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Effect of Broccoli on the Antioxidant Activity of Experimental Rats Ingested Thermally Oxidized Oil

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Abstract: The effect of broccoli powder, aqueous and methanolic extracts on antioxidant activity in rats fed on thermally oxidized oil was studied. Chemical analysis of broccoli was investigated. Five groups of rats were used; group (1) was used as a negative control, while rats of the other groups were fed thermally oxidized oil in diet. Group (2) was left as a positive control, while groups (3), (4) and (5) were administered broccoli powder, aqueous and methanolic extracts for 60 days. The obtained results revealed that broccoli contains carbohydrate, protein, fiber and ash, and also high amount of potassium, calcium, phosphorous, total phenol and flavonoids. Positive control group which consumed oxidizing frying oil in diet showed a significant decrease in body weight, Food efficiency ratio (FER) and food intake. Also, it showed a significant increase in serum cholesterol (CHO), triglycerides (TG), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and CHO/ HDLc, malodialdehyde (MDA); and liver cholesterol and total lipid but a significant decrease in serum high density lipoprotein cholesterol (HDLc), superoxide dismutase (SOD), catalase, glutathione transferase (GST), and glutathione peroxidase (GPX); and liver triglyceride in comparing to negative control group. Broccoli powder, aqueous and methanolic extract rat groups showed that the values of liver cholesterol, total lipid and triglyceride appeared within values of negative control. However, they showed a significant decrease in liver parameters and serum MDA and significant increase in serum antioxidant enzymes activity comparing to positive control group
This study concluded that broccoli have ability to increase antioxidant activity in rats ingested thermally oxidized oil. Broccoli methanolic extract give the most favorable results, therefore this study recommends to intake broccoli when consuming fried foods.
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Keywords: Broccoli; Oxidized thermally oil; Biochemistry; Rat.

1. Introduction

Oils and fats are important part of the human diet as food or as ingredients in food products which affects both growth and health. Vegetable oils are the main source of dietary fat for almost people and play important functional and sensory roles in food products and they act as carriers of fat-soluble vitamins (A, D, E and K). Degree of saturation of oil is an important factor determining the quality of cooking oils. Unsaturated fatty acids are more susceptible to lipid oxidation than saturated fatty acids and for this reason they are main source of free radicals **(Bakkali et al., 2008)**.

Frying is one of the most popular culinary processes worldwide, for both industrial and domestic food preparation procedures. During deep frying, the cooking oil is heated at high temperature with exposure to air and moisture, resulting in lipid peroxidation. This thermal deterioration generates harmful oxygen reactive species which might be deleterious to the cardiovascular system. Several studies have also demonstrated the detrimental effects of oxidized oil, including alterations in platelet function, liver dysfunction, and endothelial impairment **(Owu et al., 1989, Naz et al., 2005 and Driss et al., 2009)**. However, the tocopherols (vitamin E) are largely destroyed during frying as well. They are usually completely destroyed before the point at which the frying oil should be replaced based on the content of polymerised triacylglycerols or polar compounds **(Reblova *et al.*, 2009)**.

Evidence suggests that fruit and vegetables are the major antioxidant sources in our daily diet. Major antioxidants present in fruit and vegetables are: vitamin C, vitamin E, carotenoids and polyphenols, especially flavonoids, which all provide protection against free radicals that minimizes some of these harmful effects **(Abd El-Ghany et al., 2009 and Monero *et al.*, 2010)**. Broccoli (*Brassica oleracea)* belongs to the *Brassica* genus and is a green vegetable from the cabbage family. It is generally sold in heads, which have multiple florets branching off a central stem, and sometimes have leaves still attached. Packed with nutrients, it is best briefly steamed, stir-fried, or eaten raw. A high intake of cruciferous vegetables is associated with a reduced risk of cancer, particularly lung and those of the gastrointestinal tract **(Davies, 2000 and Piao et al., 2005)**. Broccoli is rich in vitamin C, as well as dietary fiber and also contains multiple nutrients with potential anti cancer properties such as di-indolylmethane. It contains many bioactive, including

vitamins C and E, quercetinn and kaempferol glycosides. Broccoli consumption has been also shown to be beneficial in the prevention of heart disease **(Elizabeth and Marcela 2009)**.

Therefore, the main aim of the present study was to investigate the effects of broccoli on the antioxidant activity of experimental rats ingested thermally oxidized oil for a period of 60 days.

2. Materials and methods

2.1. Plant material:

Broccoli, one kilogram of sliced sweet potatoes, and commercial food oil for ideal frying performance (Mazola) were obtained from local market in Riyadh.

2.2. Chemicals:

All the materials used for this experiment were of analytical grade. BioMeriuex Kits were purchased from Alkan Co. for Chemicals and Biodignostics.

2.3. Test animals:

35 adult male of white albino rats (Sprague dawley strain) weighing between 150-157g, provided from experimental animals' center in Medicine collage of King Saudi University in Riyadh. Rats were housed as groups in wire cages under the normal laboratory conditions and fed on basal diet for a week as adaption period. Food and water were provided *ad-libitum*. Weekly body weight gain and daily food intake were recorded.

2.4. The basal diet:

The basal experimental diet was composed of corn starch(598),casein(200),corn oil(100), vitamins mixture(10),salts mixture (40),cellulose (50) and choline chloride (2) in g/ kg diet according to **Second Report of American Institute Of Nutrition(1980)**.

2.5. Preparation of thermally oxidized frying oil:

1 kg sliced sweet potatoes was frying in 3 L of oil in a stain-less-steel wok at 200°C for 20 min. The heated oil was then allowed to cool for at least five hours then oils were heated three hours per day for five days without any addition of new fresh oil to get oxidized frying oil as described earlier by **Adam et al., 2008** with some modification. Oxidized frying oil was added to basal diet in substitution of corn oil to form oxidative diet.

2.6- Preparation of broccoli powder:

The broccoli was cut into small pieces, dried at 60°C in hot oven and crushed to a fine powder. Broccoli powder was added as 10% of basal diet.

2.7- Preparation of aquous and methanolic broccoli extract:

100 mL deionised water was added to100 grams of broccoli powdered and left at room temperature for 12 h to allow complete hydrolysis then centrifuged at 5000g for 15 min to and finally filtrated to obtain aquous broccoli extract according to the method of **Bertelli et al., 1998**. Methanolic broccoli extract was prepared by soaking 100g of broccoli powdered in 600ml of 80% methanol with constant stirring by a magnetic stirrer for 48 hr then filtered followed by removal of the solvent on the rotatory evaporator. The rat received aqueous and methanolic broccoli extract in 100 mg kg/body weight by stomach tube **(WHO 1983)**.

2.8-Chemical analysis of broccoli:

Moisture, protein, fat, fiber and ash contents of broccoli were determined according to **A.O.A.C. (2007)**. Carbohydrate content was calculated by difference as described by **Ceirwyn 1995**. Mineral contents (Ca, Fe, Mn, P&K) were determined as described by **Pearson 1993** using atomic absorption spectrophotometer. Total carotenoids and antioxidant activity were determined by the DPPH radical scavenging method of **Zhang and Hamauzu (2004)**. The phenolic and total flavonoid content of the obtained extracts was estimated by a colorimetric assay based on procedures by **Singleton and Rossi (1965) and Heimler et al., (2005)**, respectively.

2.9. Treatment schedule:

Animals were divided into five groups as follows:

Group (1): Served as a negative control group which fed on basal diet.

Group (2): Kept as positive control group which fed on oxidative diet only.

Group (3): Kept as broccoli powder group which fed on oxidative diet with 10% of broccoli.

Group (4): Kept as broccoli aquous extract group which fed on oxidative diet and broccoli aquous extract100mg/kg body weight orally by stomach tube.

Group (5): Kept as broccoli methanolic extract group which fed on oxidative diet and broccoli methanolic extract100mg/kg body weight orally by stomach tube.

The food intake was recorded daily and body weight of the rats recorded weekly. After completion of experimental period (60 days), rats were fasted overnight and sacrificed and blood was collected for various estimations. After 24 h of last dose, rats were sacrificed for obtain blood and liver for biochemical estimations.

2.10. Serum biochemical estimations:

Serum cholesterol (CHO), triglycerides (TG) and high density lipoprotein cholesterol (HDL-$_C$) were determined by using enzymatic colorimetric methods **(Abell et al., 1952, Buccolo and David 1973, and Kostener 1977)**. Activity of superoxide dismutase(SOD), catalase, glutathione peroxidase (GPX),glutathione transferase (GST) enzymes and malodialdehyde (MDA) were determined using

commercial kits according to the methods described by **Kakkor et al.,(1984), Sinha (1972)**, **Habig et al., (1974)**, **Ellman (1958) and Uchiyama and Mihara (1978),** respectively.

2.11. Liver biochemical estimations:

Liver cholesterol (CHO), total lipids and triglyceride were determined according to **Richmond (1973), Folch et al., (1957) and Scheletter and Nussel (1975),** respectively.

2.12. Calculation of some parameters:

Food efficiency ratio (FER) was determined by **Chapman et al., (1950)**. Low density lipoprotein cholesterol (LDL-c)**,** very low density lipoprotein cholesterol (VLDL-c) and CHO/ HDL-c were calculated according to **Fruchart (1982) and Castelli and levitar (1977)**.

2.13-Statistical analysis:

Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to **Artimage and Berry (1987)**.

3. Results

Gross chemical composition of broccoli showed 41.35, 25.1, 12.77, and 10.11% of carbohydrate, protein, fiber and ash, respectively in ascending manner. Data showed that broccoli contains low level of fat as the value was 3.25 % as represented in table (1).

The obtained data in table (2) showed that broccoli contains high amount of potassium, calcium and phosphorous that the values reached to 501.16, 181.11 and 165.66 mg/100g, respectively. The values of iron and manganese reached to 4.12 and 1.61 mg/100g, respectively.

Estimation of total carotenoids, phenol, flavonoids and antioxidant activity of broccoli was illustrated in table (3). Broccoli had high value of total phenol and flavonoids as the value were 87.38 and 19.96 mg/100 g, respectively. Total carotenoids in Broccoli were 7.01 mg/100 g. The antioxidant activity of broccoli was 0.201 µm.

Positive control group which consumed oxidizing frying oil in diet showed a significant decrease in body weight, FER and food intake at $p<0.001$&0.05 as the values 45.61±1.11, 0.049±0.002 and15.41±1.10 in comparing to negative control group (91.41±4.61, 0.085±0.001 and 17.81±1.20). Broccoli powder and aqueous extract rat groups showed significant lower values of body weight and FER in comparing to negative control group ($p<0.01$&0.05) but significant higher in comparing to positive control. Broccoli methanolic extract rat group showed a significant lower value of FER in comparing to negative control group ($p<0.01$) but significant higher in comparing to positive control group. Food intake in broccoli powder, aqueous and methanolic extract rat groups was within the value of negative control as recorded in table (4).

Positive control group showed a significant increase in serum CHO, TG, LDLc, VLDLc and CHO/ HDLc and significant decrease in HDLc at $p<0.001$ in comparing to negative control group. Broccoli powder and aqueous extract rat groups showed a significant increase in serum CHO, TG, LDLc and VLDLc at $p< 0.5$ &0.01 in comparing to negative control group. Broccoli methanolic extract rat group showed only significant increase in value of LDLc ($p<0.01$) comparing to negative control group but the other serum lipid profiles were within normal. CHO/ HDLc was within normal value in broccoli aqueous and methanolic extract rat groups as shown in table (5).

Positive control group showed a significant increase in liver cholesterol, and total lipid ($p<0.001$) and significantly decrease in liver triglyceride ($p<0.05$) in comparing to negative control group. Broccoli powder, aqueous and methanolic extract rat groups showed non significant difference in the liver parameters and appear within values of negative control but showed significant decrease in comparing to positive control group as shown in table (6).

Positive control group showed a significant decrease in serum SOD, catalase, GST, and GPX and significant increase in MDA at $p<0.001$ in comparing to negative control group. Broccoli powder rat group showed a significant decrease in serum catalase and GST at $p<0.01$&0.05, respectively and significant increase in MDA at $p<0.05$ while aqueous and methanolic extract rat groups showed a significant decrease in serum catalase and GST at $p<0.05$ in comparing to negative control group. Broccoli powder, aqueous and methanolic extract rat groups showed a significant increase in serum SOD, catalase, GST, and GPX and significant decrease in MDA in comparing to positive control group as shown in table (7).

Table (1): Gross chemical composition of broccoli

Protein	Fat	Fiber	Moisture	Ash	Carbohydrate
25.21%	3.25%	12.77%	7.31%	10.11%	41.35%

Table (2): Some mineral content of broccoli (mg/100g)

Ca	Fe	Mn	P	K
181.11	4.12	1.61	165.66	501.16

Table (3): Total carotenoids, phenol, flavonoids and antioxidant activity of broccoli

Total carotenoids (mg/100g)	Total. phenol (mg/100g)	Total flavonoids (mg/100g)	Antioxidant activity (µm)
7.01	87.38	19.96	0.201

Table (4): Effect of oral administration of broccoli powder, aqueous and methanolic extract on body weight gain, food intake and FER of the experimental rat groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
Body weight(g)	91.41 ± 4.61^{a}	$45.61\pm1.11^{d***}$	$66.47\pm3.61^{c**}$	$83.60\pm3.71^{b*}$	85.11 ± 3.61^{ab}
Food intake(g/w)	17.81 ± 1.20^{a}	$15.41\pm1.10^{b*}$	16.21 ± 1.01^{a}	17.71 ± 1.27^{a}	18.11 ± 1.21^{a}
FER	0.085 ± 0.001^{a}	$0.049\pm0.002^{d***}$	$0.068\pm0.004^{c**}$	$0.078\pm0.005^{b**}$	$0.078\pm0.004^{b**}$

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (5): Effect of oral administration of Broccoli powder, aqueous and methanolic on serum lipid profiles of the experimental rat groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
CHO (mg/dl)	120.61 ± 12.61^{d}	$247.41\pm29.61^{a***}$	$175.31\pm15.61^{b**}$	$150.71\pm13.21^{bc*}$	145.61 ± 14.31^{cd}
TG (mg/dl)	75.61 ± 7.21^{c}	$129.65\pm12.14^{a***}$	$96.67\pm10.12^{b*}$	$91.21\pm8.14^{b*}$	85.11 ± 7.13^{bc}
HDLc (mg/dl)	39.81 ± 3.61^{a}	$25.11\pm3.21^{c***}$	31.16 ± 4.17^{ab}	35.21 ± 5.19^{a}	36.41 ± 6.01^{a}
LDLc (mg/dl)	65.68 ± 6.22^{d}	$196.37\pm19.21^{a***}$	$124.82\pm11.14^{b**}$	$97.26\pm8.99^{c**}$	$92.18\pm9.61^{c**}$
VLDLc (mg/dl)	15.12 ± 1.19^{c}	$25.93\pm2.17^{a***}$	$19.33\pm1.61^{b*}$	$18.24\pm1.51^{b*}$	17.02 ± 1.41^{bc}
CHO/ HDLc	3.02 ± 0.66^{c}	$9.85\pm1.67^{a***}$	$5.62\pm0.88^{b**}$	4.28 ± 0.57^{bc}	3.99 ± 0.53^{c}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (6): Effect of oral administration of broccoli powder, aqueous and methanolic on liver cholesterol, total lipid and triglyceride (mg/g) of the experimental rat groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
Cholesterol	4.22 ± 0.45^{b}	$6.17\pm1.31^{a***}$	4.99 ± 0.55^{b}	4.66 ± 0.46^{b}	4.51 ± 0.37^{b}
Total lipids	35.60 ± 3.66^{b}	$48.91\pm4.81^{a***}$	40.35 ± 4.21^{b}	37.16 ± 3.50^{b}	39.61 ± 3.14^{b}
Triglyceride	3.41 ± 0.22^{a}	$2.17\pm0.18^{c**}$	2.85 ± 0.21^{ab}	3.21 ± 0.34^{a}	3.30 ± 0.33^{a}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (7): Effect of oral administration of Broccoli powder, aqueous and methanolic serum SOD, catalase, GST, GPX, and MDA of the experimental groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
SOD (mmol/l)	40.21 ± 5.33^{a}	$15.41\pm1.77^{c***}$	33.21 ± 4.29^{ab}	36.17 ± 6.11^{a}	35.14 ± 5.10^{a}
Catalase (µ /l)	270.11 ± 27.41^{a}	$125.71\pm12.71^{d***}$	$199.51\pm19.61^{bc**}$	$210.14\pm21.61^{b*}$	$212.21\pm22.21^{b*}$
GST (mmol/l)	125.77 ± 13.13^{a}	$65.14\pm7.21^{c***}$	$97.11\pm9.67^{b*}$	$95.40\pm9.61^{b*}$	$99.61\pm10.21^{b*}$
GPX (mmol/l)	63.71 ± 7.20^{a}	$27.61\pm3.66^{b***}$	55.19 ± 6.60^{a}	59.61 ± 6.09^{a}	61.11 ± 7.01^{a}
MDA (mmol/l)	7.20 ± 1.31^{c}	$13.71\pm1.66^{a***}$	$9.14\pm1.33^{b*}$	8.21 ± 1.21^{bc}	8.11 ± 1.10^{bc}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant

4. Discussion

The obtained results of chemical analysis of broccoli were in accordance with that of other authors, who had shown that broccoli is high in fiber, which aids in digestion. A tablespoon of broccoli powder has as much protein as a cup of rice or corn with half the calories. It is a good source of energy-producing vitamin B1, vitamin B3, vitamin B5, protein, and iron; bone-healthy magnesium, phosphorus and calcium. Broccoli is rich in both nutritional antioxidants; vitamins C and E, and non-nutritional antioxidants; carotenoids, and phenolic compounds, particularly flavanoids **(Lin and Chang 2005 and Faller and Fialho 2009)**. According to several studies, reported that broccoli is also rich in polyphenols, a large group of phytochemicals that are often considered the most abundant antioxidants in the diet. There is a linear correlation between polyphenol content and antioxidant function **(Borowski et al., 2008)**.

Research has shown that the heating of the oil at high temperatures produces structural chemical, physical, changes which lead to compositional diversities as a result of degradation reactions that take place such as auto-oxidation, thermal polymerization, cyclisation and hydrolysis **(Warner 1999)**. During deep frying, oxidative and thermal effects result in the formation of many volatile and nonvolatile products, some of which are potentially toxic. Results of this study showed reduction in body weight gain, food intake and FER in positive control. These results were agreed with **Alexander (1981)** who reported that consumption of the thermally oxidized oils results in decreased food consumption, growth retardation, and weight gain in organs such as liver and kidneys, as well as mutagenicity and cellular damage in various organs of laboratory animals.

It is known that elevated levels of LDL cholesterol constitute a major risk factor for atherosclerotic diseases, whereas elevated levels of HDL have beneficial effects. Atherosclerosis is increased through intake of heated oil. However, heating of fats leads to decomposition of poly unsaturated fatty acids and therefore to changes in the fatty acid composition. The dietary fatty acids influence the fatty acid composition of the LDL and therefore the susceptibility of LDL to oxidation **(Cohn 2002 and Kratz et al., 2002)**. Plasma total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol with decrease in HDL-cholesterol content were found to be significantly increased in the heated oil fed groups compared to corresponding fresh oil fed groups. Since LDL particles have atherogenic properties, the presence of elevated levels of these particles may have role in hyperlipidemia and hemostatic changes associated with atherosclerosis. The oxidation of oil produces lipid peroxides, which may be one of the reasons for the elevation of total cholesterol and influence platelet alterations that play crucial role in cardiovascular events **(Siti et al.,2008 and Chinu and Thankappan 2011)**.The exposure to the high heat repeatedly for many times can lead to deterioration of the precursor of vitamin E and destroys all the vitamins and increase in free radicals formation.Thus can injury of renal and liver tissues. Broccoli is rich in dietary fiber which can lower levels of LDL, cholesterol. This lowering of cholesterol helps protect the arteries and prevents the onset of heart disease **(Totani and Ojiri 2007, Adam et al., 2008 and Bahadoran and Tohidi 2012)**. Broccoli contain Vitamin C which protects against cell death, directly scavenges superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radicals, and acts as a lipid peroxidation chain breaking agent Vitamin C also co-operates with vitamin E to regenerate membrane-bound oxidized α-tocopherol, creating an antioxidant network **(Gliszczynska-Swiglo *et al.*, 2006)**.

Data in this study showed an increased in serum MDA concentration which may be related to the increased in free radicals. This finding was consistent with the observation that the free radicals reduced the activity of the endogenous antioxidant enzyme SOD **(Conner and Grisham 1996)**.

Broccoli provides many antioxidants such flavonoids, carotenoids lutein, zeaxanthin, and beta-carotene in significant amounts. Other antioxidants provided by broccoli in beneficial amounts include vitamin E and the minerals as manganese and zinc. Proanthocyanidin compounds and flavonoids in broccoli extract can increase antioxidative defense that caused significant increases in the levels of GPX, SOD and catalase as well as improved liver structure **(Wargovich et al., 2001, Piao et al., 2005 and Podsedek 2007)**.

Conclusion

As a conclusion, the broccoli consumption could have a protective effect against thermally oxidized food oil. Broccoli extract showed the best results in improving lipid profiles in serum and liver and enhancement of serum antioxidant enzymes

Recommendation

Consumption of vegetables especially broccoli is recommended when eating fried food. Further research is needed to increase knowledge regarding the bioavailability of antioxidant compounds from broccoli, and to confirm effects of different cooking methods of broccoli on the concentration of the antioxidant.

