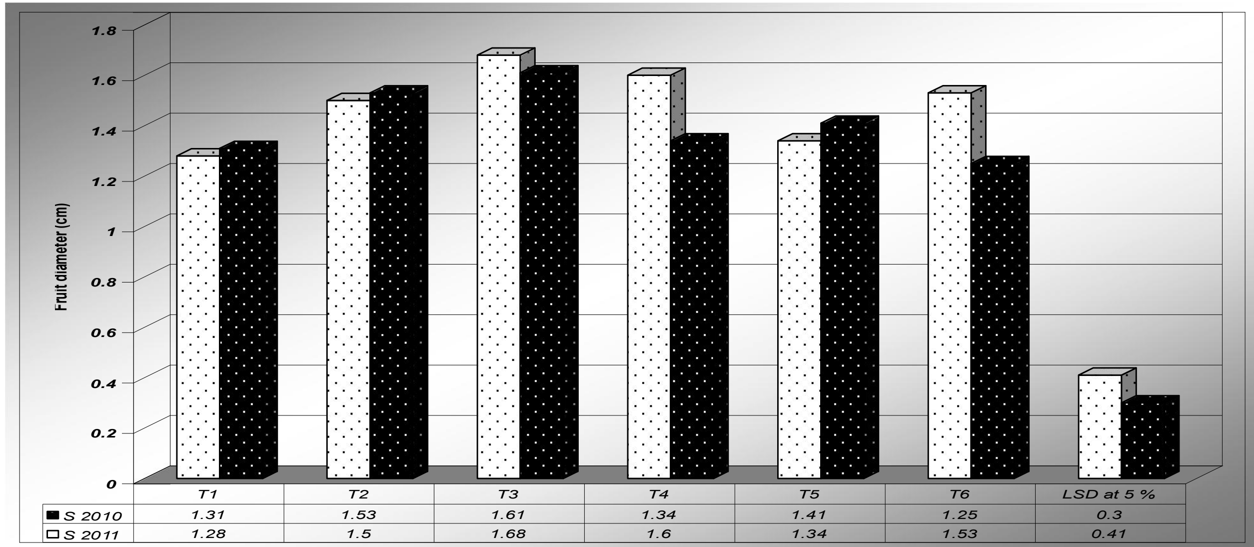


Fig.(6): Effect of GA_3 and NAA (ppm) on fruit diameter (cm) of Manzanillo olive trees in 2010 and 2011 seasons

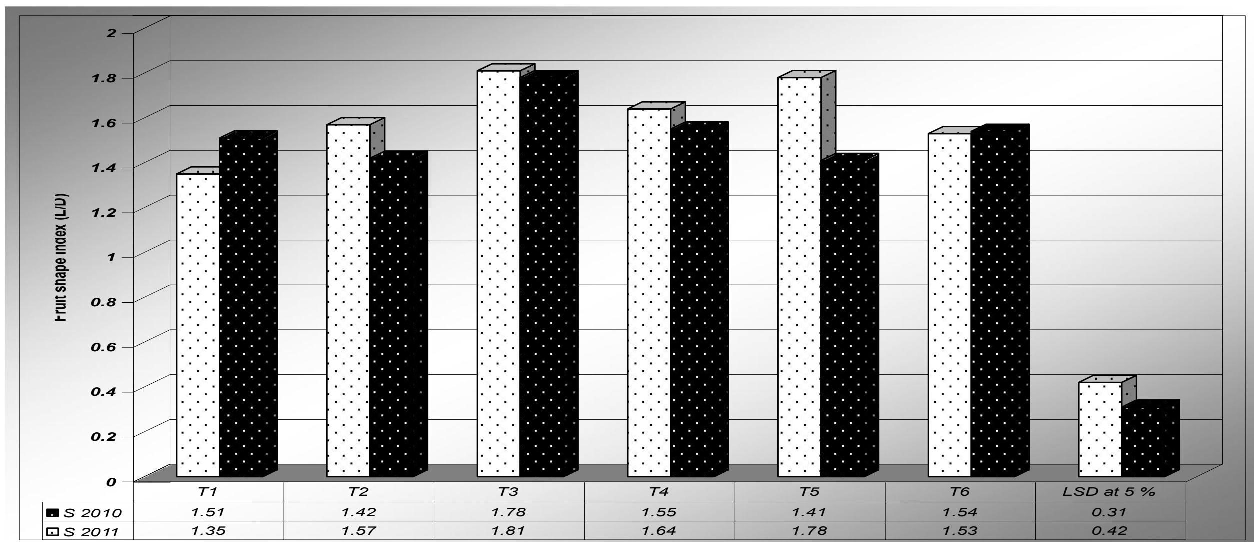


Fig.(7): Effect of GA_3 and NAA (ppm) on fruit shape index (L/D) of Manzanillo olive trees in 2010 and 2011 seasons.