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Submerged fermentation of the hybrid of *pleurotus tuberregium* and *pleurotus pulmonarius* in zobo and synthetic media

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ABSTRACT: This research work used the hybrid of ***Pleurotus tuberrigium*** and ***Pleurotus pulmonarius*** in Zobo and synthetic media, to investigate their ability to grow and produce mycelia mat during submerged fermentation. It was discovered that the ability of the hybrid of the two mushrooms in Zobo medium to form mycelia mat was lower compared to that of the synthetic medium; the mycelia mat weight in the synthetic medium and Zobo medium were 0.82g and 0.47g respectively. Moreover, the lag phase of the hybrid of the two mushrooms in the synthetic medium was shorter than that in the Zobo medium, this was shown by the turbidity of the medium on the second day while the Zobo medium became turbid on the third day. Changes in the colour of the Zobo medium showed that the hybrid of the two mushrooms has the ability of utilizing the medium for growth. There was a significant difference in the turbidity values of the Zobo medium compared to that of the synthetic medium, considering their means and t-test value of 5.58 and 7.80 for Zobo and synthetic medium respectively.
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Keyword: hybrid, Zobo medium, mycelia mat, turbidity values, *Pleurotus tuberrigium, Pleurotus pulmonarius*

INTRODUCTION

Mushrooms are highly nutritive, because they contain good quality protein, vitamins and minerals. They are low calorie food with very little fat and are highly suitable for obese persons. With no starch and very low sugars, they are the 'delight of diabetics' (Fasidi *et al.*, 1993).

There are many varieties of mushrooms, ***Pleurotus* sp.** are characterized by a white spore print, attached to gills, often with an eccentric strip or no strip at all. They are commonly known as Oyster mushrooms. ***Pleurotus tuberregium*** is a tropical and sub tropical sclerotial mushroom which has been gaining some interest in U.S. and it is common in southern Nigeria, ***P.tuberregium*** is used as both food and medicine. The sclerotium, which is hard is peeled and ground for use in vegetable soup. The sclerotium is expensive and considered a delicacy. ***P. tuberregium*** is used in some combination that are intended to cure headache, stomach ailment, cold and fever, as well as asthma, smallpox and high blood pressure (Zhang *et al*., 2001)

P. tuberregium seems to be adapted to a wide range of materials. This was proved by the work of Okhuoya and Okogbo (1991), which used slerotia to inoculate oil palm fruits fiber as spawning material; while Fasidi *et al*., (1994) used banana leaves, corn cobs, cotton wastes and rice straw for sclerotia production.

Pleurotus pulmonarius is a virtually indistinguishable from ***P. ostreatus***, and differs largely in it habitat preference for conifer woods. ***P. pulmonarius*** and ***P. ostreatus*** grow on variety of hardwoods with ***P. pulmonarius*** primarily a spring mushroom and ***P. ostreatus*** grow most prevalently in the summer fall. ***P. pulmonrius*** (Phoenix Oyster) is an aggressive mushroom that fruits easily on a wide range of substrates (Isikhuemhen *et al.,* 2000).

Much research work has been carried out on ***Pleurotus* sp** cultivation (Royse, 2002), biomass production using solid and liquid-state fermentation, medicinal properties; but so far, none about ability of the hybrid of ***Pleurotus tuberrigium*** and ***Pleurotus* pulmonarius** to grow in Zobo is known. Studies on mushroom cultivation have been focused on optimization of alternative substrate. It has been shown that a wide range of agricultural products, weeds and wastes can be used to produce food, feed, enzyme and medicinal compounds and in the degradation and detoxification of wastes (Gregori *et al.,* 2007).

Submerged fermentation with this genus of fungi can be more uniform and easy to reproduce, this is interesting for obtaining products, though this potential is yet to be recognized by Western biotechnology companies (Smith *et al.,* 2002). Sánchez *et al*. (2006) performed solid state fermentation with ***Pleurotus* sp.** on grapevine sawdust supplemented with synthetic medium and peroxidase activity was detected in all strains evaluated with the highest being ***P. ostreatus*** and ***P. pulmonarius.*** Márquez-Rocha *et al.* (1999); reported

on ***P. ostreatus***' growth in submerge liquid fermentation in a stirred tank bioreactor and showed that by varying impeller geometry and speed, aeration intensity, the growth rate and pellet size change. Also the lag phase of ***P. tubberegium*** growth in submerged liquid fermentation was shorter with glucose and fructose than with maize starch (Wu *et al.*, 2003).

Sanchez *et al.* (2002) showed the recycling of viticulture waste in solid state fermentation with ***P. ostreatus and P.pulmonarius*** yielding a high-fiber feed for limited use in ruminants. It has been shown that wheat straw supplemented with ***Lolium perenne*** grass chaff stimulated fruitification and mushroom yield of ***Pleurotus pulmonarius*** (Domondon *et al.,* 2004).

Rupak *et al.* (2005), showed that there was an increase in the biomass production of ***Pleurotus sajor-caju*** grown on whey when enhanced with plant growth hormones, 3.2g of biomass was produced, this showed an increase over the control which produced 2.5g.

Zobo drink, a non alcoholic drink produced from the petals of ***Hibiscus sabdarifa*** (Linn Roselle) by boiling and filtration (Ogiehor *et al.,* 2007). Zobo beverage has been shown to be a good source of natural carbohydrate, protein and vitamin C, which constitute the major reason(s) for consuming soft drinks and fruit juices (Okoro, 2003; Ogiehor and Nwafor, 2004).

The name 'Zobo' is derived from the local Hausa (Northern Nigeria) name for the Roselle plant that is 'Zoborodo'. The calyx contain about 8.3% moisture, 4% citric acid, 1.5% pigment (mainly anthocyanin), 6.9% protein and about 9% soluble solid with pH of 2.7 (Adenipekun, 1998).

The aim of this project work is to study the ability of hybrid of and ***P. tuberregium and Pleurotus pulmonarus*** to grow in Zobo and Synthetic media and produce mycelia mat; during submerged fermentation.

MATERIALS AND METHODS

The Organism

The hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** was produced by crossbreeding the two mushrooms on the slant. Slants of Potato dextrose agar and yeast extract were produced in MacCartney bottles, inoculated with the spawn of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** and allowed to grow at room temperature in the dark cupboard for four days.

Media

The Zobo medium was produced by boiling the dry calyx of ***Hibiscus sabdarifa*** (Roselle plant), the calyx is then filtered out and the pH regulated to 5.8. The synthetic medium was prepared by dissolving 15g of glucose, 5g of peptone, 3g of yeast extract, 1g of $MgSO_4.7H_2O$, 2g of KH_2PO_4 and 1g of Urea; all in 1l of distilled water and the pH was regulated to 5.8.

The media were sterilized in the autoclave at 121^0C for 15 minutes, and were pour into two different fermentors aseptically.

Fermentation

Two fermentors containing sterilized synthetic medium and zobo medium each were inoculated with the hybrid of the two mushrooms by washing organisms from three grown slants into each bioreactor, an aeration hose was introduced and the mouth sealed.

Growth and Harvesting of the Organisms

The fermentation process was allowed to run for 10 days at room temperature and then terminated. Organisms were harvested by filtration using Number 1 Whatman filter papers, then centrifuged and precipitated; cell debris was air dried and weighed.

RESULTS

Table 1 shows the result of the turbidity scores for the two media. The synthetic medium became turbid on the second day and the turbidity increased until the fourth day after which there was no detectable increase again in the turbidity of the medium. Zobo medium on the other hand became turbid on the third day and the growth was at the climax on the fourth day.

Table 1: Turbidity score for the two media as fermentation days increased

Day	Turbidity Score	
	Synthetic Medium	Zobo Medium
1	-	-
2	++	-
3	+++	+
4	++++	++
5	++++	++
6	++++	++
7	++++	++
8	++++	++
9	++++	++
10	++++	++

KEY:
+ : turbidity grade
-: no visible growth or turbidity

Result of the weighed mycelia mat and precipitate from the filtration and precipitation processes respectively are shown in Table 2. Weight

of the mycelia mat from synthetic and Zobo media are 0.83g and 0.47g respectively. While the weight of the precipitate from synthetic and Zobo media are 0.11g and 0.06g respectively.

Table 2: Mean weight of mycelial mat and precipitate of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** after ten days submerged fermentation

DRY MASS	SYNTHETIC	ZOBO MEDIUM
MYCELIAL MAT WEIGHT (G)	0.82 ± 0.01^{a}	0.47 ± 0.02^{b}
PRECIPITATE WEIGHT (G)	0.11 ± 0.02^{a}	0.06 ± 0.01^{b}

Values are means scores ± Standard Deviation
Mean values followed by the same superscript are not significantly different by Duncan's Multiple Range test ($P\leq0.05$)

Table 3: T-test result on the turbidity for the two media as fermentation progress

Medium	Mean Turbidity	T-Value	P value
Zobo Medium	1.50±0.27	5.58	$p\leq 0.05$
Synthetic Medium	3.30±0.42	7.80	$p\leq 0.05$

DISCUSION

Turbidity score shows the growth of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** based on the utilization of the media by the organism, and thus the turbidity. The faster growth rate of the organism in the synthetic medium compared to the Zobo medium may be in compliance with the work of Sánchez *et al.,* (2006), which reported that 'supplementing of solid media with synthetic medium produced high peroxidase activity in ***Pleurotus* sp.**.

The delayed turbidity in the Zobo medium until the third day may be in contrast to the report of Wu *et al.* (2003) that the lag phase of ***P. tuberregium*** in submerged liquid fermentation was shorter; this may be due to reduced ability of the organism to utilize the sugars available in Zobo.

The synthetic medium showed a higher yield of mycelia mat and precipitate than that of Zobo medium, but the two showed a lower yield compared to what Rupak *et al.* (2005) reported.

There was a significant difference in the t-test result of the turbidity of the two media, with a mean of 1.50 for zobo medium and 3.30 for the synthetic medium this agrees with the work of Wu *et al.* (2003).

CONCLUSION

The synthetic medium supported the growth of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** better than the Zobo medium as seen in the result. Addition of some nutrients to the Zobo medium may aid a better growth and yield of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** in this medium.

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Removal of cadmium from freshwater cultured Nile tilapia *Oreochromis niloticus* using Neem Leave Water Extract (NLWE) and Neem Leave Powder (NLP)

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Abstract: The study aimed to investigate the clinical picture, bioassay of cadmium pollution evaluating the influence of Neem Leave Water Extract (NLWE) and Neem Leave Powder (NLP) on recovery and removal of cadmium in tissues of Nile tilapia *Oreochromis niloticus* and water. *Azadirachta indica* (A. indica) leaves were obtained and Leave Water Extract (NLWE) and Neem Leave Powder (NLP) were prepared. To evaluate effect of neem leaf as water extract (NLWE) and neem leaf powder (NLP). A total of 80 apparently healthy Nile tilapia *Oreochromis niloticus,* weight (80±5) gram, fish were divided into four groups of 20 fish each, comprising three experimental groups and one control. 1st group was control simultaneously exposed to dechlorinated tap water only. 2nd group was exposed for cadmium chloride (15 mg/l). 3rd group exposed neem leaf water extract (1/10 LC50) only. 4th group exposed to cadmium chloride (15mg/l) and neem leaf powder NLP (200g/l). Clinical signs, post mortem lesions and mortality were monitored and recorded. Blood was collected from five of fish in each group after 30 days of starting the experiment. The musculature, skin, gills, liver, spleen and kidneys were collected from five fish of each group after the period of experiment for histopathological examination. From the present study, it was concluded that, Neem leave powder NLP efficiently remove cadmium from water decreasing it in tissues of fish. while NLWE remove cadmium in low degree but affect significantly the hematological, physiological and immunological state of *O. niloticus*, improving health status of fish.
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Key words: cadmium; (NLWE); (NLP); *Azadirachta indica; Oreochromis niloticus;* Clinical signs; physiological; immunological.

Introduction:

There is a great concern about the toxic impacts and environmental pollution caused by heavy metals especially in aquaculture all over the world. Toxic heavy metal ions get introduced to the aquatic streams by means of various industrial activities viz. mining, refining ores, fertilizer industries, tanneries, batteries, paper industries, pesticides etc. and posses a serious threat to environment. (**Dhiraj Sud *et al*.,2008**). Cadmium accumulates in the different organs causing fish death in many cases due to the more susceptibility to the bacterial infections and impairment of the immune system (**Baldisserotto *et al*., 2006)**.

Conventional techniques have their own inherent limitations such as less efficiency, sensitive operating conditions, production of secondary sludge and further the disposal is a costly affair (**Ahluwalia and Goyal, 2005a**). Another powerful technology is adsorption of heavy metals by activated carbon for treating domestic and industrial waste water (**Hosea *et al*., 1986).** The utilization of sea weeds, moulds, yeasts, and other dead microbial biomass and agricultural waste materials for removal of heavy metals has been explored (**Bailey *et al*., 1999; Sudha and Abraham, 2003).**

Recently attention has been diverted towards the biomaterials which are by products or the wastes from large scale industrial operations and agricultural waste materials.Neem, Azadirachta indica (*A. indica*) is one of the most promising medicinal plant, having a wide spectrum of biological activity, well known mainly for its insecticidal properties (**ICAR, 1993**). Every part of neem tree have been known to possess a wide range of pharmacological properties, especially as antibacterial, antifungal, antiulcer, repellent, pesticidal and detoxifying agent (**Biswas *et al*., 2002; Das *et al*., 2002; Mousa *et al*., 2008**). One of the most promising natural compounds is azadirachtin (AZA), an active compound extracted from the neem tree (*Azadirachta indica*), whose antiviral, antibacterial and antifungal properties have been known for 2000 years **(Isman *et al*., 1990; Harikrishnan *et al*., 2003)** Neem has been used successfully in aquaculture systems to control fish predators and treatment of large numbers of bacterial and parasitic fish diseases (**Dunkel and Ricilards, 1998; Mona *et al*., 2011).**

Present study aimed to investigate the clinical picture, bioassay of cadmium pollution evaluating the influence of Neem Leave Water Extract (NLWE) and Neem Leave Powder (NLP) on recovery and removal

of cadmium in tissues of Nile tilapia *Oreochromis niloticus* and water with monitoring some blood, physiological parameters and histopathological alterations due to exposure to cadmium and Neem water extract (NLWE) and Neem Leave Powder (NLP).

Material and methods:

Experimental fish:

A total of 100 apparently healthy Nile tilapia *Oreochromis niloticus*, weight (80±5) gram obtained from private fish farm and acclimated for 2 weeks in aquaria supplied with dechlorinated tap water with continuous aeration.

Preparation of Neem Leaf Water Extract (NLWE) and Neem Leave powder (NLP):-

Azadirachta indica (*A. indica*) leaves were obtained from nurseries of agricultural ministry, Giza, Egypt dried and finely chopped, grounded in blender then amount of 500g was soaked in tap water, (liter of water) for 24 h at room temperature as described by **Cruz *et al.*, (2004)**. The mixture was filtered and the extract (500 g/l) was used immediately in the experiments as. (NLWE). While the reset of grounded leave used as it is as (NLP).

Clinical and post mortem examination:

Clinical and post mortem examination was carried out to the fish (*O.niloticus*) exposed to cadmium and neem each alone and cadmium and neem (neem water extract and neem leave powder) together according to **(lucky, 1977**).

Determination of 96-h LC50

Static toxicity tests were run to determine lethal concentrations (96-h LC50) of neem leaf water extract to *Oreochromis niloticus* fish, tests were conducted in 30 L glass aquaria, 6 fish per aquarium, containing neem leaf extract diluted in tap water to the following concentrations: 0 (control group), 1, 2,4,6,8,10, 12, g /l. Each treatment had 3 replicates. All laboratory conditions were maintained constant. Deaths and abnormal behavior fish were recorded every 3 h for the 1st day, then every day for other 3 days. The value of 96-h LC50 were estimated.

Experimental design:

To evaluate effect of neem leaf as water extract and neem leaf powder. A total of 80 apparently healthy Nile tilapia *Oreochromis niloticus*, weight (80±5) gram fish were divided into four groups of 20 fish each, comprising three experimental groups and one control. Each group was placed in separate glass aquaria. 1st group was control simultaneously exposed to dechlorinated tap water only. 2nd group was exposed for cadmium chloride (15 mg/l) (**Osman *et al.*, 2009**). 3rd group exposed neem leaf water extract (1/10 LC50) 0.2 ppm only. 4th group exposed to cadmium chloride (15mg/l) and neem leaf powder NLP (200g/l). The experiment was carried out in static systems. Clinical signs, post mortem lesions and mortality were monitored and recorded during treatment.

Table 1: Cadmium exposure and treatment with (NLWE) and (NLP)*** (experimental design)**

Groups*	Cadmium exposure	Concentration of Neem
1st (negative control)	0	0
2nd (positive control)	15	0
3rd Treated group NLWE	15	0.2 ppm
4th Treated group NLP	15	55 g/l

* Each group 20 fish

**NLWE= Neem water extract

***NLP= Neem leave powder

Blood sampling:

Blood was collected from the caudal vein of five fish in each group after 30 days of starting the experiment. The liver, kidneys, musculature and gills were also collected from five fish of each group after the period. The blood sample was divided into two portions. The first portion was kept as a whole blood in heparinized tubes for hematological examination. Serum was separated from the second portion for biochemical analysis. Tissue homogenates were prepared from liver, kidneys, musculature and gills were digested as described by **Cottenie (1980)** for determination of cadmium residues.

Tissue Cadmium Determination:

Cadmium concentration was determined in the tissues according to **Jackson (1973)**.

Hematological Examination:

Packed cell volume (PCV), hemoglobin (Hb) concentration and red blood cell (RBC) count were examined in the whole blood as described by **Stoskopf (1992).**

Biochemical Analysis

Serum:

Total protein level in serum was determined according to **Cannon *et al.* (1974)** Serum albumin concentration was measured as described by **Gustafsson (1976)**. Blood serum globulin was calculated by subtracting the concentration of albumin from that of the total protein and albumin/globulin ratio (A/G ratio) was calculated by dividing albumin

concentration over that of globulin **Coles (1986).**

Histopathological examination:

The macroscopic lesions of internal organs (Liver, kidneys, musculature and skin of all groups were collected and fixed in 10% formal saline for histopathological examination). Small pieces of suspected lesions of the organs were taken and preserved in 10% formalin for 24 hrs. Paraffin section (5-10 microns in thickness). Liver and musculature of infested fish after fixation in 10% formalin solution. Sections were stained with haematoxylin and eosin stain (H&E). **Bancroft *et al.* (1996)**.

Statistical Analysis:

Data were presented as mean±standard error (S.E.) and the significance of differences was estimated using ANOVA test (**Senedecor, 1964**).

Results:

96-h LC50 for *O.niloticus:*

96-h LC_{50} for *O. niloticus* exposed to NLWE was 2 ppm while NLP was 550 gm (table 1).

Table 1. Lethal and sublethal concentrations of Neem leaf aqueous extract NLWE (ppm) and Neem leaf powder NLP (gm) for Nile tilapia,

Neem form	96-h LC_{50}(g/l)	$^{1}/_{10}$ LC_{50}(g/l)
Neem leaf as water extract NLWE	2 ppm	0.2 ppm
Neem leaf powder NLP	550 gm	55 gm

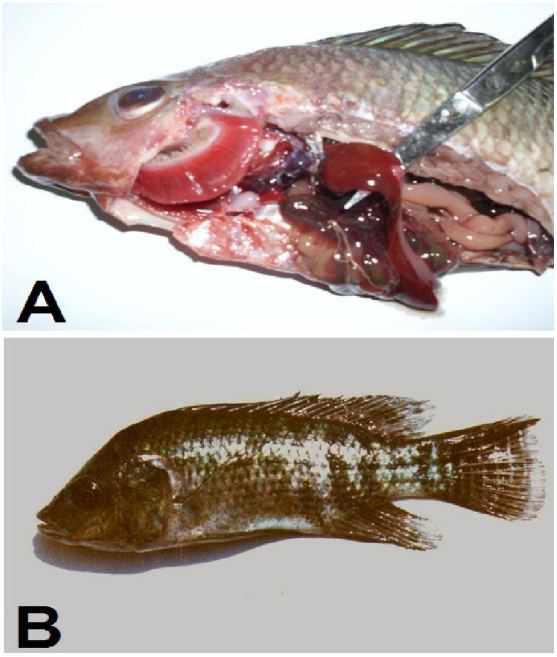

Figure 2: Showing (A) *O. niloticus* exposed to cadmium showed paleness and excessive mucous on gills with inflammation and enlargement of spleen, and liver (B) *O. niloticus* showing dark metallic skin with excessive slimness exposed to cadmium chloride

Clinical signs of fish:

O.niloticus exposed to NLWE showed respiratory distress, gasping, gulping the atmospheric air erratic swimming with some nervous manifestations in the form of fish moved in all directions of aquaria and some of them swam in circular directions were noticed during determination the LC50 of NLWE, while fish exposed to NLP being normal without any abnormal sings, cadmium showed slimy body with dark skin colour (fig1,B), with signs of restlessness some fish suffered from asphyxia and jumped outside water, finally loss of appetite, escape reflex and settle down to the bottom, sluggish movement.

Post mortem of fish:

O.niloticus exposed to NLWE showed congested gills, distended, enlarged gall bladder, while fish exposed to NLP showing no lesions while fish exposed to cadmium showed paleness and excessive mucous on gills with inflammation and enlargement of spleen, enlargement and distended gall bladder with spotted inflammatory patches in the liver (fig1,A).