3.3.2. Fruit chemical properties:

TSS %:

Data in Fig.(8) showed that TSS percentage of Manzanillo olive cultivar significantly increased by increasing GA_3 in the two seasons in comparison with control treatment. Maximum value of TSS percentage was obtained by spraying the olive tree with GA_3 at 75 ppm. The data in Fig. (8) also cleared that using NAA individually at 75 ppm led to a significant increase in TSS compared with control. The additive treatment with GA_3 at 75 ppm plus NAA either at 50 or 75 ppm resulted a significant increase in TSS% in comparison with control. Insignificant difference in TSS % was noticed between these additive treatments and GA_3 treatments. These results are in agreement with those of **Abdrabboh, (2009**) working on olive who reported that TSS% of olive fruits was increased by spraying the trees with some growth regulators. **Hifny *et al.,(*2009)** reported that the increase in TSS% of fruit at harvest might be attributed to the intensive photosynthesis in trees previously treated with growth regulators.

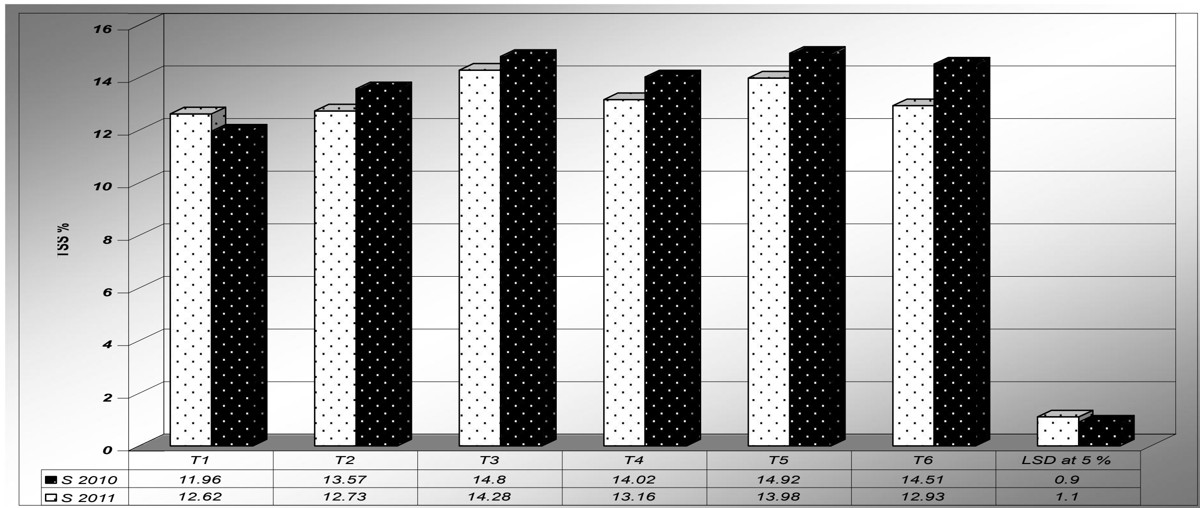


Fig.(8): Effect of GA_3 and NAA (ppm) on fruit TSS % of Manzanillo olive trees in 2010 and 2011 seasons.

Total acidity%:

Regarding the total acidity of fruit juice, data in Fig. (9) showed an opposite trend of that of TSS percentage in all treatments when compared with that of control in the two seasons. Spraying Manzanillo olive trees 10 days after fruit set with GA_3 and/or NAA either individually or in combinations at all tested concentrations resulted a decrease in total acidity% in comparison with control. In this regard, the combination of NAA at 50 ppm supplemented with 75 ppm of GA_3 treatment recorded the least total acidity % in olive fruits in comparison with control and other treatments. The significant decrease in total fruit acidity could be attributed to the promotion occurred in fruit maturity, whereas the fruit ripened earlier than those of control trees. (**Hifny *et al.*, 2009**).