Histopathological Examination:

The histopathological alterations were observed in musculature as suffered from hyalinization of some muscular bundles with infilteration of lecuocytic inflammatory cells with diffused deposition of melanin pigmented cells (fig 3 A &B). gills suffered from advanced stages of hyperplasia, odema of the core of primary gill lamellea (fig 3C&D). Spleen was manifested as hemosiderin was detected in the congested red pulps focal melanin pigment cells deposition was observed in the white pulps and in the perivascular tissue of the dilated and congested blood vessels (fig4, E).

The kidney was manifested as focal haemorrhage in between the degenerated and necrosed tubules associated with dilatation and congestion in the blood vessels with perivascular deposition of melanin pigmented cells (fig 4, F) The liver was manifested as melanin pigmented cells with leucocytes inflammatory cells infiltration were observed in the portal vein associated with congestion in the central vein (fig 4,G &H).

Table 2: Cadmium residues (mg/g wet weight) in the organs of *Oreochromis niloticus* exposed to 15 mg/L cadmium and treated with NLWE and NLP

Organ / group	liver	kidney	muscle	gills
Control	0.041±0.002 A	0.043±0.002 A	0.031±0.001 A	0.061±0.002 A
Cadmium	6.280±0.170 aB	3.215±0.128 aB	1.286±0.067 aB	1.276±0.073 aB
Cadmium+ 0.2 mg/l NLWE	5.940±0.164 aC	2.995±0.106 aC	1.178±0.072 aC	1.210±0.039 aC
Cadmium+ (55 gm) NLP	2.990±0.105 abc	1.592±0.067 abcd	0.432±0.013 abcd	0.764±0.023 abcd

Each value represents mean ± S.E.; N=5.
Small letters a, b, c and d in the same column represent a significant change against capital letters A, B, C and D respectively by LSD using ANOVA at P= 0.05

Table 3: Some hematological prarameters in *Oreochromis niloticus* exposed to 15 mg/L cadmium and treated with NLWE and NLP

Parameter / group	RBC count (x10^6/mm)	Hemoglobin	Pcv %
Control	3.20±0.10 A	8.32±0.30 A	25.60±1.41 A
Cadmium	1.70±0.07 aB	5.52±0.17 aB	17.62±1.29 aB
Cadmium+ 0.2 mg/l NLWE	1.80±0.08 aC	5.83±0.19 aC	19.65±1.26 aC
Cadmium+(55 gm) NLP	2.15±0.08 bc	7.18±0.24 bc	24.40±1.19 bc

Each value represents mean ± S.E.; N=5.
Small letters a, b, c and d in the same column represent a significant change against capital letters A, B, C and D respectively by LSD using ANOVA at P= 0.05

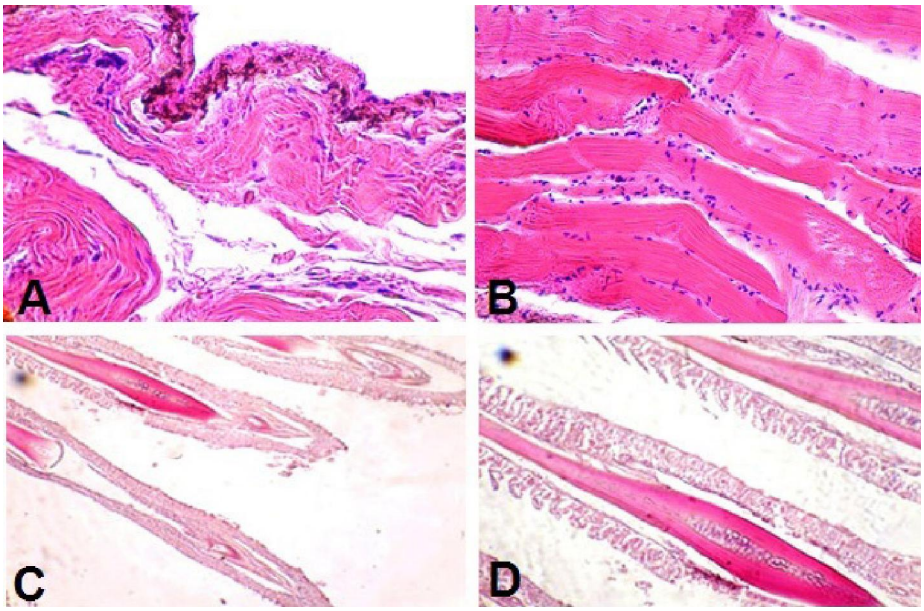

Figure 3; Showing (A &,B) skin and musculature of *O.niloticus* exposed to cadmium: there were diffused melanosis with inflammatory cells infiltration in the dermis, in association with hyalinization of some skeletal muscle bundles (C& D), hyperplasia of secondary gill filaments with edema of the core of primary gill lamellae

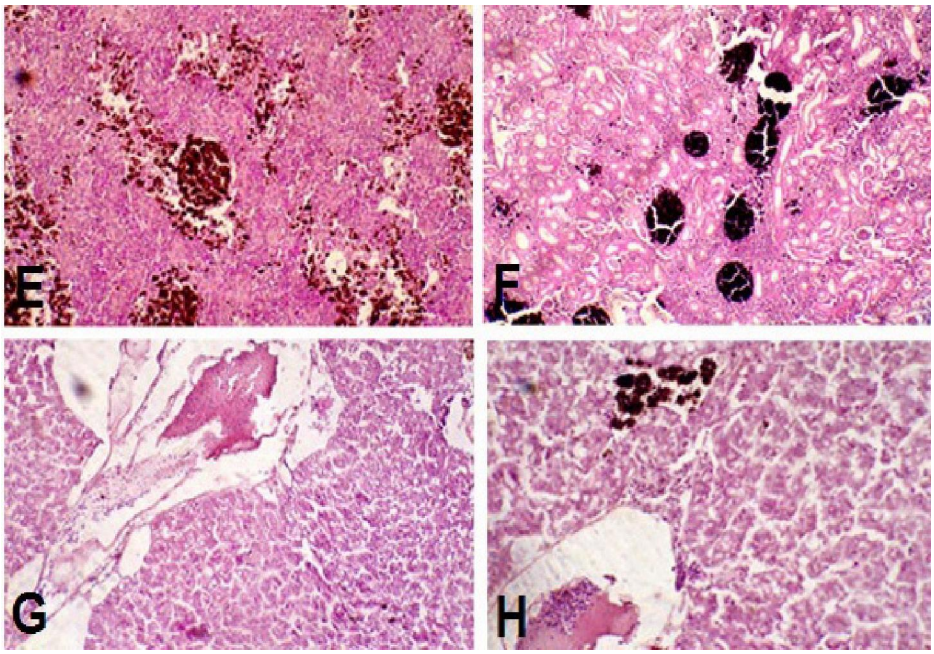

Fig 4: Showing (E& F) spleen and kidney suffered from focal melanosis and necrosis of cells of spleen and kidney, (G&H), liver of *O.niloticus* exposed to cadmium showing congestion in the central vein and sinusoids with hemosiderosis, with necrosis and degenertation in the hepatocytes,

Table 4: Blood serum proteins levels in *Oreochromis niloticus* exposed to 15 mg/l cadmium for 30 days and treated with NLWE and NLP

Parameter / group	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
Control	4.75±0.28 A	1.63±0.08 A	3.12±0.15 A	0.59±0.03 A
Cadmium	3.15±0.14	0.88±0.04 aB	2.27±0.10 aB	0.32±0.02 aB
Cadmium+0.2mg/l NLWE	3.30±0.15	0.96±0.05 Ac	2.34±0.10aC	0.37±0.02 aC
Cadmium +(55 gm) NLP	4.64±0.25	1.56±0.08 bc	3.08±0.13 bc	0.55±0.04 bc

Each value represents mean ± S.E.; N=5.
Small letters a, b, c and d in the same column represent a significant change against capital letters A, B, C and D respectively by LSD using ANOVA at P= 0.05

Discussion:

present study investigate efficacy of Neem leave water extract (NLWE) as detoxifying agent and Neem leave powder (NLP) as adsorbent agent in removal of cadmium in water and *O. niloticus* tissues and as immunostimulants in improving the physiological and immunological status of *O.niloticus*. Neem leaves being economic and ecofriendly due to their natural origin, availability in abundance, renewable, low in cost and more efficient are seem to be viable option for heavy metal remediation. Studies reveal that various agricultural materials such as rice bran, rice husk, wheat bran, wheat husk, saw dust of various plants, bark of the trees, groundnut shells, coconut shells, black gram husk, hazelnut shells, walnut shells, cotton seed hulls, waste tea leaves, etc has been tried (**Annadurai *et al*., 2002; Mohanty *et al*., 2005; Dhiraj Sud *et al*.,2008**) development of modern drugs from neem should be emphasized for the control of various diseases. An extensive research and development work should be undertaken on neem and its products for their better economic and therapeutic utilization (**Schmutterer, 1995 & Ketkar and Ketkar, 1995**). **Bhattacharyya and Sharma (2005)** and **Serafini Immich *et al*., (2008)** investigates the efficiency of neem tree leaves powder in the removal of Remazol Blue RR present in aqueous solution, they studied color removal from textile effluent through adsorption processes using Neem leaf powder (Azadirachta indica) as an adsorbent. They reported that there was high efficiency of Neem leaf powder (NLP) in the color removal process. **Serafini Immich**

***et al*., (2008)** added that The results given showed that the natural adsorbent Neem, without the extract, is not toxic toward the aquatic environment Therefore, this adsorbent can be used in the adsorption processes without adverse or toxic effects on aquatic organisms.

Concerning to the clinical sings and post mortem lesions appeared on fish after exposure to cadmium and neem leave water extract each alone *O.niloticus* exposed to NLWE showed respiratory distress, gasping, gulping the atmospheric air erratic swimming with nervous manifestations in the form of fish moved in all directions of aquaria and some of them swam in circular directions were noticed while fish exposed to cadmium showed slimy body with pale skin with signs of restlessness some fish suffered from asphyxia and jumped outside water, finally loss of appetite, escape reflex and settle down to the bottom, these results nearly agree with the results obtained by **Mousa *et al*., (2008); Osman *et al*.(2009) and Mona *et al*.(2011).**

Regarding to the histopathological alterations due to exposure to cadmium pollution, the study revealed that the affected liver was manifested as melanin pigmented cells with leucocytes inflammatory cells infiltration were observed in the portal vein associated with congestion in the central vein. Beside that kidney was manifested as focal hemorrhage in between the degenerated and necrosed tubules associated with dilatation and congestion in the blood vessels with perivascular deposition of melanin pigmented cells. In addition, the affected gills was manifested with destruction in most of the lamellae in other filaments and hyperplasia in some other filaments and lamellae. There was diffuse haemorrhage allover the arch, while the racker showed goblet cells formation and oedema in the connective tissue core, musculature was manifested as hyalinization in some muscular bundles and spleen was manifested as hemosiderin detected in the congested red pulps focal melanin pigment cells deposition was observed in the white pulps and in the perivascular tissue of the dilated and congested blood vessels these results nearly agree with **Oliveira Ribeiro *et al*. (2002), Thophon *et al*. (2003); Gupta and Srivastava (2006); Kaoud and El-Dahshan (2010).**

Concerning the concentration of cadmium in fish tissues it is observed that cadmium concentration in liver, kidney, musculature and gills was significantly higher in fish exposed to cadmium for 30 days than control group and the elevation in cadmium concentration is more drastic at the end of 30 days exposure. The treatment with 55 g/l NLP or 0. 2 g/l of NLWE concentrations significantly decreased the concentration of cadmium in tissues but not to the level of control. Present results indicate that 200 g/l NLP is effective in decreasing the adverse effect of Cd pollution removing it from water reducing Cd bioaccumulation in fish,its effect was pronounced than NLWE. Particulate organic matter can scavenge metal from water and help to reduce metal from fish. These results are in agreement with **Santachi (1988)** who found that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish. Cd accumulation in liver, gills and musculature of fish exposed to Cd alone was higher than that of NLP treated group and NLWE treated group. These results suggest that NLP and NLWE could remove Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues. Besides, that neem in its two forms can eliminate more amount of Cd from the body through secretions of feces and urine.

Regarding hematological parameters, cadmium exposure for 30 days significantly diminished RBC count, PCV and hemoglobin concentration in *Oreochromis niloticus* in comparison with control. Treatment with NLWE at concentrations 0.4 or 0.2 g/l significantly elevates these parameters near the control values. The reduction of these parameters in Nile tilapia, *O. niloticus* exposed to cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemosynthesis that affected by pollutants **Wintrob (1978).** Also, the decrease in RBCs count may be attributed to haematopathology or acute haemolytic crisis that results in severe anemia in most vertebrates including fish species exposed to different environmental pollutants **Khangarot and Tripathi (1991)** or may be the decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in sever aneamia **(James and Sampath 1999**; **Kaoud *et al*.,2011**). Also, **Gill** and **Epple (1993**) found a significant reduction in the RBCs, Hb and Hct in American eel Anguilla rostrata after exposure to 150 µg Cd/L. **Karuppasamy *et al.* (2005**) found a significant decrease in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, *Channa punctatus* after exposure to sublethal dose of Cd (29 mg CdIL).The addition of NLWE increased in concentration the haematological parameters (RBCs, Hb and Hct) which indicating to the capability of NLWE to chelate Cd from the water and fish subsequently, the Cd pollution was reduced. These results are in agreement with **Osman *et al*.(2009)** who observed that *Oreochromis niloticus* exposed to cadmium along with Humic acid as chelating agent showed a significant improvement in blood parameters due to reduction of cadmium level in water and fish.

Respecting the serum protein, cadmium exposure for 30 days significantly declined the levels of total

protein, albumin and globulin as well as A/G ratio comparing with control. Decreases in serum protein concentration and the albumin/globulin ratio in the blood may indicate some liver dysfunction. When exposed to stressors, the gills become "leaky" to water and ions, often resulting in osmoregulatory imbalance **Mazeaud *et al*. (1977).** So the decline in serum total protein, albumin and globulin may be also due to a degree of haemodilution under the stress of pollution. The A/G ratio is an index used to track relative changes in the composition of serum or plasma **Jacobes *et al*. (1990).** A drop in A/G ratio can indicate a shift from albumin production to globulin proteins in response to stress. The treatment with 0.4 or 0.2 g/l of NLWE in the aquarium water significantly elevates the values of these parameters to nearly the values of control. These may be due to that presence of NLP as a chelating agent reducing cadmium level from water as well as fish tissues improving physiological status and enhancing immune response of fish. **(Khan and Wassilew, 1987)** who reported that the aqueous extract of neem bark and leaf also possesses anticomplement and immunostimulants activity and Neem oil has been shown to possess activity by selectively activating the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge. The results of the present study nearly agree with **Chandra and Khuda-Bukhsh (2004)** who mentioned that the $Cdcl_2$, is a common pollutant, and Aza, a natural product of the neem plant used extensively as an 'ecofriendly' agent for many purposes, had any genotoxic effect of their own on nontarget aquatic organisms of economic importance; and second, if Aza could have any ameliorating effect on $Cdcl_2$-induced genotoxicity in *O. mossambicus* tissues. As compared with distilled water-treated controls, both $Cdcl_2$ and Aza induced genotoxicity in *O. mossambicus,* the former in greater quantity than that produced by Aza. However, Cd-induced toxicity in *O. mossambicus* appeared to be ameliorated to some extent by Aza.

From the present study, it was concluded that, Neem leave powder NLP efficiently remove cadmium from water (as adsorbance) decreasing it in tissues of fish improving aquaculture an ecosystem of fish, it is not toxic toward the aquatic environment Therefore, this adsorbent can be used in the adsorption processes without adverse or toxic effects on aquatic organisms while NLWE remove cadmium in low degree but affect significantly the hematological, physiological and immunological state of *O. niloticus*, improving health status of fish. So Neem as natural product is a promising tool for controlling cadmium pollution in aquaculture when it used in two forms NLP or NLWE., Also, they significantly reduces cadmium level in fish tissues including musculature.

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The Impact of Land Use Consolidation Program on Agricultural Productivity: A Case Study of Maize (*Zea mays* L.) Production among Households in Nyabihu District, Western Rwanda.

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Abstract: The high population growth in Rwanda has resulted in land fragmentation and poor crop yields in many rural farms hence necessitating the government to introduce the land consolidation policy in the country with the aim of boosting crop yields and effecting rural household development. The purpose of this study was to determine whether the land consolidation policy is effective in boosting maize production yields among the maize growers in the sector. Specifically, the research analyzed maize production before and after the land consolidation policy. To achieve the objective of the study, a survey was conducted with 40 households selected randomly from Mukamira Sector where the policy was implemented from 2004. The results showed that maize production in the Sector increased by 347% from a mean yield of 2027.5 kg to 9071.9 kg per household per season as a result of the policy (p=0.05). According to chi-square test there is a significant relationship between maize income as a dependent variable, fertilizer use and land size as independent variables (p=0.05). However, two major constraints were observed that hamper maize production and these were the low output prices and small land holdings. This study concludes that land consolidation policy has had great impact in the sector due to increased use of mineral fertilizers, certified maize seeds and better crop husbandry practices. To overcome the low prices of maize grains, there is need for farmers to market their produce as cooperatives rather than as individuals so that they may consolidate their bargaining power as cooperatives. Input suppliers need to open businesses near the farmers to try and provide good service.

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Key words: Bargaining power, Land fragmentation, Land consolidation policy, Impact, Collaboration, Input suppliers, and Chi-square test

1. Introduction

Rwanda's economy is largely agrarian. More than 80% of the Rwanda's projected population of 10.5 million depends on farming. The total land area of the country measures 24,700 square kilometers. Although about 79% of the country's land is classified as agricultural, only 11% of the land represents permanent crop land. The remaining agricultural lands are covered with forests, marshlands and marginal lands in the hillsides where permanent and routine cultivation of crops are not tenable. Of the total arable land of 2,294,380 ha, 1,735,025 ha is cultivated with food and cash crops and the remaining represents pastures and bushes. Over 80% of the population live in rural areas and subsist on smallholder farming.

With an average of 407 persons per square Km, Rwanda represents the most densely populated nation in the continent. Hence the land distribution is highly fragmented and skewed in Rwanda Land in Rwanda is the most valuable, productive and contested asset. Proper management of land is therefore a must. However, most of the laws governing land administration and management in the country had been formulated by the colonialist and have remained the same till 1990's. Several reforms and policies are under implementation in Rwanda, among these, the Land Use Consolidation policy is the key for agricultural transformation.

Land consolidation is sometimes incorrectly interpreted to be only the simple reallocation of parcels to remove effects of fragmentation. In reality, land consolidation has been associated with broader social and economic reforms from the time of its earliest applications in Western Europe. The first consolidation initiatives of Denmark in the 1750s were part of a profound social reform to free people from obligations to noble landlords by establishing privately-owned family farms. The consolidation of fragmented holdings did result in improved agricultural productivity but this was not the only objective of these reforms. This chapter illustrates the

wide range of rural development objectives, ranging from agricultural improvement to village renewal and landscape development and protection, which can be addressed through land consolidation projects. It describes various land consolidation approaches and concludes with an overview of conditions that should be put in place before land consolidation projects can be undertaken (FAO, 2003).

The overarching strategies of economic development and poverty reduction in Rwanda that envisions social transformation through agriculture require shifting from such subsistence farming to commercial oriented agriculture. In Rwanda, the growing demographic pressure on land and continued fragmentation of household's plots by inheritance forced the land use patterns to be inevitably re-organized. Volume of food crop production is a function of physical land area and the productivity of land under cultivation. Crop productivity, often measured as the ratio of farm outputs to inputs, reflects the efficiency of usage of inputs. However the efficiency of the inputs depends on the size of the farm land. Land fragmentation thus affects productivity and competitiveness of smallholder farms. Furthermore, the inherent difficulties in mechanizing farm chores in small farms also impede public and private investments.

The Land Use Consolidation Policy was enunciated in 2004 by the Government. The process of land consolidation, the method of reversing the action of land fragmentation, is not new in the World countries. In Britain land consolidation took place so long ago, that many writers and even experts tend to forget that it took place at all (Simpson, 1976). Some of the earliest attempts at land consolidation, as a method of land reform, took place in Scandinavia, particularly in Finland (FAO, 2003), Sweden (Osterberg and Petterson, 1992) and Denmark (Bins, 1950) in the 18th and 19th centuries. According to Clout (1987), at least half of Western Europe's farmland was considered to need consolidation in the 1950s, a time when Europe had pressing needs of reconstruction after the Second World War.

Land use consolidation had been also implemented in Central and Western European countries since 1989 as part of an overall strategy of transition from centrally planned agriculture to privatization and market development in order to increase farmer's revenues. It was also implemented in Latin America, Asia and Southern Africa to mitigate land fragmentation. In Kenya, the customary land tenure failed to meet the needs of an expanding population which then resulted in low subsistence levels and influenced land reforms needing land consolidation to stop further fragmentation in Kikuyu, Kiambu and Maranga Districts (Mackenzie, 1993).

The land use consolidation policy was implemented for the first time in 2008 by the Government of Rwanda, through the Ministry of Agriculture, as part of the Crop Intensification Program (CIP). The CIP was initiated by the same Ministry in September 2007 with a goal to increase agricultural productivity of high potential food crops and to provide Rwanda with greater food security and self-sufficiency. The implementation of this program involves various components, including Land Use Consolidation as the main pillar, the proximity advisory services to farmers, inputs (seeds and fertilizers) distribution and post-harvest technologies (e.g. driers and storage facilities).

The program is also supported by other initiatives like land husbandry, irrigation and mechanization infrastructure development to bring more land under production, avoid dependency on rain-fed farming system and use of farm power in the context of a market-oriented agriculture. The LUC policy is in line with Rwandan Government efforts to mitigate hunger and poverty. It correlates not only with CIP but also with the "Villagisation" known as new resettlement program "Imidugudu" which started earlier in 2004. Therefore, its implementation process involves various stakeholders (e.g. Ministries, NGOS, Civil Society Organizations and the Private Sector).

The implementation process of LUC Policy in Rwanda Land use consolidation is a multi-sector process. Although the technical plan for land use was drawn by MINAGRI through its implementing agency Rwanda Agriculture Board in conjunction with local administration authorities. Based on the agro ecological potential and the land area available in each district, the CIP estimates the consolidated area that can be grown with priority crops in each district. Through negotiations with district authorities, target figures are agreed and captured in the performance contracts of the respective districts. The district -and sector agronomist, IDPs in cells and Farmer Promoters (abajyanama bubuhinzi) in villages then mobilize the farmers for growing the priority crops in a consolidated fashion. At national level, stakeholders under the IDP steering committee framework include MINAGRI, MINALOC, MINIRENA, MININFRA, NGOs, Private Sector, Province and District authorities (RGB, 2012).

Eight priority crops (Irish potato, cassava, beans, maize, wheat, rice, banana and Soybean) have been selected for promotion under land use consolidation policy. The rotation system is based on comparative advantage, crop suitability in a specific agro-ecological zone and its contribution to the overall

food security. Crops like Irish potato, cassava, beans and maize have shown a competitive advantage with a positive trade balance, according to the recent cross - border trade study (MINAGRI, 2010).

In an effort to address both marketing and post-harvest challenges, the Government of Rwanda (GOR) has decided to establish driers and food storages facilities where land has been consolidated. Consolidated use of lands allows farmers to benefit from the various services under CIP such as: (i) efficient delivery of inputs (improved seeds, fertilizers), (ii) proximity extension services, (iii) post harvest handling and storage facilities, (iv) irrigation and mechanization by public-and private stakeholders and (v) Concentrated markets for inputs and outputs.

Maize is one of the major crops in Rwanda and is ranked fifth among food crops and second among cereals after sorghum. In 2000, approximately 32% of the land allocated to cereals production was occupied by maize MINAGRI (2001). Maize is currently cultivated in the whole country and is essentially intercropped with beans. Consumption of maize is consistently increasing and maize is becoming an important cash crop for small-scale farmers, especially in the maize growing regions. According to an earlier survey by the department of statistics, MINAGRI (1990), volume traded for the rural areas during that period was estimated at about 5,000 tons while in the year 2000 this figure was estimated to be about 50,000 tons. Maize supplies a high quantity of carbohydrates to the population. The crop has become popular especially in urban areas amongst manual laborers and is targeted by the ministry of Agriculture (MINAGRI), to contribute to the nutritional status of the population.

According to MINAGRI (2010), maize is currently fourth after bananas, sweet potatoes and white potatoes in providing energy per capita. Maize has more uses than any other cereals. It is used mainly as food for human consumption, but it is also the number-one feed grain in the country, being the main source of calories in animal feeding and feed formulation. Maize is one of the priority crops that have been chosen by the government of Rwanda in its effort to increase household incomes and the nutritional status of Rwandan people through increased production and marketing.

The statistics from the Ministry of Agriculture indicate that consolidated lands have been increasing since the policy's adoption, from 28,788 Ha in 2007 to 254,000 Ha in 2010 and 502,916 Ha in 2011. The same statistics show that maize and wheat production have increased six-fold. They also show that production of Irish potato and cassava has tripled while the production of rice and beans increased by 30% in the past 4 years. A survey conducted by the National Institute of Statistics Rwanda (NISR) revealed that poverty in the country has dropped by 11.8 percent since 2006. However, data to show the before and after the LUC progamme are minimal and hence the justification for this research in Nyabihu district one of the districts chosen by MINAGRI to implement the policy.

2. Material and Methods

This study was carried out in Mukamira sector (division), Nyabihu district, in Northern Rwanda in 2012. Nyabihu district is divided into 12 Sectors, namely, Bigogwe, Jenda, Jomba, Kabatwa, Karago, Kintobo, Mukamira, Muringa, Rambura, Rugera, Rurembo, and Shyira which are further subdivided into 73 Cells (Uwihanganye, 2008). The surface area of the district is 512 km^2 with a population of 280,210 and a population density of 541 people per km^2. Using simple random sampling method, a sample of 40 households was drawn from a sampling frame of the households in Mukamira sector. Using this sample, a household survey was conducted on each household and data collected. Data was analyzed using descriptive statistics and Friedman Test with the help of SPSS computer program. The demographic situation of the district is presented in Table 1.

Table 1: Basic indicators of health and hygiene in district

No.	Indicators related to the health	National indicators	Situation in 2007	Situation in 2012
1	General population	-	280,210	-
2	Life expectancy	55	49	**55**
3	Child mortality/000	50	20	**10**
4	Pregnancy mortality/0000	200	750	**500**
5	**Malnutrition of children%**	**10**	**20**	**10**

Source: Nyabihu District report (2007)

Through land use consolidation policy, Nyabihu District identified the paths in particular to develop irish potatoes, maize, and wheat, because these crops are specially adapted to the soil and climatic condition. Table 2 depicts the yields of crops planted in 2007 season A under land use consolidation.

Table 2: Production, yield and surface cultivated (Season 2007A).

Crop	Surface in ha	Yield in kg / ha	Productions in tons
Sorghum	0	0	**0**
Maize	5181	968	**5.013**
Wheat	3549	746	**2.647**
Rice	0	0	**0**
Beans	2667	715	**1.908**
Pease	192	661	**127**
Ground nuts	0	0	**0**
Soya beans	0	0	**0**
Banana	2569	6436	**16,534**
Irish potatoes	8741	7786	**64,167**
Sweet potatoes	1558	5836	**9,093**
Yams	821	4675	**3,838**
Cassava	690	6142	**4,238**
Market gardening	2318	7761	**17,991**
Fruits	1471	8186	**12,041**
Total	**29257**		**137,597**

The sample size for this study was based on the population cultivating maize under the land consolidation program. The following formula
n c = N X n / N+ n (Bouchard, 1995) was used to determine the sample size of 70 beneficiaries in Mukamira Sector, where N is the total population of the district; n is the selected sample from the study sector; and n c is the sample size. Therefore, n c= 70x96/70+96= 40.

The following formula n i= Ni x n/N was used to determine the sample size at cell level; where: n.i= the sample size proportion to be determined; n=the sample size; N=the total population and Ni= the population in stratum i. Interviews were conducted in the local language (Kinyarwanda) and data collected using a structured questionnaire. The questionnaire was written in English and translated to Kinyarwanda language to enable smooth administration of interviews.

The collected data were processed and analyzed using the SPSS programme (Statistical Package for Social Sciences version 17) to achieve both descriptive and inferential statistics. The Statistical tests applied include paired sample test, Chi-square test and Friedman Test.

3. Results

In Table 3, many farmers cultivated maize in Mukamira Sector. Maize has been chosen by Nyabihu District leaders in the LUC because that crop adapts easily to climate. About 51% of male and 429% of females (93%) of households surveyed cultivated maize crop. The varieties of maize cultivated before LUC is depicted in Table 2.

Table 3: Number of respondents who cultivated maize by gender in the sector

Products	Male		Female		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Maize	22	51.2	18	41.9	40	100
Total	22	55	18	45	40	100

Table 4: Varieties of crops grown in Mukamira sector before LUC

Varieties	Frequency	Percentage
Nyirakagori	5	11.6
Manyiginya	14	32.6
Mayizeri	19	44.2
Others	2	4.7
Total	40	100

In Table 4, about 12% of households cultivated Nyirakagori, 32,6% (Manyiginya), 44,2% Mayizeri and 4,7% grew other varieties.

The types of fertilizers used by households growing maize are depicted in Table 5. In Table 5, only two types of fertilizers were used; organic manures and inorganic fertilizers. From Table 5, 23.3% of respondents used organic manures only as fertilizer before land consolidation program.

Table 5: Fertilizer types used

Fertilizers	Gender			
	Male	Female	Total	Percentage
Organic Manures	3	7	10	23.3
Mineral fertilizers	0	0	0	0
Both	0	0	0	0
Total	3	7	10	23.3

The trend of maize production before LUC is depicted in Fig 1.

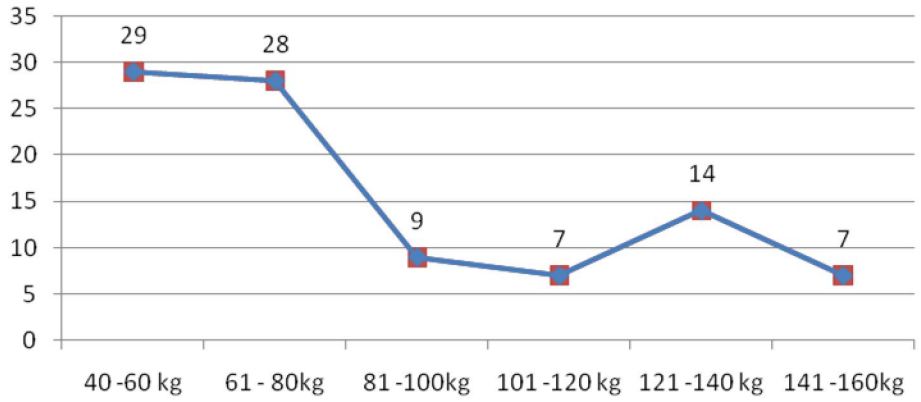

Figure 1: Situation of maize production before land consolidation

Figure 1 depicts maize yields produced by the respondents: 29% of households surveyed produced 40– 60 kg; 28% produced 61 – 80 kg; 9% produced 81– 100 kg, 7% (101-120 kg), 14% (121-140 kg) and 7% (141-160kg). Total production before land consolidation was 81,100kg for all farmers.

The varieties of maize after LUC are depicted in Table 6.

Table 6: Varieties of maize grown after land consolidation (LUC) by gender

Varieties grown	Gender			
	Male	Female	Total	Percentage
Tamira	3	4	7	17.5
Hybrids 6 x 18	8	7	15	37.5
Hybrids 6 x 3	8	4	12	30
Pool 9	3	2	5	12.5
Others	0	1	1	2.5
Total	22	18	40	100

Table 9 shows that four varieties of maize crop were cultivated after land consolidation in Mukamira Sector. A significant proportion grew hybrids 18 and 24. This indicates the increased access to selected seeds under the program**.** The proportion of households using organic manures, mineral fertilizers and both fertilizers is shown in Table 7.

Table 7: Fertilizers used after land consolidation program

Fertilizer types	Gender			
	Male	Female	Total	Percentage
Organic Manure	9	3	12	30
Mineral Manure	11	10	21	52.5
Both	2	5	7	17.5
Total	22	18	40	100

From Table 7, more than half of the households are using inorganic fertilizers (52.5%); 30% are using organic manures only and 17.5% are using both fertilizers.

The maize yields under LUC program is depicted in Fig 2. Figure 2 above indicates that 25% of households produced 100- 200 kg and 201 -300 kg; 17% (310-400 kg), 15% (401-500kg), 10% (501-600kg), 3% (601-700kg), 3% (701-800kg), and 3% (above 800kg). Total production of maize was 362,875kg for the 40 farmers.

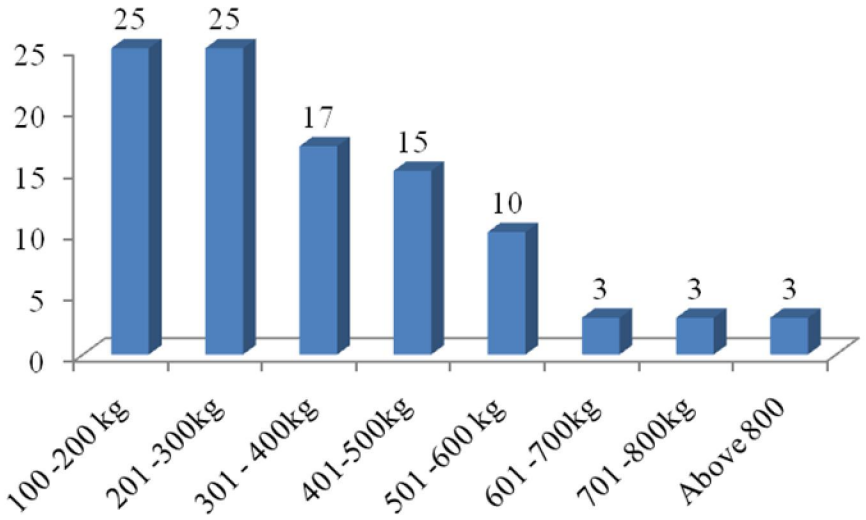

Figure 2: Maize yields after consolidation program

The average production of maize after land consolidation policy program shows that there is a 347% yield increase which indicates that land consolidation policy was very effective ($p<0.05$).

Table 8: Yield before land consolidation and after land consolidation

Kilograms	N	Average yield(kg)/household/season
Maize yield before	40	2027.5
Maize yield after	40	9071.875

4. Discussion

Land consolidation has always been regarded as an instrument or entry point for rural development. Early concepts of rural development were virtually the same as agricultural development because of the predominant role of agriculture in rural areas at the time. Improving the agrarian structure was viewed as being identical to maintaining the social viability in rural areas; what was good for the farmers was good for rural areas. An overall objective of early projects was thus to increase the net income from land holdings by increasing the volume of production and decreasing its costs. With this focus on agricultural development, these projects served to consolidate parcels and enlarge holdings and included provisions such as irrigation and drainage infrastructure to improve water management, construction of rural roads, land leveling, soil improvement measures and changes to land use such as converting agriculturally inferior land into forest land or wetlands (FAO, 2003). Such agricultural improvements are still essential but rural space is now no longer regarded as one of agricultural production alone. Concepts of rural development have become much broader and have expanded to include increased environmental awareness and a wide range of nonagricultural applications. The emphasis of land consolidation projects has shifted from a focus on restructuring

agriculture to one of achieving more efficient multiple use of rural space by balancing the interests of agriculture, landscape, nature conservation, recreation and transportation, especially when land is required for the construction of major roads.

The results of the survey indicate that Land Consolidation program has achieved some benefits to communities in the district through increased land use management awareness, adoption of improved maize varieties and fertilizers and thereby increasing substantially the per hectare household yields of maize.

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The Religious intellectualism of soft movement in Iran

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Abstract: Once in Iranian society, religion has been influenced by of political authority in associated with the public sphere undoubtedly increases public expectations of the effectiveness of political religion; but when the demands not fulfilled by the politicized religion, As a result Sense of religious restraint, is particularly prevalent in society. Since the Iranians are among religious people from the past to today, As a result, it is possible some have changed their religion as a negative struggle or at the same Religious, be neutral in the face of political religion. Consequently in response to a religious crisis, a religious intellectual movement is willing to act of criticism and ideas in the software against the religious property in the mental space of society. They are trying to influence people in the community with a sense of religion and their religious beliefs until Strongly Politicized religion will propel to socialization.
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Keywords: Religious intellectual movement, Religious base, religious reform movement, the Iranian religiosity, Software movement

1. Introduction

Political or social movements may be similar to each other in the contemporary world. But, essences of every movement will Identity proportional to its associated culture. However, in most developing countries and ideological movements is based on its cultural origins, can be identified in response to political or social conditions. In addition, Software movement is created in cyberspace by the development of information technology and modern media; which can be seen in the as Social perception with features of cultural identity. These movements do not have aspects of the populist, but also seek to change the attitudes in the Society that its success can be seen in the intellectual attitude of the next generation. Since, a sense of insecurity in the Iranian political culture Due to Political despotism and other factors in the Iranian society.

Obviously, the formation of the religious intellectual movement in the software, a response is due to the growth of the middle class and development of new media to depoliticize and Socialization of religion.

The political system does not control on the ideology of this media. Hence, Iran's religious rulers of some politicians consider the proceedings of religious intellectual movement as a soft revolution. The religious intellectual movement in Islamic communities, especially in Iran, It is not a legitimate form but it is based on the society needs as the flowing stream. So, in this era religious traditions have been placed in front of it. In other words, whenever the religion is been impasse about the subject and cannot respond to the demands of religious people; as a result, in response to this problem, it turns critiquing until can respond to social and political problems of religionists. This event is still continuing.

There has always been seen on History; but once religion is unable to respond, unintentionally will repeat and imitate. Especially when has been placed the management of religion in the hands of power holders and only it has an instrumental role intent to influence; in particular, the essence of religion Is connected to Tiki, ultra and the invisible Thus may be widespread superstition about it(Abbas Milani. (2004).

Thus the religious intellectuals will prevent this crisis. It defends the religion proportional to religious crisis. But the problem of religious enlightenment is most people who are seeking to profit from the religion. Since the group's identities are dependent on religion so combats any change and transformation that would threaten their interests and have no fear of violent behavior. Consequently, at this stage, the religious intellectuals will be faced with the kind of bellicose. Therefore, sometimes the religious intellectual movement it takes exorbitant costs, for religious criticism. It should be noted, Religious intellectuals may be wrong after successful and it is not religious trustee. Perhaps, the intellectuals have played a role in change and rationalization of religion and this can prevent to achieving their goal.

Thus it is necessary that the religious intellectuals, defines strategy as implementation of a project and clarify plans. So the religious intellectual movement should attempt t make out the religion from monopoly of custodians and provide the conditions for citizens up to religion, be accepted

without intermediaries. Among most important task of the intellectual movement of Religion, after idea generation, this is which tries up to change the dynamics of religion in the realm of modernism away from its custodians in the various forms of cultural, artistic and media to a rationally discourse. It means that tries using by literature, cinema, theater, music and so expands the discourse within the community (Hinton, William. 1972).

It should be emphasized that the religious intellectual When will become a movement the followers of religious traditions continue to repeat and imitate; In addition, their custodians would have defensive and adversarial postures against each new phenomenon. At this stage, therefore, the religious intellectual movement based on social conditions will become a revolutionary and try to change Religion based on the public interest in the development and modernism. It also reduced the incidence of social violence. At this point, the focus should be in the public sphere because if people do not perceive the importance rationally change, undoubtedly religious intellectual movement will be ineffective. The religious intellectual movement should not restrict the scope of its activities to entourage and tries to make relationship with the people, Could cause the public to be receptive to the reform and renewal of religion in society.

People will incline to the movement when they reached the bottleneck of the religious traditions; Especially In the religious development and justice Become doubt of custodians; importantly, the incapable of "social mobility". But the conditions for the occurrence of the religious intellectual movement will be provided when it has seen Change religion as combat the negative and create an optional approach, the dominant religion in society.

Therefore, it does not make religious intellectual movement in response to the religious traditions of society; when is formed in the community which wants to fight against a confined intellectual system with its intellectual critique. Because it is different Religious intellectual movement features Compared with other political movements – religious and will act more like software and intellectual movements; Because of this, more than anything else, has the essence of the mind. But it should not Virtue of its elite, has been no social base among the people. Especially when the society is composed of the religion, Otherwise Movement in a society where people are predominantly religious or religion-oriented would not be able to prove a relationship with the public. So the religious intellectual movement by religion can be acceptance and legitimacy to reform a system of traditional religious in public opinion; and however, it has been introduced itself to the public as a sectional-flow and explain is done to reform due to the crisis. Therefore, after the removal of religious crisis, would pay critical thinking to the dynamics of religion. Because it should be take care of the intellectual and critical position in every situation.

It does not act such a conventionally political or social movement. These movements that seem instead of streets and public should be formed in the minds of society. It continues to criticize the religion via modern and traditional media that will prevent the growth of superstition and religious despotism; and more than anything, to generate ideas to pay criticism until religion, has not been politics; Of course it is without impairing the religious sphere. In this way, the religious intellectual movement can continue in the field of social thinking, because this movement is created in the light of social connections and media.

Despite the different interpretations of it, Should not ignore the historical and cultural origins of the movement. Hence, it can manifest itself as mental and software support of Political and social movements in society; and give cohesiveness to civil and even political movements. However, the religious intellectual movement is bound to be critique of the use of religion as a tool. Secondly, it is necessary to influence thoughts is generated, based on cultural and religious.

Thirdly, constantly to prevent superstition among the people teach them to criticize religion. Fourthly, after the essential approaches; should be targeted for justice, spirituality and ethics. Then the religious intellectual movement could spread to the political - religious authority. It can even be hidden in the Authority to gradual reform. Phenomenon which is a part of religious intellectual movement and it could form the religious authority after the Islamic Revolution in Iran. In this way, the intellectual movement has been able to advance its religious reform of the power (Farhad Khosrokhavar. (2004).

Even if the religious intellectual movement wants have adhered to the concepts of Western intellectual, it is better that is not separated from the structure of thought and Iran - Islamic culture. Otherwise it will lose opportunities with the public (Farhad Khosrokhavar. (2004). According to the historical and cultural experiences should offer religious ideas of Religious intellectuals based on spirituality, especially social religion to cut human suffering in the light. In addition, instead of engineering religion will attempt to resolve the properties of religion and popular religion (Walter L. Hixson. 1997).