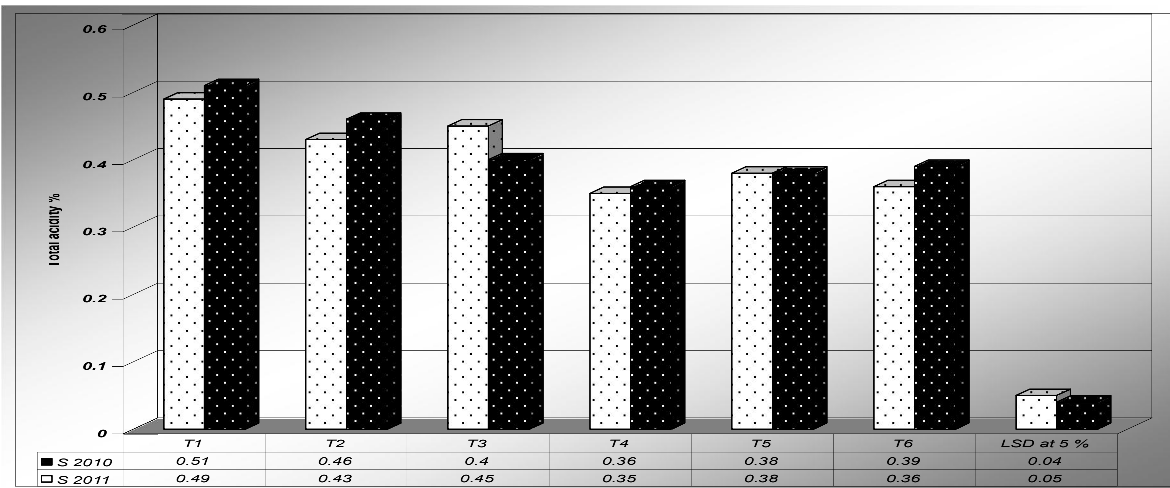


Fig.(9): Effect of GA_3 and NAA (ppm) on fruit total acidity % of Manzanillo olive trees in 2010 and 2011 seasons.

TSS/ acid ratio:

Data in Fig. (10) showed that TSS/ acid ratio significantly increased by increasing GA_3 concentrations in the two seasons in comparison with control treatment. Maximum values of TSS/Acid ratio were achieved after spraying the olive trees with 75 ppm of GA_3. Regarding NAA, data in Fig. (10) also cleared that spraing olive trees with NAA at 75 ppm caused a significant increase in TSS/Acid ratio compared with control. The data also indicated that adding GA_3 at 75 ppm to NAA either at 50 or 75 ppm led to a significant increase in TSS/Acid ratio in comparison with control. Insignificant difference in TSS/Acid ratio was noticed between these treatments and GA_3 treatments. These results are in agreement with that of **Brahmachari *et al (*1996**) on Guava and with **Hifny *et al.*, (2009**) on olive who reported that TSS/ Acid ratio of fruits were increased while fruit total acidity were decreased by spraying the trees with some growth regulators.