It should make a religion out of Specificity until to avoid confusion with the religious intellectual at the same simplicity (Gheissari, Ali. 1998). Most

importantly must try to separate the sacred from the non-sacred. Overall religious intellectual movement needs to determine their religious bases for people in the process of social and political communication.

And so does not use independent intellect or Kantian wisdom. For a critique of religion does not use only the independent intellect, as a result of this religious intellectual movement will lead to a social mass crisis with religionists? So the first step is to advance the religious intellectual movement in Iran, Movement must move from the Religion base and the first, its argument is equipped with the religious intellect and Iranian culture until the religious intellectuals to be able to influence the process of social interaction. Hereby can engineer public opinion in favor of the religious reform; since criticism base has been evaluate more difficult to criticism (Hassan Mahmoud Khalil. 1994).

Therefore, if religious intellectual has not religious belief, in religious intellectual movement will not be able to establish a relationship with the audience. Otherwise, the same intellectuals of the Constitution (1906) will be lost Sincerity of the religious intellectual movement in the audience. Although some religious intellectuals, it is necessary the movement criticize modern religion and prevalent religious traditions in all spheres of social. It will not help the movement communication process. However, Even if you believe the criticism as a process of gradual; first, the critical tools should be the base of the religion until it is effective in causing doubt in the public sphere. Moreover if you want to criticize religious texts from the perspective of modernity, so "the acidity of reason" has been changed religious identity as the ruins. In these conditions will deny any ideology.

In the event, should see the principle of social solidarity. These matters are not testable. In that case, the intellectual movement involves the transformation and change in the time process. In other words, what is the last product of the religion such as Justice, morality, Spirituality of religious do not have need to test. On the other hand, the nature of the intellectual movement is calculated Apart from the person of the intellectual (Noel W. Thompson. 2006).

In religious intellectual movement, it is necessary to consider many things such as ideology but the intellectual is not required to follow it. The tendency of the intellectual to ideology can be considered a commitment.

After 34 years of the Islamic Revolution in Iran, Have not seen integrated intellectual movement due to ideological aversion of intellectuals; if they sometimes have led to the uprising of civility, it is generally based on an individually idea. For example, the attitudes of Soroush led to the emergence of the reform movement in the middle-class society, especially religious. It was the reformist movement that was created in the 23 may 1997 with the election of President Khatami. But reform could occur from the perspectives "Soroush". Because, Soroush could design new religious ideas, the society has ready for criticism. While the before it was less dare to criticize religion. However, Soroush as a religious intellectual and philosopher of religion is first could establish a reformation to the revolution. After the Soroush views expressed, some religious intellectuals have ventured to follow him to criticize religion and surrounding; As far as they have criticized of Soroush. However, Soroush was successful because not only he had dominated the traditional areas, but always reformation was considered as his concern. Religious intellectual movement, needs to internal correlation. Hence, it is not possible for Iranian religiosity.

Unless it is done on the basis of the religion bases, otherwise, the historical and contemporary experience has shown that intellectual could not be solidarity. This implies that the Iranian society is “Religious “in terms of cultural. Thus coherence of Intellectual, between religious intellectuals is easier than secular intellectuals. Thus, the secular intellectuals want to work as an intellectual movement in society, they are compelled to start from the rationality of religious at the first step as the transition period; Of course, if they want to have the protection of their intellectual middle class especially, the majority of religious people. Due to this, today, the role of religion is growing with a description of the intellectual and spiritual despite harsh modernization invasions in the early 21st century.

As a result of the religious intellectual movement, has been created for religious reform according to human need and wants to improve religious capacity to its exploit. It wants to use the potential of the constitution of the Islamic Republic of Iran, follow religious reformation to realization of democracy. In addition, it is fighting with some profiteers who are dependent on religion (Johnson, Paul. (1988).

However, today religious jurisprudence has more important than religion. Therefore, it is necessary to change them for sustainability; As a result, it spread as a religion in today's Iranian society it is the jurisprudential that it has an unclear relationship with religion. Even in same, the jurisprudence has been positively wallowing by their custodians the doctrine of Ijtihad in the Shia religion. The " Ijtihad " can modernize the jurisprudence according to time and The intellect may be more

highlight than in Shari'ah; Particularly, is created modern jurisprudence in Iranian society. While, The part of Iranian intellectuals, according to intellectual dogmatism want without any regard to (Evolutionary Quantization) are corrupted by independent intellect, whatever as a religious beliefs of the community ever formed. This means ignoring the beliefs that which is located in the popular during the many years.

Consequently, the influx of modern intellect, without notices, Religious believers will get defensive against the arrival of each of modern reason. Some religious intellectuals and secular intellectuals Attempt to sociological research on religion to localizing of modernity and at least want not cause the religious sensibilities of the public sphere in the first step.

When Islam was entered to Iran by the Arabs during multiple attacks and when the Arabs were trying to pervasive Islam in all parts of Iran, It lasted nearly two centuries. Since then, Iranian intellectuals have begun modifying Islam based on the insight that they were gained as Iranian style. After this is that was born, the golden era Or the Islamic Civilization in Iranian society. Because the Iranians, with attitude Cyrus and the Achaemenid era have offered a new interpretation of Islam that it has led to the universal religion. So that in terms of civilization has been far more perfect than its origin in Saudi Arabia. Thus, from the third century AH on that becomes identity Islamic civilization against the other civilizations. As far between Shia and Sunni occurs confrontation and hostilities in the political arena especially during the Seljuk (fifth century AH); and then continue fearsomely instability by the Mongols up to the tenth century AH. Finally, the advent of the Shia in the Safavid state, The National unity is created in Iranian lands. Since the Shia is powerful and it finds itself in conflict with the Sunni Especially in the Ottoman Empire Undoubtedly, for self defense, takes place defensive postures. In this case, has been withdrawn tolerance and moderation religious from The Shia; and to protect itself against the Sunni of religious attacks Comes to dogmatic.

Although previously during, the fifth century AH coincided with Eleventh century AD has been weakened Ijtihad and rationalism in Islam in the political conflict between Shiites and Sunnis. Since the Muslims were suffering an epistemic discontinuity who are suffering due to the loss of the Islamic rationality. It was far from Mu'tazilah doctrine and philosophers like Farabi and Ibn Khaldun (Majid Fakhry, *Al-Farabi,* (2002).

After this period that Islam gradually became the dogmatic nature due to conflict between Shiite and Sunni. In this regard, it is noted that Arab intellectual's abed al-Jabri, However, the classical period of Islam, six of the first century AD, can be compared with the European modernity (Contemporary Arab Thought. 2004).

So that "Jabery" underscores in this era the Islamic intellect is more tangible than the intellect of "Arab ethnicity". Despite the domination of the Seljuks and the Mongols on the regions of Iran, before the formation of the Safavid stat, Religious thought, was not so dogmatic as compared with the Safavid era to the present. In other words, the authority of the jurisprudence has been created in the community from the Safavid period onwards and will make a stop at the idea production and even contemplation. Before the Safavid dynasty, Shiite imams were no such attributes, infallibility…

Thus, the Middle Age that Frye refers to as the Islamic civilization could be the first critical base for religious intellectuals of today's Iranian society. At that time, scientists have been able to express new characters: the Sheikh al-Rumi, Hafiz, Saadi, Ibn Sina, Suhrawardi and that the result is an interpretation mystical – Spiritual which is seen as a religion of toleration and Indulgence. Some religious intellectuals also expressed that the religious intellectual project can not be completed by a means of such rationality. Therefore, by using of interpretations is about religion in the "golden era" Can be examined in terms of its epistemic. In this regard, Mostafa Malekian, Including intellectuals and philosophers, is divided Islam into three levels: "Islam Level One", which was formed during the lifetime of the Prophet Muhammad. "Islam Level Two" by scientists and other Islamic areas was created in the middle era. "The third level of Islam" is the practical experiences of Muslims from Islam in history. Islam is prevalent among the people as ritual; such as the religious delegations, shrines, mourning rituals, clerics, mosques, etc. Hence can be criticized the third level Islam by Islam Level Two that for religious intellectuals, because of religious Iranians, Not only is regarded as the suitable base for ideas but also can criticize the religion.

Because of lack of religious base is costly for the intellectual, Behalf of the religious people and religious authority will impose religious intellectuals. Unless, religious intellectuals do want to communicate with the general populace; But when it wants to become an intellectual movement consequently needs to relationship and influence on the intellectual and religious people; hence, it can not consider the public interests. Some religious and secular intellectuals that can research just about Religion, They believe that should not sacrifice fact to interests and religion even if it reduces the suffering of the people. However, not only this is a

common "truth" with the intellectual movement; but also it is indefinable.

So the intellectual currents - both religious and secular - they want to make rational and epistemic changes in the society as a rise of software and intellectual; must support the part of the population that these people could be middle class that are able to establish the mentally relationship with the intellectual. On the other hand, it should be common awareness among the intellectuals and the mass of the people, especially in the form of software movement.

Otherwise, an intellectual due to the inability to establish a relationship gets: autonomy, fantasies and narcissistic. Thus, religious intellectuals in addition to the religious beliefs of its members, Based on the inappropriate experiences of secular intellectuals – have been created the proper identity Based on Understanding of the culture of the society, It has gradually become the rise of software and epistemic in society by the development of new media till to establish effective relationships with people will change Political religion to social religion. Secular intellectuals have failed to eliminate the role of religion by modern rationalism. For this reason, today the intellectual movement considers necessary to strengthen spiritual - social religion against Political religion in the attitudes of the public. Whatever the religion will the more political takes defensive mode against the pressures and threats of modernism.

Because they had to maintain their status, is encountering By Any other opinion and the relationship as adversarial. In addition, in the minority and threats to the religion various aspects such as Sunni, The reasons are for dogmatism in Shiite religious after the Safavids. So that becomes institutionalized in the Iranian culture. However, in the Shiite culture, "insecurity" is of these attributes of religion in the period (Dahl, Robert. 2000).

Although the origin of Shiism is primarily based on the nature of the opposition movement and this opposition is determined by the political leadership – religious. Which is defined later the struggles and political views of Shia as the concept of the "the Hidden Imam".

Thus, Shia is waiting for the Mahdi In order to establish their ideal government. They want to make justice by political authority on the society. However, in response to this problem, some religious intellectuals in Iran's Shiite of society attempt to cover the stature of Shiism by Modernity till reduce the political aspects of religion (Jahanbakhsh, Forough. 2001).

Because Ali Shariati, including religious intellectuals was in Iran before the Islamic Revolution, Could give cohesiveness between students and middle-class society by Social aspects of Shia In order to Cultural Revolution; However this type of religious intellectuals with Marxism and modern structures.

He criticized of modern Shia beliefs (which is attributed to the Safavid era and later) by the Shia Alawite doctrine (before the formation of the Safavid state). Such an approach in Iran's religious community has emerged as a powerful which tends to Clergymen of the Shiite to the religious intellectual movement. So, today, those are among Clergymen who have joined the religious intellectual movement and always defend the social religion against political religion. Although between the current Shia Clergymen - including clerics - who are also upset that the Shiite government forming.

However some senior clerics and Clergymen oppose with the politicization of the Shia religion, especially government forming by Clergymen the; but this is not the intellectual attitude. This approach seeks to preserve the traditions of the past. Apparently they want to freeze religion in a range of traditional. In case religious intellectuals, including talented seminarians, are seeking to modernize religion and they try to criticize Shia Sharia from the bases of religion, which is based on the kinds of reciting of the Safavid dynasty, in jurisprudential reasoning.

They want to promote religious Indulgence and tolerance to protect the Shia religious community in Iran by modern jurisprudence and Ijtihad. These seminarians think that Disagreements between Shia and Sunni is unfounded According to theological and historical. For this reason, historically conflicts between the two major religions of the world have the political aspect that gradually extends to religious beliefs that are not based on the intellectual and religious. Because most of their religious texts belong to the median age was not composed Safavid rule.

That is why they are called Shi'ite Alawite. Ali Shariat is the first religious intellectuals that describe time classifications in the formation of Shiites: as Alawite Shia and Safavid Shia. Not only this kind of division of the Shia is not true of traditional Clergymen, but some of them have distorted interpretations of religious and intellectuals In terms of theological tradition and Shiite jurisprudence and are convinced that this is influenced by other of schools of thought in the Islamic world and philosophical Rationality. According to Ayatollah ... Mojtahedi, the major seminary in Tehran, This group of intellectuals Clergymen that they are intermediate between Shias and Sunnis that were removed from Shia theological principles and into Sunnis have similar (Mill, John Stuart. 1984).

Thus, from the perspective of leading Shiite clerics and even some politicians of Iran, they are similar to the Wahhabi. Today, several factors have provided the necessary conditions for criticism such as: The mass media, Massive sources of religious information in the Internet spaces. Moreover, some intellectuals are conservatism in the exploitation of religious texts of the religious society. Perhaps, the studies issue of secular intellectuals would be the religious according to social necessity, not only do not take a serious revival of religious enlightenment, But, according to Iran's political culture of conditions, it is considered a stage of transition to modernity. So do not think in any approach except for the religious (Ahmad Sadri and Mahmoud Sadri).

For this reason, their attitude toward religion is instrumental aspects to make modernism. Because the consensus of many religious intellectuals (Including religious believer and non-believer) which indicates that it is possible for any social reform of Iranian society by the religion, Especially in order to make modernity and democracy; Otherwise, according to historical experience, will not reach result both religious and non-religious intellectual movement. Because of any particular social movement, political or cultural relations will be required to religious people. It may be that there are many of intellectuals and political currents in religious intellectual movement Because of transition period and Glasnost.

There may be various groups: Some do not believe in religious Sharia, or they know separate Sharia from religion. Some may believe that modern religious Shari'a and somehow are looking to modernize the jurisprudence and other intellectuals are merely their studies on religious beliefs and religious culture of Iran, Therefore, it is likely that the person will not have of religious belief; But they all in a movement known that Any modernization process Should be carried out within the context of religion, due to "religiosity" of Iranian. Their religiosity in Iranian culture has caused some groups opposed to the Iranian regime while belief of modernity, are used Shiite to achieve social justice and democracy. Even other opposed groups, Such as social groups, etc., are looking to modernize by the Shiite Iranians. Before the Islamic Revolution These groups were known as the Islamic Marxists because they knew Shia religion lack of Modernity.

Although today they have not forgotten the Marxist approach However, the modern Shia belief is considered, as well as the intellectual foundation for the group. This implies that we can not find any aspect of culture that is not the result of the presence of Iranian religion. Even first thing as the Constitutional Revolution (1906) occurs in European style, Religious considerations are undoubtedly the greatest obstacle to the formation of the secular intellectuals. Therefore, they are forced to consider the issue of religion in the process of modernization society. Quoted from "Hamid Algar," Malcolm Mirza Khan, the first generation of Iranian secular intellectuals have always tried, to introduce modernity in a way that is not opposed by the Shiite religious scholars, Introduction of modernity in a way that is not opposed by the Shiite religious scholars (Mohammad M. Shabestari. (2004).

Therefore the secular religion that was cognitive of the Iranians religious, Emphatically Are advised to the approach of religious community. Despite the religious content in Relevance and effectiveness of the method, is not deemed unreasonable any changes and tactical movement; especially method can be changed according to the time (Mirsepassi, Ali. 2006).

However, Ijtihad and inferences are different methods of Jurisprudence That the Jurisprudence of Iranian society has neglected. That this method could be used intended to provide religious indulgence and negligence as exit out of as religious ideology with dogmatism. Thus, the secular intellectuals were considered in the constitutional jurisprudence using a device for advancing their own goals. Hence, Yousuf Khan Mostasharoldoleh, was explaining the rules of the west and French with the religious appearance and trying to promote the idea of modernity with religious rules till has not been their thoughts in conflict with the religious beliefs of the majority of people (Seyyed Hossein Nasr, 1996).

So, sometimes Mirza Malcolm Khan would recommend his intellectual friends Attempt to refer to the statements the Islamic sources when explaining the principles of Western modernity. However, Intellectuals religious expression, it was explained in the Constitutional Era Apparently, based on religious beliefs and religious people. But in fact, Religion was not only instrumental aspect for secular intellectuals. Undoubtedly, are considered as a way of establishing a relationship with the people, But after the Islamic Revolution Religion is an instrumental aspect. But religion is considered their beliefs. However, they are trying to bring religion into politics came out of the process of secularization and in accordance with the conditions of society, must be present in the social field, Should be limited to the private sphere or of individuals.

The religion of Islam is capable of the most severe social crisis better than any other unifying element is done to maintain strong social cohesion. Even the people and communities torn apart in the same geographical and cultural distances can to respect to a subjective unit of society against an

enemy. In addition, in the following Islamic religion, there are religions that developed in the social and political crisis and are consistent with the conditions, so that is still alive and strong. This resistance is due to its religious origin; such as Shia has always been overly influenced by the space of instability and insecurity that existed in the history of Iran. In other words, a Shiite has specific their religious psychology that is influenced by multiple factors in the course of history and political culture. Unintentionally, the defensive aspect can be seen as compatibile. So the kind of sense of insecurity is in its religious culture. Apparently, Shiite has played a role in the political and social solidarity; Because Shia hardly is being formed within the individual. Basically Iranian human individuality has been identified in historic continuity from the Safavid period in the religious community. Iranian intelligentsia, both secular and religious, could to act successful when is familiar with Iranian traditions, both religious and national orientation.

It can change Iranian cultural conditions with a sense of tradition and modernity. After twenty centuries was undermined religion by modern, therefore, it is important the enlightenment, but twenty-one centuries, show that religion is returning with new interpretations. This event is not coincidental, thus, the question is posed in what is the reason for the return of religion even in modern developed societies? Numerous responses may be given; But the most important reason that can be raised, new media to dealing with religious matters in the world today (George B. de Huszar, ed. 1960).

Apparently, religion is for people far more tangible than in the past. Some also believe that religion is returning to pre-Secular. This assumption is currently growing. So, today the religious intellectual movement in Iran, In addition, Iranian religious display, indicates the expression of the religion in the global arena. For this reason, Soroush believes is not been modified in any society, unless of religion. Hence, this reason is defined as the failure of secular intellectuals in Iran. The problem is not so serious religion and if they show a significant, it is not necessary to understand the scope of religious traditions. Religious intellectuals, it is essentially that has been religious character as the socio – political reaction till order to offer improved in the realm of mind and social environment (Mahmoud Sadri. 2001).

Moreover, the reform is effective that while criticizing to the political - religious system in the society should be Communications. There is speculation that because of the multiplicity of ethnic, religious, cultural in Iran, It has been shown that it is possible to threaten and endangered the territorial integrity and national identity of by secular lifestyle (Mahmoud Sadri. 2001).

This rule is based on religious beliefs of the most religious community could provide grounds for emotional solidarity. This is as historical experience and is able to inhibit the degradation the country. Therefore, it is possible to remove religion from society may have consequence of the identity crisis. Unless the generational replacement, is performed simultaneously with the evolution of culture; Otherwise National Solidarity should be protected from the Reformation and modern interpretations. Religious intellectual movement may not Unless reform is inherent in society by instruments and art forms such as film and television, theater, literature, music and ...

That's why, even with the removal of authoritarian regimes in the Middle East, including Egypt, Tunisia, Libya, Iraq, Afghanistan, Yemen and ... has been Observed for their modern constitution have a deep interest in religion. This shows that Islamic societies are not assured to western modernity Due to the subjective awareness of the colonialism (Riccardo Bavaj. (2010).

yet some secular intellectuals are insisting that Religious intellectuals are detrimental for their Iranian intellectuals. But such an approach, intellectuals will fall into the trap of ideological. The intellectual who wants to rely on his Religion Or is believed to religious paradigms such as Jalal Al Ahmad and Ali Shariati (before the emergence of Islamic Revolution of Iran) again falls into the anti-West. As far as the religious intellectual movement will be forced to start again from scratch; In other words it should experience the mind of the West again (Mirsepassi, Ali. 2006).

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Carbon Footprint for Paddy Rice Production in Egypt

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Abstract: Emissions resulting from rice cultivation are estimated in this paper including emissions from mechanical operations, field burning and N fertilization. The estimates are constructed using data and procedures from the IPCC guidelines for emissions estimation Coupled with Life Cycle Analysis procedures. The results show that the larger amounts of emissions come from Lower Egypt (Nile Delta). The regions with higher emissions are located as a rice belt in the Northern of the Nile Delta, Methane emission from the flooded rice fields are the main source of GHG emissions, contributing about 53.25 % of the total emissions. Rice straw burning after harvesting is the second largest source contributing 35.82 %. Nitrogen fertilization contributes out 9.92% and mechanical activities contribute about 1%. Finally, the carbon footprint for paddy rice is 1.90 Kg CO_{2eq} / Kg paddy rice.

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Abbreviation:

Carbon footprint (CFP) – also named Carbon profile - is the overall amount of carbon dioxide (CO_2) and other greenhouse gas (GHG) emissions (e.g. methane, nitrous oxide, etc.) associated with a product. The carbon footprint is a sub-set of the data covered by a more complete Life Cycle Assessment (LCA) (**ISO, 14040**)

1.Introduction

With the accumulating evidence on climate change, there has been interest in examining the greenhouse gas (GHG) contribution of production practices and products as a mean of identifying intensive emitting options that could be target of GHG mitigation actions. Such a GHG emission level estimation is often called a carbon footprint[1]. Agriculture is one target of such activity as emission levels are about13% of the annual GHG emissions that are related to all human activities (**Olivier *et al.*, 2005 and Harada *et al.*, 2007**).

Rice cultivation is one activity that has received attention as a GHG emitter (IPCC, 2007). Rice is important in Egyptian agriculture, with Egypt being the largest rice producer in the Near East region (**Abdulla, 2007**). Total area used for rice cultivation is approximately 600 thousand ha or about 22% of all cultivated area in Egypt during summer (**Tantawi and Sabaa, 2001**). The average yield is 8.2 tons/ha with an approximate straw production of 5-7 tons/ha (**Sabaa and Sharaf, 2000; Badawi, 2004**).

Rice is an important emitter of methane (CH_4), one of the major greenhouse gases (GHG). According to the Intergovernmental Panel on Climate Change (IPCC), the warming contribution of CH_4 is 19–25times higher than that of CO_2 per unit of weight based on 100-yr global warming potentials **(IPCC, 2007**).

Agricultural activities are responsible for approximately 50% of the anthropogenic emissions of CH_4, with rice paddies contributing over 10% (**Scheehle and Kruger, 2006; USEPA, 2006**).

The Intergovernmental Panel on Climate Change (IPCC, 2007) estimated the annual global emission rate from paddy fields averages 60 Tg/yr, with a range of 20 to 100 Tg/yr. This is about 5-20 per cent of the total CH4 emissions from anthropogenic sources. This figure is mainly based on field measurements from paddy fields in the United States, Spain, Italy, China, India, Australia, Japan and Thailand (**IPCC,1997**).This carbon foot print is mostly composed of the methane production from flooded rice (67%) and the deforestation effect (29%) due to the persistence of 149 000 ha of hill side slash-and-burn land use change for rice production (**Bockel *et al.*, 2010**).

Observed seasonal rice methane emissions from around the world show large ranges, reflecting the effects of local as well as regional differences in agricultural, biological, and climatic factors. (**Wassmann *et al.*, 2000)** compute an average median emission value of 27.23 g m^2, with a range from less than 1 g m^2 to 155 g m^2.

The burning of rice residue is a another emission source yielding carbon dioxide (CO_2),

methane (CH_4), and nitrous oxide (N_2O), plus pollutants such as carbon monoxide (CO), particulate matter (PM), and toxic polycyclic aromatic hydrocarbons (PAHs)(**Lemieux *et al.*, 2004 and Duan *et al.*, 2004**).

Emissions of N2O may also occur. Direct sources include emissions from cultivated and fertilized soils. Indirect emissions result from transport of N from agricultural systems into ground and surface waters with subsequent emission as ammonia or nitrogen oxides **(Xu*et al.*, 1997; Mosier *et al.*, 1998)**. Methodologies for calculating both direct and indirect emissions of N_2O related to agricultural production take into account anthropogenic N inputs including synthetic fertilizers, animal wastes and other organic fertilizers, biological nitrogen fixation by crops, cultivation of organic soils, and mineralization of crop residues returned to the field (**IPCC, 1997**).

With reference to CO_2 emissions, agricultural practices may be grouped into primary, secondary and tertiary sources (**Gifford, 1984**). The main sources of farm level CO_2 emissions are either due to cropping operations (e.g., tillage, sowing, harvesting and transport) or stationary operations (e.g., pumping water, grain drying). Therefore, reducing emissions implies enhancing use efficiency of these operations by conserving inputs used in the operations, and using other CO_2-efficient alternatives (**Lal, 2004**).

The aim of this study was to estimate the GHG emissions from Egyptian rice fields in terms of the emission from rice cultivation, mechanical operations(irrigation pumping, tillage, harvesting), nitrogen fertilization and burning rice straw. Finally we calculate the carbon footprint taking into account all GHGs associated with paddy rice (kg-CO_{2eq} / Kg paddy rice)

2. Material and Methods

2.1 Study area

This study focus on the major rice cultivation areas in Egypt especially that along the Northern Coast. This study considers emissions in four major regions Lower, Middle, and Upper Egypt plus lands out of the Nile Valley. The cultivated area of each governorate was collected from the statistics of the Ministry of Agriculture and Land Reclamation forth years 2008 to 2011. Rice in Egypt is planted as a summer season crop generally under flooded conditions. Urea and synthetic fertilizers are predominantly applied with significant organic matter application (about 15 -20 cubic meters of cattle manure per hectare). Rice straw is normally left in the fields after harvest in September and October, and most of it is burned. Greenhouse gas emissions from rice occur during the growing season and upon burning rice straw.

2.2 Methane emissions from rice cultivation

The annual amount of CH_4 emitted from rice is a function of the number and duration of crops grown, water regimes before and during the cultivation period, and organic and inorganic soil amendments **(Neue and Sass, 1994; Minami, 1995; Harada *et al.*, 2007)**. Soil type, temperature, and rice cultivar also affect CH4 emissions. Therefore, the basic equation to estimate CH_4 emissions from rice cultivation is shown in Equation (1) Based on **IPCC (2006)**. CH4 emissions are estimated by multiplying daily emission factors by cultivation period of rice and annual harvested areas.

$$CH_{4\,Rice} = \sum_{i,j,k} \left(EF_{i,j,k} * t_{i,j,k} * A_{i,j,k} * 10^{-6} \right) \quad (1)$$

Where:

$CH_{4\ Rice}$= annual methane emissions from rice cultivation, in Gg CH4 yr^{-1}

EF_{ijk}= a daily emission factor for *i*, *j*, and *k* conditions, in kg CH4 ha^{-1} day^{-1}

t_{ijk} = cultivation period of rice for *i*, *j*, and *k* conditions, in days

A_{ijk}= annual harvested area of rice for *i*, *j*, and *k* conditions, in ha yr^{-1}

i, *j*, and *k* = represent different ecosystems, water regimes, type and amount of organic amendments, and other conditions under which CH_4 emissions from rice may vary

Emissions for each different region considered are adjusted by multiplying a baseline default emission factor by various scaling factors as shown in Equation (2). The calculations are carried out for each water regime and organic amendment separately as shown in Equation 1.

$$EF_{ij} = EF_c * SF_w * SF_{pj} * SF_o * SF_{s,r} \quad (2)$$

Where:

EF_{ij} = adjusted daily emission factor for a particular harvested area

EF_c = baseline emission factor for continuously flooded fields without organic amendments

SF_w = scaling factor to account for the differences in water regime during the cultivation period (Continuously flooded = 1, error range= 0.79-1.26 based on??)

SF_{pj} = scaling factor to account for the differences in water regime in the pre-season before the cultivation period (less than 30 days= 1.90, error range=1.65-2.18 source)

SF_o = scaling factor that accounts for differences in both type and amount of organic amendment applied (from Equation3) source

$SF_{s,r}$ = scaling factor for soil type, rice cultivar, etc.,

On an equal mass basis, more CH4 is emitted from organic amendments containing higher amounts of easily decomposable carbon and emissions also increase as more of each organic amendment is applied. Equation (3) and the default conversion factor for farm yard manure present an

approach to vary the scaling factor according to the amount of farm yard manure applied. (IPCC,2007).

$$SF_o = (1 + \sum_i ROA_i * CFOA_i)^{0.59} \quad (3)$$

Where:

SF_o = scaling factor for both type and amount of organic amendment applied

ROA_i= application rate of organic amendment *i*, in dry weight for straw and fresh weight for others in tonne ha^{-1}

$CFOA_i$= conversion factor for organic amendment *i*(in terms of its relative effect with respect to straw applied shortly before cultivation)

According to IPCC 2006, Guidelines for National Greenhouse Gas Inventories, the default conversion factor for farm yard manure is equal 0.14 with an error range of 0.07-0.20.

2.3 Greenhouse gases emission from field burning

Based on 2006, IPCC Guidelines, the emission factors for burning of rice residue can be estimated using equation 4.

$$L_{fire} = RB * EF * 10^{-3} \quad (4)$$

Where: L_{fire} the burning emissions in Mg ha^{-1} is the amount of emission from burning of rice residue; RB (Mg) is the amount of rice residue on a dry matter basis that is burned in the field in kg ha-1; EF (g kg-1 dm) is emission factor. The default emission values for rice straw burning of different greenhouse gases are tabulated in Table 1.

Table(1): Default value for emission factors for rice residues open burning.

	Gef (g kg^{-1}dm)
CO_2	1185
CO	113.2
CH_4	2.7
N_2O	0.07
NO_x	3.1
$PM_{2.5}$	27.63
PM_{10}	13
Black Carbon	0.69

According to 2006 IPCC Guidelines

2.4 Greenhouse gases emissions from Fertilizer application

The average nitrogen fertilizer application for cultivated rice is about 285 kg. N / ha. The emission of N2O from rice field was estimated following **Bouwman (1996)**, using the following equation for N_2O emissions from agricultural soils:

$$E = 1 + 0.0125X F \quad (5)$$

Where E is the emission rate (kg N2O-N ha-1), the 1 gives the background emission rater and F is the fertilizer application rate (kg N ha-1 y-1).

There is also one ton CO_2 per ton of N applied that is generated in manufacturing.

2.5 Greenhouse gases emission from fuel consumption

Egyptian agricultural engineers have compiled average values for power requirements and fuel used per hectare for specific farming tasks in those regions as shown in Table 2 (**Grisso *et al.*, 2004**) these figures assume typical conditions and average working depths and may be used to make fuel estimates for the indicated operations.

Predicting fuel consumption for a specific operation can be estimated using the following calculation according to **ASAE (1998)**:

$$Q_i = Q_s \text{ x } P_{db} \quad (6)$$

Where:

Q_i = estimated fuel consumption for a particular operationin $L.h^{-1}$

Q_s = specific fuel consumption for the given Tractor L/Kw.h

While, a specific fuel consumption (Q_s) estimate may be calculated from the equation as follows(**Grisso *et al.*, 2004**):-

$$Q_s = 2.64 \text{ x} + 3.91 - 0.203 (738 \text{ x} + 173)^{0.5} (7)$$

Where; (x) is the ratio of equivalent PTO power required by an operation to that maximum available from the PTO, this ratio depending on draft and speed of implement.

Power requirements for thresher and mower:

To estimate the engine power during threshing and mowing operation, the fuel use was measured immediately after each treatment. The following formula was used to estimate ending used engine power (EP) according to **Hunt Donnell (1983).**

$$EP = [\ f.c\ (1/3600\ )PE \text{ x } L.C.V \text{ x } 427 \text{ x } \eta thb \text{ x } \eta m \text{ x } 1/75 \text{ x } 1/1.36\ ] \quad (8)$$

Where :

$f.c$ = The fuel consumption, (L/h)

PE = The density of fuel, (kg/L) (0.823 kg/L)

L.C.V = The lower calorific value of fuel, (11000 k.cal/kg)

ηthb = Thermal efficiency of the engine, (35 % for Diesel)

427 = Thermo-mechanical equivalent, (Kg.m/k.cal)

ηm = Mechanical efficiency of the engine, (80 % for Diesel)

Table(2): Average energy-use rates and fuel requirements for farming tasks

Operation	Energy-use rate, PTO hp-hrs/acre	Diesel fuel, gal/acre	Diesel fuel Liter/ha
Chisel plow	16	1.1	13.4
Combine, small grains	11	1	12.2
Mower	25	1.8	21.6
Thresher	20	1.4	16.8
Water pump (8 hp)	24	1.7	20.4

3. Results and Discussion

3.1 Distribution of rice cultivation in Egypt:

Total rice cultivated and burned from 2008—2011 is tabulated in Table 2. Note the burned rice residue is smaller with composting, manufacturing and other uses being employed on about 40% of the land (according to **EEAA, 2009**). We assumed that the amount burned is stable during the studied period (Table 3).

The largest rice cultivation area a occurs in the Behira, Kafr_El Sheikh, Dakahlia, and Sharkia governorates and these area Northern Coastal zone Governorates in the Egyptian "rice belt".. After that region, the Lower Egypt region (Nile Delta) has the next largest rice cultivation area.

The highest total rice cultivation was recorded at 2008 at about 739 thousand hectares, these area was decreased by about 170 thousand hectares in 2009 years (after a new policy regarding flood irrigation). The rice cultivation area decreased again at 2010 to be about 456 thousand hectares, but this area increased again at 2011 to be about 588 thousand hectare, but then the rice cultivation area increased in 2011 perhaps due to the 25 January revolution and a lack of government enforcement.

3.2 Annual CO_2 Emission from Machinery activities:

Table 3 shows the calculation results for annual CO_2 emission from machinery activities from 2008 till 2011. Most (76%) of the CO_2 emission production result from irrigation water pumping using diesel pumps.GHG emissions from mower activities contributes about 7.7 % of the total machinery emissions while thresher and combine together contribute about 10 %. The highest annual machinery emission was recorded in 2008 due to the high amount of rice cultivation area. Lower Egypt has the highest GHG emissions because has the largest rice cultivated area (Table 4).

In Egypt flood irrigation predominates for rice production, water is poured into a paddy field until reaches a certain height relevant to plant stage of development. Periodically the irrigation is repeated until the crops are mature and ready for the dry harvest. The roots are kept under water for most of the crop life. The energy required to pump water depends on numerous factors including the water flow rate and the pumping system efficiency (**IPCC, 2006**). The energy use depends on the water table depth or the lift height. The diesel pump system could be as close as possible to the water source or be made floatable to be moved along the irrigation canal. The overall irrigation efficiency is higher as less percolation and drainage losses occur along the open ditch conveying systems. This system need slots of pumping energy and thus pumping uses the most fuel (**Abdulla, 2007**; **Tantawi and Sabaa, 2001**).

3.3 Annual CH_4 and CO_2 Emissions from rice cultivation:

Data in Table 5 illustrate the annual emissions of methane and carbon dioxide from flooded rice field from 2008 till 2011 for different regions (Lower Egypt, Middle Egypt, Upper Egypt and Out the valley). Regarding to CH_4 emissions, the flooded rice fields are a significant source of atmospheric CH_4. The emission is the net result of opposing bacterial processes, production in anaerobic micro environments, and consumption and oxidation in aerobic micro environments, both of which can be found side by side in flooded rice soils. The annual CH_4emissions from the cultivated area was estimated at 285323 Tonnes for 2008, withCH_4decreasingduring 2009, 2010 and 2011 due to smaller cultivated area. Normally, the decomposition of organic matter in soil is caused by microbiological activity with wetlands soils showing rapid decrease in oxygen due to heavy microbiological activity during growth (**Cabangon *et al.*, 2002**). Hence, the soil in wetlands is identified as anaerobic, a condition affecting the chemical and biochemical processes when compared to aerobic soils (**Lemieux *et al.*, 2004 ; Duan *et al.*, 2004**). The minus value results from the anaerobic condition of soils that have been long used for rice cultivation and results in conditions of oxygen deficiency, greatly reducing the oxidation reduction potential (**Wassmann *et al.*, 2000 ; Badawi, 2004**; **Bockel *et al.*, 2010**).

3.4 Annual N_2O from applied nitrogen fertilizers:

Estimates of N_2Oemissions from nitrogen fertilization are presented in Table 6. We again find the highest N_2O emissions during 2008 again due to highest cultivated area of rice. Table 5 also shows the total nitrogen used under the assumption of a constant application rate of 285 kg N per hectare. In turn the highest N_2O emission was also in Lower Egypt. Direct emission of N_2O produced naturally in soils through the microbial processes of nitrification and denitrification, has been shown to

be influenced by agricultural management, such as water regime, organic amendments and cropping type (Jiang *et al*., 2003).

3.5 Annual CO_2 Emission from burning rice straw:

Annual output of rice straw per hectare in recent years is almost stable with a value of about 7-8 Tons per hectare, while the total national output differs due to changes the total rice cultivated area (Table2). The estimated annual emissions from rice straw burning are presented in Table 7.

The highest GHG emissions again occur in the 2008 season and in Lower Egypt, These findings are in line with estimates in **Gupta *et al.* (2004).** The major constraint in reducing these emissions is the short time available between rice harvesting and sowing of next crop.

3.6 Total Annual CO_2 Emission and carbon footprint:

The estimated levels of CO_{2eq}across all sources (Machinery, Cultivation, Nitrogen fertilization and rice straw burning) are tabulated in Table 8. Again here the highest total $CO_{2eq}$was occurred in 2008 season and in the Lower Egypt region. The carbon footprint was also estimated at 1.90 Kg CO_{2eq} / Kg is the same in all regions and years because of the assumptions of equal quantity of water and nitrogen fertilizer application in all regions as well as the assumption of constant yields (8.0 tonnes rice grain per hectare and 6.6 tonnes rice straw per hectare).

The carbon footprint of a product is the quantity of greenhouse gases (GHG), expressed in carbon dioxide equivalent (CO_{2eq}) units, emitted across the supply chain for a single unit of that product. Indeed, CFP is a mean for the government to sensitize citizens and industrials to climate change and to reach its GHG reduction target. Moreover, it has a significant advantage for private companies to label their product with the government support since they increase their credibility (**Gerber *et al.*, 2010**). Measuring the carbon footprint of a product across the supply chain is a recent trend that has several benefits. By giving consumers the choice to turn consumption toward more carbon effective products and by advising them on their own reduction opportunities, CFP labels sensitize the population in order to switch to a low carbon economy. Thus, standards systems such as carbon foot printing, potentially can contribute to a low carbon economy through (i) market differentiation, (ii) driving performance and (iii) platforms for discussion and synergies (**Brenton *et al.*, 2010**).

3.7 The contribution of GHG emission sources for rice production

Figure1 shows the percentage contributions from the different aspects and field practices. Methane emissions are the main source of emissions contributing about 53.25 % of the total. Rice straw burning is second contributing 35.82 %, while the machinery activities contribute about 1%. Moreover, nitrogen fertilization contributes about 10% of total GHGs.

Mitigation may be possible and perhaps could generate tradable, income enhancing carbon credits (**Tsuruta *et al.*, 1997**). To reduce emissions one could replace burning of rice straw with some other use, decrease ploughing and take steps to slow organic decomposition and increase photosynthesis. For methane reduction, agriculturists could reduce fertilization, improve soil quality by increasing aeration and drain water from the paddies prior to the panicle formation stage. For N_2O reduction, farmers can add organic fertilizer instead of chemical fertilizer (**Chun *et al.*, 2003, Scheehle and Kruger, 2006; USEPA, 2006**).

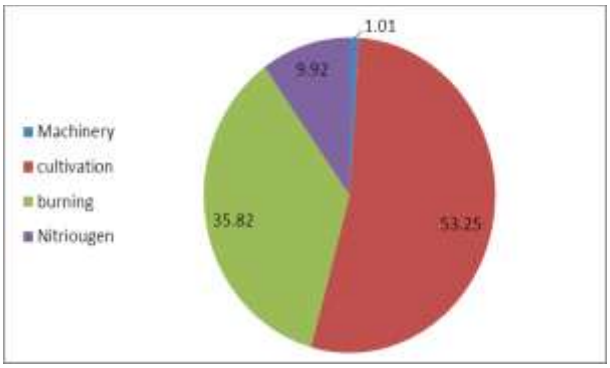

Fig 1: The average percentage of different sources of the GHG resulted from different field practices of rice production in Egypt during the studied period from 2008to 2011.

Uncertainty in emission estimations

Several factors may affect the accuracy of the estimation of emission estimates above. The calculations rely heavily on inferences from limited statistical information and extrapolations of emission factors from limited literature.

Conclusions

This paper presents a detailed calculation of GHG emission from Egyptian rice production. The main sources are methane releases, field burning and nitrogen fertilization. Lower Egypt is the region with the largest emissions. Additionally the carbon footprint per kg paddy rice was computed and some possible mitigation strategies discussed.

Table (3): Distribution of the rice cultivation in Egypt from 2008 – 2011.

Governorates	Total Cultivated area /ha				Total Burnt area/ ha			
	2008	2009	2010	2011	2008	2009	2010	2011
Alexandria	1870	850	955	1059	1122	510	573	635
Behera	97056	83432	64513	87869	58234	50059	38708	52721
Gharbia	74378	52840	43705	51377	44627	31704	26223	30826
Kafr_El Sheikh	149293	135262	115183	123549	89576	81157	69110	74129
Dakahlia	203940	149875	119732	175675	122364	89925	71839	105405
Damietta	30831	26968	23522	28830	18499	16181	14113	17298
Sharkia	140995	106807	77874	98522	84597	64084	46724	59113
Ismailia	1968	1648	1346	2269	1181	989	808	1361
Port Said	8924	8408	6481	9337	5354	5045	3889	5602
Suez	0	0	0	0	0	0	0	0
Qalyoubia	11300	4142	2200	6903	6780	2485	1320	4142
Cairo	14	4	3	0	8	2	2	0
Lower Egypt	720568	570235	455514	585390	432341	342141	273308	351234
BeniSuef	700	209	60	148	420	125	36	89
Fayoum	12605	0	0	0	7563	0	0	0
Middle Egypt	13305	209	60	148	7983	125	36	89
Assuit	81	5	0	0	49	3	0	0
Upper Egypt	81	5	0	0	49	3	0	0
Within the valley	733954	570450	455574	585538	440372	342270	273344	351323
New Valley	4498	378	1135	2830	2699	227	681	1698
Noubaria	726	55	53	108	436	33	32	65
Out the valley	5225	432	1188	2938	3135	259	713	1763
Total	**739178**	**570882**	**456762**	**588477**	**443507**	**342529**	**274057**	**353086**

Table (4): Emissions of carbon dioxide from different mechanical operations during 2008 – 2011.