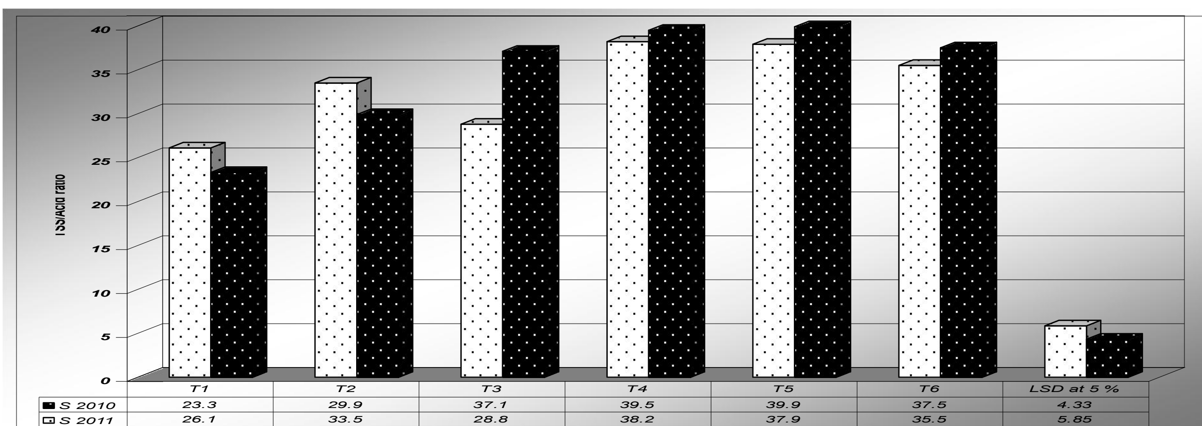


Fig.(10): Effect of GA_3 and NAA (ppm) on fruit TSS/Acid rati of Manzanillo olive trees in 2010 and 2011 seasons.

Effect of growth regulators on oil content (% of dr. wt):

The results in Fig. (11) also showed similar trend to that of TSS percentage of fruit juice in the two seasons. The data showed that oil content of Manzanillo olive trees (% of dr. wt) increased by increasing GA_3. GA_3 at concentration 75 ppm had increased the fruit oil content more than those were produced under the effect of 50 ppm ppm GA_3 or those under control in both seasons. The results are in agreement with that obtained by **Abd El-Naby *et al.* (2012)** working on olive trees, who reported that GA_3 greatly increased fruit oil percentage compared with control. Data presented in Fig. (11) also cleared that oil content of Manzanillo olive trees significantly increased by spraying the trees with NAA either alone or in combination with 75 ppm of GA_3 compared with control. Similar results were obtained regarding NAA where **Eris and Barut (1993)** working on olive trees reported that NAA treatments greatly increased fruit oil %. The improvement in fruit quality could be attributed to the effect of NAA as a thinner whereas it decrease the number of retained fruits, so decrease the degree of competition between fruits in obtaining more photosynthesis product. **Martin *et al.* (1980) and Lavee (2006)** reported that NAA positively influenced fruit quality i.e., fruit size, flesh/pit ratio and oil content.

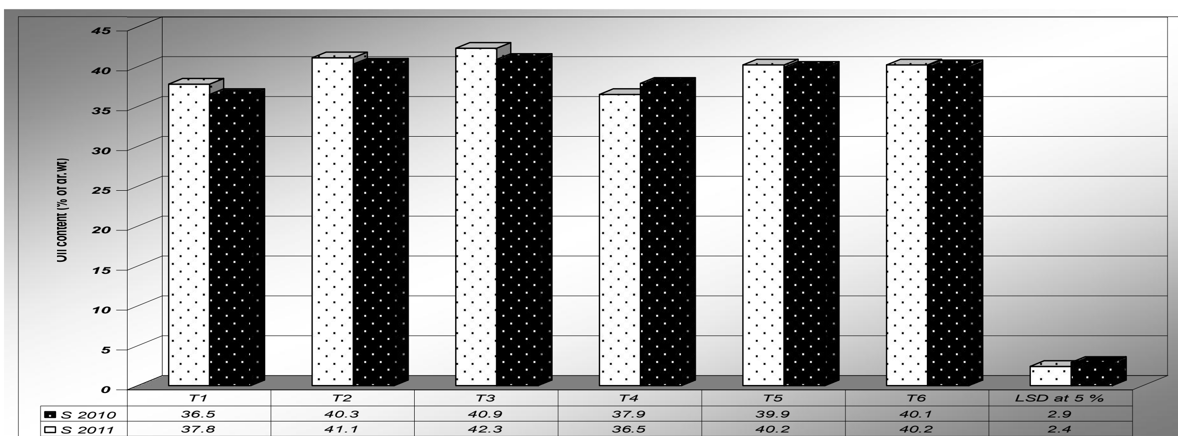


Fig.(11): Effect of GA_3 and NAA (ppm) on fruit oil content(% of dr.wt.) of Manzanillo olive trees in 2010 and 2011 seasons.

Corresponding author: Dr. Gamal Abdrabboh, Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Nasr city, Cairo, Egypt.
E-mail: Gabdrabboh65@yahoo.com

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