Region	Area	Irrigation	Chisel plow	Mower	Thresher	Combine	Total
	ha	Tonnes CO_2					
2008							
Lower Egypt	720568	84775	8298	8641	7272	2263	111249
Middle Egypt	13305	1565	153	159.6	134	42	2054
Upper Egypt	81	10	1	1.0	1	0.25	13
Out the valley	5225	615	60	0.6	53	16	745
Total	**739179**	**86964**	**8513**	**8802**	**7460**	**2322**	**114060**
%		76.24	7.46	7.72	6.54	2.04	100
2009							
Lower Egypt	570235	67088	6567	6838	5755	1791	88039
Middle Egypt	209	25	2	2.11	0.38	0.66	30
Upper Egypt	5	0.6	0.06	0.05	0.01	0.02	1
Out the valley	432	51	5	4.36	0.79	1.36	62
Total	**570881**	**67164**	**6574**	**6845**	**5756**	**1793**	**88132**
%		76.21	7.46	7.77	6.53	2.03	100
2010							
Lower Egypt	455514	53591	5246	5463	4596	1431	70327
Middle Egypt	60	7	1	0.1	0.1	0.2	8
Upper Egypt	0	0	0	0	0	0	0
Out the valley	1188	140	14	2.2	1.3	4	161
Total	**456762**	**53738**	**5260**	**5465**	**4597**	**1435**	**70495**
%		76.23	7.46	7.75	6.52	2.04	100
2011							
Lower Egypt	585390	68871	6741	7020	5907	1839	90378
Middle Egypt	148	17	1.7	0.2	0.5	0.5	20
Upper Egypt	0	0	0	0	0	0	0
Out the valley	2938	346	34	3.2	10	9	402
Total	**588476**	**69234**	**6777**	**7023**	**5917**	**1848**	**90800**
%		76.25	7.46	7.74	6.52	2.04	100

Table (5): Emissions of Methane and carbon dioxide from rice fields during 2008 – 2011.

Region	Area ha	CH_4 Tonnes	CO_2eq * Tonnes
2008			
Lower Egypt	720568	278139	5840924
Middle Egypt	13305	5135	107850
Upper Egypt	81	31	657
Out the valley	5225	2016	42354
Total	**739179**	**285323**	**5991785**
2009			
Lower Egypt	570235	220110	4622325
Middle Egypt	209	80.674	1694
Upper Egypt	5	2	41
Out the valley	432	166	3502
Total	570881	220360	4627561
2010			
Lower Egypt	455514	175828	3692396
Middle Egypt	60	23	486
Upper Egypt	0	0	0
Out the valley	1188	458	9630
Total	**456762**	**176310**	**3702513**
2011			
Lower Egypt	585390	225960	4745171
Middle Egypt	148	57	1200
Upper Egypt	0	0	0
Out the valley	2938	1134	23815
Total	**588476**	**227152**	**4770186**

- CO_2eq: the value of CH_4 multiplied by 21

Table (6): Emissions of nitrous oxide and carbon dioxide from rice field during 2008 – 2011.

Region	**Area ha**	**Total applied N kg**	**N_2O kg**	**CO_2 Tonnes**
2008				
Lower Egypt	720568	205361880	2567025	1087842
Middle Egypt	13305	3791925	47400	20087
Upper Egypt	81.0	23085	290	122.3
Out the valley	5225	1489125	18615	7888
Total	**739179**	**210666015**	**2633329**	**1115939**
2009				
Lower Egypt	570235	162516975	2031463	860884
Middle Egypt	209	59565	746	316
Upper Egypt	5.0	1425	18.8	7.5
Out the valley	432	123120	1540	652
Total	**570882**	**162701085**	**2033768**	**861859**
2010				
Lower Egypt	455514	129821490	1622770	687689
Middle Egypt	60	17100	215	91
Upper Egypt	0.0	0.0	0.0	0.0
Out the valley	1188	338580	4233	1794
Total	**456762**	**130177170**	**1627218**	**689574**
2011				
Lower Egypt	585390	166836150	2085453	883763
Middle Egypt	148	42180	528	223
Upper Egypt	0	0	0	0
Out the valley	2938	837330	10468	4435
Total	**588476**	**167715660**	**2096449**	**888422**

Table (7): Emission of CO_2, CO, CH_4, N_2O, NO_x, PM2.5, PM10 and black carbon from rice straw during 2008 – 2011.

Region	Area	Tonnes								
	ha	CO_{2eq}	CO	CH_4	N_2O	NOx	PM2.5	PM10	Black carbon	Total CO2
		2008								
Lower Egypt	432341	3381337	380079	7704	19.97	8846	79697	37095	197	3929399
Middle Egypt	7983	62435	7018	142	0.37	163	1472	685	3.64	72555
Upper Egypt	48.6	380	42.7	0.87	0.00	0.99	8.96	4.17	0.02	441.7
Out the valley	3135	24519	2756	55.9	0.14	64.14	577.90	268.98	1.43	28493.0
Total	**443507**	**3468671**	**389896**	**7903**	**20**	**9074**	**81755**	**38053**	**202**	**4030889**
2009										
Lower Egypt	342141	2675885	300783	6097	15.81	7000	63070	29356	156	3109604
Middle Egypt	125	981	110	2	0.01	2.57	23.12	10.76	0.06	1139.7
Upper Egypt	3.00	23	2.64	0.05	0.00	0.06	0.55	0.26	0.00	27.3
Out the valley	259	2027	228	4.6	0.01	5.30	47.78	22.24	0.12	2355.8
Total	**342529**	**2678916**	**301124**	**6104**	**16**	**7008**	**63141**	**29389**	**156**	**3113127**
2010										
Lower Egypt	273308	2137545	240271	4870	12.63	5592	50381	23450	124	2484007
Middle Egypt	36.0	282	31.6	1	0.00	0.74	6.64	3.09	0.02	327.2
Upper Egypt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
Out the valley	713	5575	627	12.7	0.03	14.58	131.40	61.16	0.32	6478.4
Total	**274057**	**2143401**	**240929**	**4884**	**13**	**5607**	**50519**	**23514**	**125**	**2490813**
2011										
Lower Egypt	351234	2747001	308777	6259	16.23	7186	64746	30136	160	3192247
Middle Egypt	88.8	695	78.1	2	0.00	1.82	16.37	7.62	0.04	807
Upper Egypt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
Out the valley	1763	13787	1550	31.4	0.08	36.07	325	151	0.80	16022
Total	**353086**	**2761482**	**310405**	**6292**	**16**	**7224**	**65087**	**30295**	**161**	**3209076**

Table (8): Carbon footprint for paddy rice based on the estimation of the total emission of CO_2 from different rice production activities during 2008 to 2011.

Region	Area	CO_2	CO_2
	ha	tonnes	Gg
2008			
Lower Egypt	720568	10969414	10969
Middle Egypt	13305	202546	203
Upper Egypt	81	1233	1.23
Out the valley	5225	79480	79.48
Total	**739178**	**11252673**	**11253**
Carbon footprint Kg Co_2eq / Kg paddy rice			1.90
2009			
Lower Egypt	570235	8680852	8681
Middle Egypt	209	3180	3.18
Upper Egypt	5	76	0.08
Out the valley	432	6572	6.57
Total	**570882**	**8690679**	**8691**
Carbon footprint Kg Co_2eq / Kg paddy rice			1.90
2010			
Lower Egypt	455514	6934420	6934
Middle Egypt	60	912	1
Upper Egypt	0	0	0.00
Out the valley	1188	18063	18.06
Total	**456762**	**6953395**	**6953**
Carbon footprint Kg Co2eq / Kg paddy rice			1.90
2011			
Lower Egypt	585390	8911560	8912
Middle Egypt	148	2250	2
Upper Egypt	0	0	0.00
Out the valley	2938	44674	44.67
Total	**588477**	**8958484**	**8958**
Carbon foot print Kg Co_2eq / Kg paddy rice			1.90

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Antioxidants, prevention and treatment of cancer

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Abstract: The instinct to maintain the kind and love to stay has been the impetus for man since ancient times to protect himself from multiple hazards of various accidents and diseases. Cancer is one of the diseases suffered by man since long time ago and he tried hard to identify its causes and resist it in various ways. All efforts have been made till now to completely overcome it have failed, and this dreaded disease continues to be one of the most important causes of death in most countries of the world. Several studies have been conducted in various parts of the world to know the mechanisms of cancerous tumors, their causes and possibility of preventing them before they occur. Cancerous tumors occur as a result of the availability of two factors: genetic predisposition and the presence of an external factor that stimulates tumor occurrence; such as radiation and oxidation-causing substances inside the body. To prevent the tumor as a result of oxidation causing materials tended the efforts of researchers to know the importance of natural antioxidants (natural antioxidants found in fruits, vegetables, herbs, etc.) to prevent tumor Of the most important natural antioxidants, which play an important role in preventing tumors are some vitamins like (vitamin C and vitamin E) and multi-phenols. In this paper we review the most important antioxidants and their sources, discussing mechanisms of cancerous tumors; we also review the most important studies carried out on the relationship between antioxidants and tumors and their role in the treatment and prevention of this disease.
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1. Introduction

Plants and herbs and their derivatives have been used since ancient times to combat many aches and pains afflicting humanity and many herbs used as anti bacterial and pain relievers such as eucalyptus leaf and willow leaf. The last five Years have come full of information from laboratories around the world confirming the positive impact of diet on human health. Many of attention has focused a lot on oxidative activity and anti-oxidant and its relationship to aging and degenerative diseases such as cancer, cardiovascular diseases and diabetes[1].

It has been found that many of the chronic diseases, including heart disease and coronary artery occlusion in addition, occurrence and emergence of many types of cancer depend on the conversion of the large cellular molecules and cancer generators in vivo to special forms of from oxidizing interactive materials. So, nutrition supporting health should include consuming daily five to ten varieties of tomatoes, vegetables and fruits, fruit juices and tea. These substances rich in micronutrients with anti-oxidation properties.

Such as multi-phenols, vitamin C, vitamin E, beta-carotene and Licopin[2]

Also, the low incidence of certain types of cancer in the countries of the Mediterranean attributed to the high rates of consumption of olive oil which contains multiple compounds such as phenols hydroxy Trizol and Alinin 2.

Some of the poly phenols found in many plant foods have been studied and the extent of their effect as anti-carcinogenesis, they were shown that they are anti-carcinogenesis and anti-mutations, also qirosummen (of poly phenols), a pigment found in the spices like turmeric and Lagic acid (which is also a poly phenol), found in fruits especially strawberries, berries, nuts and vegetables which contain large quantities of it and other poly phenols, shown to provide protection against chemicals that cause carcinogenicity in animals 3.

Recent studies have shown that the antioxidants in grape juice, red grapes, strawberries, raspberries, peanuts show influential chemically blocking of carcinogenesis in rats have shown recent studies that tea that contains multiple vehicles of phenols showed protection against many types of diseases, including cancer[3].

To understand how oxidants can treat and prevent cancer tumors and other diseases, we shall review and discuss the mechanisms of oxidation in the following paragraph.

Mechanism of oxidation

Information obtained from recent researches on cancer and cardiovascular disease, inflammatory

disease and the aging process indicate that these incidents or symptoms are strongly associated with each other by a general process, which is to organize the oxidation process at the level of a living cell. A considerable attention has been given to the impact of certain types of nutrients on antioxidants status. To understand the impact of natural antioxidants (found in food) on the oxidative balance, it is necessary to clarify the process of oxidation through its relationship with cellular metabolism[1].

Oxygen is necessary to sustain life, but may have negative effects if the numbers of oxygen-containing radicals (Highly Reactive Oxygen) exceed the need of the cell or not adequately seize[4].

Metabolic activity produces free radicals which are unstable molecules that are able to interact with electron donors to neutralize their charge. This interaction is useful for the synthesis of nucleic acids, hormones and proteins in the presence of a catalyst such as iron, copper, manganese and molybdenum. The free radicals are also produced naturally to confront the invading (attacking) organisms like microbes and viruses[1].

Oxidative balance/Antioxidant is highly regulated, but oxidative stress induced by overproduction of the types of reactive oxygen (Reactive Oxygen Substances, ROS) leading to interfere with cellular functions. The types of intensity of reactivity of oxygen vary from high reactivity as in the radical of the hydroxyl (OH) and ferric ion (Fe+2O) or Copper hydroxide (Cu(OH)2) to low reactivity as in the positive ion to the super oxide radical (O2) and root Alberoxyl (ROO) and hydrogen peroxide (H2O2) singular oxygen (O2) [1] peroxyl acid (HOCl) and nitric oxide(NO -) and nitrite peroxide (ONOO-) and epcoxyl radical (RO-), also, free radicals breed by external factors such as toxic compounds.

And microbial attacks, ozone and ultraviolet radiation, cigarette smoke or by intensive exercise[1].

Free radicals in the cells are affected by modulating the activity (DNA) or the production of energy, proteins and hormones. Free radicals also change unsaturated fatty acids in the membranes of cells which reduces their ability to protect the contents of the cell during the exchange with the external environment. Protein oxidation have been diagnosed by free radicals as an important cause of the aging process and tissue damage arising from radiation of the oxidation proteins bearing functional groups (SH-) may lead to severe damage because of their metabolic importance and as enzymatic active groups in respiratory processes. Oxidation of nuclear bases in (DNA) stimulates the genetic mutations and increases the risk of cancer. The oxidation of fats and proteins increases the complications of vascular hardening, diabetes and low immune function[1].

Cell combats the toxic activity of free radicals by multiple internal mechanisms of antioxidants in which detoxification via the enzyme system in which the phase enzymes (I) activates molecules (cytochrome, P450, NADPH) and convert them to water-soluble compounds attracting electrons before connecting the free radicals with anti-venom molecules (glutathione, UDP - Glukoroncel, amino acids) in order to disable and remove them. Glutathione, vitamin C, vitamin E and coenzyme Super Dismutase oxide are the basic internal defenses of the cell[1].

Although cells possess the means of enzymatic and non-enzymatic reactions against free radicals, but the antioxidant nutrients contribute to the overall protection from oxidative activity of free radicals for the safety of the cell and strengthen immune function[1].

It is difficult to assess the impact of various mixtures of food ingredients and their action on the metabolism of the cell, it has been concluded that ascorbic acid and tocoferolate are one of the best protective factors against a wide range of oxidizing molecules. The relationship was well studied original mechanism for prevention by GSH. Carotenoids which include alcoppinate also work as antioxidants to protect cell membranes and DNA integrity and multi phenols (flavonoids) found only in plants play an important role in the defense against oxidation. It is believed that the presence of fruits, vegetables and herbs in food protect against cancer and many other diseases because of their antioxidant properties. In addition they are good sources of vitamins A,C and E and the factors associated with mineral enzymes. Mineral nutrition, including adequate selenium, copper and zinc are very important in the ideal activity of the enzyme system against oxidation [1].

How to configure cancer and tumors

The beginning, encourage and progress of cancer development are linked to a number of factors related to metabolism, food and the external environment. The accumulation of types of reactive oxygen in the cell and modifications in DNA synthesis and enzymatic activity and mechanical defense all affect the development of configuration of cancer disease. Accordingly, the antioxidants can play an important role in the prevention and control of cancer progression [1].

A - The beginning of cancer

Primary generators of cancer that are activated by phase (I) enzymes such as cytochrome P450 capable of modulating DNA in the genetic

content and stimulation of tumor as a result of the accumulation of and activity of cancer generators such as reactive oxygen species. They also may be formed as a result of the presence of carcinogenic compounds such as Nitrosamine (a cancer Generators present in tobacco) or as a result of formation of heterocyclic amines from fish or cooked meat and they are toxic to the genetic content, which leads to activation of enzymes of phase (II) and the occurrence of genetic mutations 5-7.

B – Promotion of Cancer

When genetic mutations affect the DNA they may lead to the formation of malignant tumor cells, this stage is controlled by a series of steps of conversion the mark that include factors NFKB (nuclear CAPA B factor) and AP-1 (Activator protein I), which is regulated by the capability for oxidation and reduction so they are sensitive to oxidants / antioxidant state in the cell[8-11].

C- Progress of cancer

Cancer progress occurs as a result of growth of malignant tumor cells with cell division in the phase due to bonding of growth factor with the receptor and conversion of the generator's sign to division [12-13].

The Urokinase enzyme (an enzyme that helps the proteolysis) plays an important role in growth and secondary spread of cancer via interference with the ability of enzymes to recognize their primary sources. Cancer progress and invasion of cells and creation of secondary cancer spread may facilitate due to excretion and activity of colagenase of the mineral enzymes that analyze protein complexes, exposure of multi- saturated fatty acids in immune cell membranes to oxidation due to oxidation activities of free radicals promotes cancer development[14-15].

Effect of antioxidants in the protection against cancer tumors.

Primarily, for the substances (antioxidant) to play an effective role in the prevention of oxidation, they must be able to donate electrons and fix the charge and non-dual electrons on the body of the substance (antioxidant)[3].

The mechanisms of antioxidant may be summarized in that3:

1. Prevents mutants and toxicity of (hereditary) gene.
2. Stops and inactivate and develops vital chemical indicators of the tumor.
3. Working as anti-toxics for enzymes.
4. Track the products of active metabolites of carcinogens.
5. Actively act as antioxidant and cleaner of free radicals.

Action of antioxidants at the beginning of cancer

Antioxidants inhibit cytochrome enzymes P450 and they directly neutralize primary generator of cancer via their strong activity in the capture of oxygen before cell damage 5-7 various phenol compounds that inhibit Nitrosamine compounds in vitro and inhibit formation of varied rings amines during cooking of meat and fish[16].

Antioxidants such as Flafenodate, showed the highest protection against genetic mutations, non-enzymatic cutting and intervention of negative ions of super oxide. They also lead to a significant reduction in reverse mutants induced by different mutations' generators. Antioxidants promote deflationary death of cancer cells. And increase resistant of lymphoid cells to oxidative damage. They intervene (supporting) enzymatic defense mechanism in cells 1.Some poly-phenols[17-19] (EGCG poly-phenols in tea) inhibit the action of phase (I) enzymes and prevent stimulate cancer generators and stimulate phase (II) enzymes that connect active generators of cancer and lead to their obstruction[17.19].

Action of antioxidants at the stage of cancer promotion

Antioxidants halt work AP-1 and inhibit division signal converters which are responsible for cell division, inhibit interactions between proteins and compounds that are bonded by closure and prevent them from bonding[20] stimulates vacuum articulated contact between the cells and prevent inhibition due tumors' promoters 16 it has been found that (EGCG) inactivates the activity of telomerase (the enzyme opens the lock ability of division in cancer cells) thus stimulates aging and reduces the life span of tumor cells in leukemia and solid tumors[21] caffeine also shows inhibiting effect on the mechanical repair of deadly damage in the cells of tumors sensitive to radiation, which improves the sensitivity to radiation therapy[22].

Action of antioxidants in the stage of cancer progress.

Recent studies have indicated that multi-phenols inhibit the growth of malignant tumors and stimulate deflationary death even in tumor cells resistant to deflationary death[23-25] also it was found that (EGCG) showed inhibition of reaction to enzyme Urokinase and halted division oral cancer cells in phase (GI) While curcumin substance has stopped cell division at the stage of S/G2M, when used together showed a synergistic effect[26] the compound (EGCG)can also kill mutated cells in a specialist manner because of the adenovirus[27], antioxidants also disable the synthesis of DNA in the cells of liver cancer, cancer of the blood cells and cancer of lung cells[28] and can inhibit the adhesion of tumor cells in

the lungs of mice which stop the progress of cancer[29] also oxidation of multi fatty acids prevents unsaturation in immune cell membranes, helping them to resist the tumors. The Filafinoedat inhibiting the release of (Tumor necrosis factor Alpha TNF-α) and reduce tumor promotion 30 they also seems to inhibit frequent production of the gene responsible for multidrug resistance and modify the enzyme Topoizomirase which has to do with the growth of the tumor 31 they carry out a specialized inhibition Of elected enzymatic activities and targets the anomality in DNA and repair it[31].

Effect of antioxidants on different types of cancer

1- Skin Cancer

Some studies have indicated that the topical use multiple or consumption of poly-phenols lead to inhibition of the beginning of the tumor and its promotion by chemical cancer generators or ultraviolet radiation in experimental rats 32-35 more recent study has shown that caffeine may be of benefit in the chemical prevention of stimulated cancer generate due to ultraviolet UV [36-37] also poly-phenols inhibition of the growth of skin tumors very steady note, it was possible to note decline in the tumor. It has been observed the full decline in about 4% of the tumor-bearing mice papiloma virus (out of 346 rat)[35].

2- Leukemia

Some studies have shown that poly-phenols are of benefit to the mucous layer lining the mouth. Studies have shown that there is an inverse relationship between the rates of eating this type of antioxidants and rates of formation of pimples 38 and that tumor patients who took poly-phenols decreased the number of pimples compared with patients who did not take poly-phenols. This study indicates that eating antioxidants not only has protective properties but also has therapeutic properties.

3- Esophageal cancer

Studies did not show any relationship between eating phenol juice with the presence of or low incidence of cancer of the esophagus 39.

4- Pancreatic Cancer

Pancreatic cancer is one of the most cancerous tumors that cause death. A study in China has shown that there is an inverse relationship between pancreatic cancer and the rates of consumption of poly-phenols[40] a decrease was found in infection rates between 12% to 53% among men and women respectively 40 Another study in Poland showed that there is a statistically significant decline with morality up to ($p < 0.001$) with the increase in the rates of poly-phenols consumption in food and tea[41].

5- Lung cancer

When treating mice with poly-phenols they have observed decline in the number of lung cancer tumors after chemical stimulation to generate lung tumors[32-42] and the same result was obtained when consuming Nitrosamine NNK for tobacco[43-44] or when tumors were allowed to self evolve[45] it stopped an increase in the compound 8-hydroxy deoxy guanosine in mouse lung and a compound 6-methylguanine. Each of these bases are derived from purine necessary to generate mice lung tumors[43-44].

6- Cancer of the gastrointestinal tract

High doses of Tiaflavin and multi-phenols have led to inhibition of tumor proliferation when presented to rats[32,46] also drenched green tea inhibited chemically stimulated anterior stomach cancer in mice. One study reported that the multi-phenols failed to reduce the incidence or the proliferation of colon cancer. While another study reported that a high dose of phenols led to the reduction of primary malignant lesions. little inhibition has been observed of the anomaly focus glandular cavity stimulated by ozomethane in rat colon[46-47] also found that multiple phenols which provided in low concentration led to a significant reduction in the incidence of the large bowel tumor 46-48 in tumors induced by dissimilar ring amines, multi-phenols have led to stimulate phases I and II that remove toxicity for the rapid secreting generators of genetic mutations 49 also inhibition of liver cancer was observed in rats and mice 48-49.

7- Mammary gland cancer

Recent study on the development of mammary gland cancer could not notice any significant impact on the generation of mammary gland cancer in rats that were fed on normal diet[32,50-51] this effect was also observed in clear auto-formation of the tumor of mammary gland deciduous in mice that have been fed on poly-phenols with aluminum hydroxide[32] It was found that injection of(EGCG) within the peritoneal cavity led to growth inhibition and reduction in size of tumors generated by cell lines of breast cancer in humans and attached inside the mice and rats[52-53] it has been found hat poly-phenols may be helpful in chemotherapy, oral consumption of which the by Dropsical IRISH tumor-bearing mice with Doxyrobicin has increased efficiency of chemotherapy 54 as they fully inhibit the development of mammary gland tumors in mice with a mixture of Tamoxiphene 55 and Tininat have led to promote the activity of anti-tumor and proliferation in mice infected with secondary ovarian cancer 56

8- Prostate cancer

Seems to be that the risk of cancerous tumors inversely proportional to the increased intake of antioxidants. Daily communion antioxidants significantly reduces the incidence of prostate cancer (approximately 11%) 57.

9- Cancer of the bladder

It was found that there was a 50% reduction in the incidence of bladder cancer in women58, but did not notice any significant decrease in bladder cancer for men, though increased survival rates of five years in men patients when consuming with poly-antioxidants[59].

In conclusion, it is useful to recall that many chronic diseases, such as atherosclerosis and many of cancerous tumors depend on in vivo conversion of large cellular molecules.

Or cancer generators to special interactive active forms. For this reason precisely, health support nutrition must include consuming daily between 5 to 10 varieties of vegetables, fruit, juice, red grapes and tea are rich sources of micronutrients with antioxidant properties such as vitamin C, E and Alapta carotene (vitamin A) tomato also contains lycopene and many vegetables contain Kwersethein and various phenolic compounds. Tea contains Flavinoids also red grape juice contains on Receveratrol. Antioxidants are necessary for the body to control the oxidation reactions with potential negative consequential in the body where oxidation of cholesterol and low-density fats LDL lead to the production of compounds detrimental to the vascular system or blood vessels[60] So low consumption of saturated fats to reduce cholesterol in the blood in addition to communion enough antioxidants is the ideal way to reduce the risk of heart disease and hardening of the arteries 60 stomach cancer which may be caused by consumption salty pickled food which produces cancer generators with direct influence, can be disabled or inhibition by vitamins. The cancers of the colon, breast, prostate and pancreas caused by a new class of cancer generators which are varied ring amines that are formed during grill or frying foods containing creatinine, such as meat and fish 60 of these types of cancer generators can be inhibiting their formation and impact by antioxidants present in many fruits and vegetables such as soybeans, tea or by vitamin c.

Conclusions and Recommendations

The oxidation reactions can be disabled or inhibited by antioxidants found in fruits, tea and tomatoes and Alkhaddoat. Even Alchkhokhh and aging in good health will be supported by the available sufficient quantities Mnamadadat the different oxidation. Any that prevent the formation and impact of interactive products by antioxidants such as those found in fruits and vegetables, Tatm and fruit juices, juice, tea grapes Alokhmr and holds great importance to public health by reducing the risk of diseases and their prevention.

1 – to maintain health and the prevention of cancerous tumors resulting from oxidation reactions of free radicals, it is recommended to consume from 5 to 10 varieties of fresh or cooked vegetables in addition to tea and coffee instead of soft drinks that contain dyes and preservatives only.

2 - The Libyan habits of cooking meat and fish after seasoning with various spices such as turmeric, thyme and other wild herbs are good habits and must be maintained.

3 - Cooking of meat with vegetables is suitable method to reduce and prevent the formation of a heterogeneous ring amino compounds that generate cancer.

4 - Avoid and limit the intake of fried meat and fish, or at least add lemon juice to them when frying is useful in minimizing opportunities of contracting malignant tumors.

5 - There are many herbs and wild Libyan plants that were directly eaten or used in Libyan food such as Altefaf, and Agazul, Alkitoot, Ahumaidah, Alkhubes, Alhereg, Alasloz, Almiramia, etc. can have significant benefits. Therefore, I am of the view of further studies on these herbs and wild Libyan plants whether or not they contain vitamins and antioxidants.

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A study to find thyme oil dose that kill 50% of mice and minimal dose that kill all mice and maximum non-lethal dose

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Abstract: Thyme plant is one of the natural plants that have Aromatic Perfumes liked by human being, and one of the natural resources that sought by lot of researchers and scientists to use it in scientific research and studies of perfumes and pharmaceuticals and used as flavour in making a lot of industrial products. Components of thyme oil sold as a spice in the Libyan market extracted by steam distillation, the ratio of aromaticvolatile of thyme oil was 2.548% and specific gravity up to 0.9609, while the optical refractive index 1. 5111 where thyme oil extracted will be used as input in some food products for human consumption, the study required conduct some laboratory experiments with respect to toxicity to find the dose that kills 50% of the experimental animals, and then to prove that it has no damage when used repeatedly, in this study, the dose that kills 50% of mice and minimal dose lethal to all mice and maximum non-lethal dose was reached, the use of Libyan volatile thyme oil because of its great and direct relationship, especially its use in the manufacture of soft drinks, we are confident now that the use of 0.1 ml of thyme oil to prepare 1 liter of soft drink for human consumption is safe even if used repeatedly by man every day, and the dose reached was 3.84 mg, which killed 50% of mice, weighing 25 grams. This experiments was conducted at faculty of Medicine in Libya.

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Keywords: study; thyme; oil; dose; kill; mice; maximum non-lethal dose

1. Introduction

Thyme is Herbaceous plant of the platoon species, grows in mountainous areas, used as a beverage instead of or with tea, added to some food to give it an acceptable flavor, the plant is used in folk medicine frequently where it is prescribed to treat mouth infections, stomach, intestine and airways, coughing and gastroenteritis and expel intestinal worms, as well as to strengthen the heart [1]
Thyme oil can be separated easily from the plant, and this oil contains phenolic substances such as thymol, as well as carfikrolsubstance and impurities such as resins, in addition to the substances listed, the plant contains tannin and many other materials [1].

Experiments has shown that thyme has anantibacterial effect [2] has loosening effect on stomach muscles[3-4], trachea[4],strengthening the heart[5], lowers blood pressure and analgesic[6], lowering blood cholesterol[7], anti-oxidant[8],and is useful for the treatment of certain skin diseases[9]it was found that the muscles loosening substances in thyme is a flavonoid [3].

Experiments conducted on laboratory rabbits using aromatic oils extracted from some plant species have proved that 1 mg / kg of solution at a concentration 0.5 -5% is safe and led to heart activation and lower blood pressure [10].

As the thyme oil has an acceptabletaste, it can be added to soft drinks, and although thyme is used frequently as a beverage, yet, it is necessary to conduct some experiments to find the dose of thyme oil added to the drinks, which kills 50% of the experimental animals, and then to prove that it has no damage when frequently used.

Experiment:

In the initial study, the simplified method was used[11], where 2 mice were taken (weighing between 22 to 25 grams) that is for each group, were injected with a volume of solution of 0.1 ml per mouse, exponentially concentrations of dilutions in the Peritoneal cavity to reach the dose that kills all the rats and emphasize a higher dose, then immediately mice were observed in terms of behavior and return to normalstate when placed on its back and the presence of any thrills or shakes and breathing for a period of 5 hours then every hour for 12 hours and then at 16 hoursand then at 24 hours and registration of death if occurred from doses given,the dose that killed 50% of the mice and a dose lower than the dose larger ones were selected and give these doses into three groups of mice each composed of a number 8 mice, and thus determine the dose that kills 50% of the mice in a group larger in number than theprevious.

Since the thyme oil does not dissolve well in water, a drop of TWEEN 80 was addedto 1 ml of solution Which is non-toxic and has no effect of any

kind, knowing that thyme oil was tested by dissolving in alcohol and water is added to dilute, but found that ethanol at the added concentrations to a solution of the oil in itself has a detrimental effect on mice and it was replaced with TWEEN 80 .

Summary of Results:

1- Solution diluted 5 times: lead to loss of coordination and consciousness in less than 15 minutes and death before 5 hours.
2- Solution diluted 10 times: lead to poor movement before half an hour followed by head falling on both sides during walking then death before 8 hours.
3- Solution diluted 20 times: lead to poor movement and death before 9 hours, when repeated with 8 mice, all died before 16 hours, this was the minimal concentration killed all mice.
4- Solution diluted 30 times: walking was somehow abnormal; no death at 24 hours or even after a week, the experiment was repeated with 8 mice, this was the maximal concentration tolerated by mice with no death.
5- Solution diluted 25 times: this experiment was made with 8 mice, 4 of which died before 16 hours and 4 remained alive even after 24 hours and even at one week, this was the dose that killed 50% of mice.

Summary: from this experiment it turned out:

1- The least lethal dose is thesolution diluted 20 times.
2- Dose that kill 50% of mice is the solution diluted 25 times.
3- Maximum non-lethal dose is the solution diluted 30 times.

As oil density was 961 g/l this means that 1000 ml of oil contains 961g, if the solution diluted 25 times, then 25000 ml contains96100 mg

i.e. 25 ml contains 961 mg

if 0.1 ml contains 961 X 0.1/25 = 3.84 mg this is the dose that killed 50% of mice weighing 25 g

Therefore : dose for every 1g is 3.84 X1000/25 =153.6mg/kg

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Brief Review on the Application of Histochemical Methods in Different Aspects of Plant Research

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Abstract: Histochemical methods are employed in the identification, density of accumulation and distribution of chemical compounds within biological cells and tissues in different organs under microscopes using the color-stain-reaction technique and photographic recording. These include the preparation of fixed variably stained specimens and then the examination under the microscopic devices. In immuno-histochemistry, antibodies are mainly used to visualize antigens in sections of tissue under either the light or the electron microscope. Histochemistry reperesent elusive documents to be used for identifying cellular chemical processes, cellular traffics, metabolite sorting, and in investigating several diseases, mainly related to cancerous activities. It is successfully applied in gene expression studies, detection and localization of cellular components of active cell constituents such as proteins, carbohydrates, lipids, nucleic acids, and a range of ionic elements occurring in the cell solutions, in addition to identifying the characterization of secretory structures and the chemical nature of the secreted compounds. Ion homeostasis could be assessed, while constituents of the signal transduction cascades could be identified through metabolic amplification. The methods played a role in describing and tracing the ultrastructure development during different plant growth stages so as the genetic bases of plant physiological and biochemical processes could be further elucidated. The penetration process and defense reactions (hypersensitive response, oxidative burst and cell wall fortification) of various organisms could be studied histochemically. This help in comparing the resistant and susceptible plant cell lines. It also assists in indicating mechanism of pathogen invasion and hypersensitive responses. To examine the supposed pollution and contamination in a given location, histochemical methods are efficient in detecting the existence of certain injurious metals via samples of plants taken from the inhabiting area. It is however concluded that such methods are proved as good tool to be rapidly and efficiently employed in different vital aspects of biological research.

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Key words: Biological, cellular, chemical, histochemical, methods, plant, tissues.

I-Introduction

Histochemistry is devoted to study the identification and distribution of chemical compounds within and between biological cells, using stains, indicators and light and electron microscopy (Wick, 2012). To understand the definitions of histochemistry, it may be helpful to recall the definitions of histology as the microscopic study of the structure of biological cells and tissues, whereas, chemistry is the science of matter and the changes that occur between and via different chemical reactions. Thus, leading to the changes occur in molecules and cell components.

While histochemistry has broad interface with life sciences as one of the most objective methods in biology and medical research, botany was the principal scientific discipline in which such technique was evolved. While botanists retained a basic interest in the cellular chemical processes that were illumined by histochemistry, zoology-oriented histologists and histochemists used microscopy and staining technique primarily to further the development of microanatomy, taxonomy and nosology (Wick, 2012).

The methods are found to be efficient tools for analyzing, localization and distribution of molecules in cells and tissues. This include proteins, carbohydrates, lipids, nucleic acids and a range of ionic elements occurring in cell solutions. These methods are essential in pathologic diagnosis (Kiernan, 1999). Histochemists pioneered the use of small-molecule cellular stains, labeled molecules such as antibodies, and enzyme-mediated detection and signal amplification (Kiernan, 2008).

The technique is also employed to study time course of deposition and distribution of major storage compounds such as protein, lipid, starch, phytin, and minerals such as calcium, potassium and iron in rice grains (Krishnan *et al*., 2003; Krishnan and Dayanandan, 2003). Immunohistochemistry utilizes antibodies to visualize antigens in sections of tissue under the microscope in conjunction with light and /or electron microscopic examination. Light microscopy often possesses sufficient resolution to determine the distribution of an antigen between tissues and cell types (Famiani *et al*., 2000). Therefore, immunohistochemistry provided valuable information

about how the metabolism is compartmentalized between different tissues in plant structures (Walker *et al*., 2001).

II - Material and Methods:

1.Preparation of the plant tissues and fixation:

The plant tissue under investigation is collected, washed thoroughly under current water, cut (segments of 0.5-1cm) and preserved in fixative solutions for at least 48hrs.

The universal fixative solutions are formalin, acetic acid and alcohol (FAA). Chrom-acetic solution (for fresh algae and for fungi), Carnoy's solution (for chromosomes) and Erliki'sfluid (for mitochondria) are also used (Johansen 1940; Jensen 1962).

2.Tissue dehydration, paraffin infiltration and sectioning:

Following fixation, tissue samples passed through serial concentrations of dehydration solutions of ethanol and absolute alcohol. The dehydrated sections are then immersed in a mixture of absolute ethanol and xylene; followed by embedding in paraffin wax Uniform sections are prepared using microtome. The sections are affixed to slides using the Haupt's adhesive, which contain gelatin and glycerin. Before staining, the wax is to be dissolved by passing slides through xylene followed by a mixture of xylene and absolute ethanol. The sections are then transferred to a successive down series dilutions of ethanol from absolute ethanol. The methods are described in detail by **Johansen**, 1940; Jensen, 1962 and recently by (El-Awadi 2001).

3.Staining:

Table (1) illustrates some stains that are commonly used in histochemical tests.

Table 1. Stains and the cell structures` color reactions under the microscope (Jensen, 1962; O'Brien and McCully, 1981).

Stain	Investigated materials or tissues	Colour
Safranin-fast green	Cuticle, lignin, chromosome, nuclei	Red
Safranin-aniline blue	Gymnosperm	Blue
Tannic acid ferric chloride-safranin	Stem and root apical region Cuticle, lignin, chromosome, nuclei, cell wall	Red
Heidenhain's iron haematoxylin-orange G	Cytoplasmic particles and cell wall	Light orange
	Chromosome nuclei, plastids mitochondria	Dark blue
Delafield's haematoxylin	Cytoplasmic particles (chromosome, nuclei, plastids, mitochondria)	Deep blue black
	Cytoplasm	Blue gray
Toludine blue 0	Lignin	Blue green to turquoise
	Other phenols	Bright blue to dark violet
	Pectin and other celluloses	Pink to reddish
Phloroglucinol- HCl	Lignin	Red violet

4.Mounting the cover slips:

After staining, the tissue is dehydrated, and a cover slip is mounted over it. The cover slip makes the preparation permanent. The cover slip is held in place with a mounting medium. The medium should have the same refractive index as the glass index and should not affect the stained tissue. The traditional mounting medium is Canada balsam (Johansen, 1940; Jensen, 1962).

5.Microscopic Examination:

The prepared stained sections can be examined under the as Light Microscope, Electron Microscope, Stereoscopic Microscope and Fluorescent Microscope.

Fluorescent Microscope

A fluorescent microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic and / or inorganic substances (Lakowicz, 2006). It uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescent microscope, or a more complicated design such as a confocal microscope.

Fluorescence

Fluorescence is the emission of light by a substance that absorb light or other electromagnetic radiation. The emitted light usually has a longer wavelength, and therefore lower energy than the absorbed radiation. However, when the absorbed electromagnetic radiation is intense, it is possible for one electron to absorb two photons. Such two-photon absorption can lead to emission of radiation having a shorter wavelength than the absorbed radiation. The emitted radiation may also be of the same wavelength as the absorbed radiation, termed "resonance fluorescence (Skoog *et al*., 1997). The color indications of the autofluorescence of unstained and stained tissues (with fluorescent stains) under different illumination light sources are shown in Table (2).

Table 2. Colour indications of the autofluorescence of unstained and stained tissues (with fluorescent stains) under different illumination light sources

Stains	**Light**	**Fluorescent color**	**Materials**	**Reference**
Unstained plant sections	Ultraviolet (UV -2A)	Bright blue to whitish	Lignins and/or phenolics	Brammall and Higgins (1988); M El-Awadi 200…thesis
		Yellowish to brownish	Condensed tannins	
	Blue (B-2A)	Bright yellow	Lignins and/or phenolics	
	Green (G-2A)	Bright red	Phenolics	
Fluorol yellow 088	Ultraviolet (UV-2A)	Bright yellow	Lipid	Brundett *et al*. (1991)
Sudan III&IV	Blue (B-2A)	Bright red	Suberins	O'Brien and McCully (1981)

III-Examples of the Application of Histochemistry in Plant Research.

1. In gene expression

Green fluorescent protein (GFP) from the Jellyfish *Aequorea victoria* and its homologs from diverse marine animals are widely used as universal genetically encoded fluorescent labels (Giepmans et al, 2006).

Efforts on identification and development of fluorescent proteins with novel characteristics and enhanced properties, led to a powerful toolkit for visualization of structural organization and dynamic processes in living cells and organisms. The diversity of available fluorescent proteins covers the entire visible spectrum, providing numerous alternative possibilities for multicolor labeling and studies of protein interactions (Chudakov *et al*., 2010).

Sidorov *et al.* (1999) described a reproducible plastid transformation system for potato and regeneration of plants with uniformly transformed plastids. Plastid-expressed green fluorescent protein was used as a visual marker for identification of plastid transformants at the early stage of selection and shoot regeneration (Fig. 1).

The establishment of a plastid transformation system in potato offers new possibilities for genetic improvement of this important crop.

The detection of plant transformation (using β-glucuronidase; GUS assay in *Lilium* are carried out via the application of histochemical methods.

β-glucuronidase (GUS) assay is used to assess transient expression of the GUS gene using 5-bromo-4-chloro-3-indolyl β D-glucuronide (X-Gluc) as the substrate.

Six days after co-cultivation, samples of 0.1g of callus collected from each treatment are subjected to transient histochemical GUS assay.

The transformation efficiency of calli are evaluated by counting the number of blue spots, using stereomicroscope, showing GUS enzyme activity on each callus sample (Stomp, 1992). The methods / technique are described by Azadi *et al*., (2010) as shown in Fig. (2).

The use of the histochemical methods in iron and ferritin gene expression in transgenic indica rice (*Oryza sativa* L. cv Pusa Basmati) proved as efficient in such an investigation. Perl's Prussian blue staining of transgenic rice grain sections show distribution of iron accumulation (blue compound of ferric ferrocyanide) throughout the alureone and subaleurone layers and in the central region of the starchy endosperm (Fig. 3-b). Whereas, in the nontransgenic grains, blue colour formation indicating iron accumulation was restricted to the aleurone layer and the intensity of color was also very low (Fig. 3-a).

Transverse section of the transgenic rice grains indicated the high iron accumulation in embryo as well as in the endosperm (Fig. 3-d), in comparison to the nontransgenic ones. In the latter, iron appeared restricted to the embryo and aleurone layer in which the intensity of color detected in the embryo was very low (Fig. 3-c). This histochemical analysis of iron in rice specifically showed temporal and spatial deposition of storage iron (Sivaprakash *et al.,* 2006).

Advances in histochemistry and cytochemistry made it possible to retrieve quantitative data from 2D and 3D microscopic images. In this way, valid quantitative results can be regenerated (e.g. gene expression data at the mRNA, protein and activity levels) from microscopic images in relation to structures in cells, tissues and organs in 2D and 3D. Volumes, areas, lengths and numbers of cells and tissues can be calculated and related to these gene expression data while preserving the 2D and 3D morphology (Chieco *et al*., 2013).

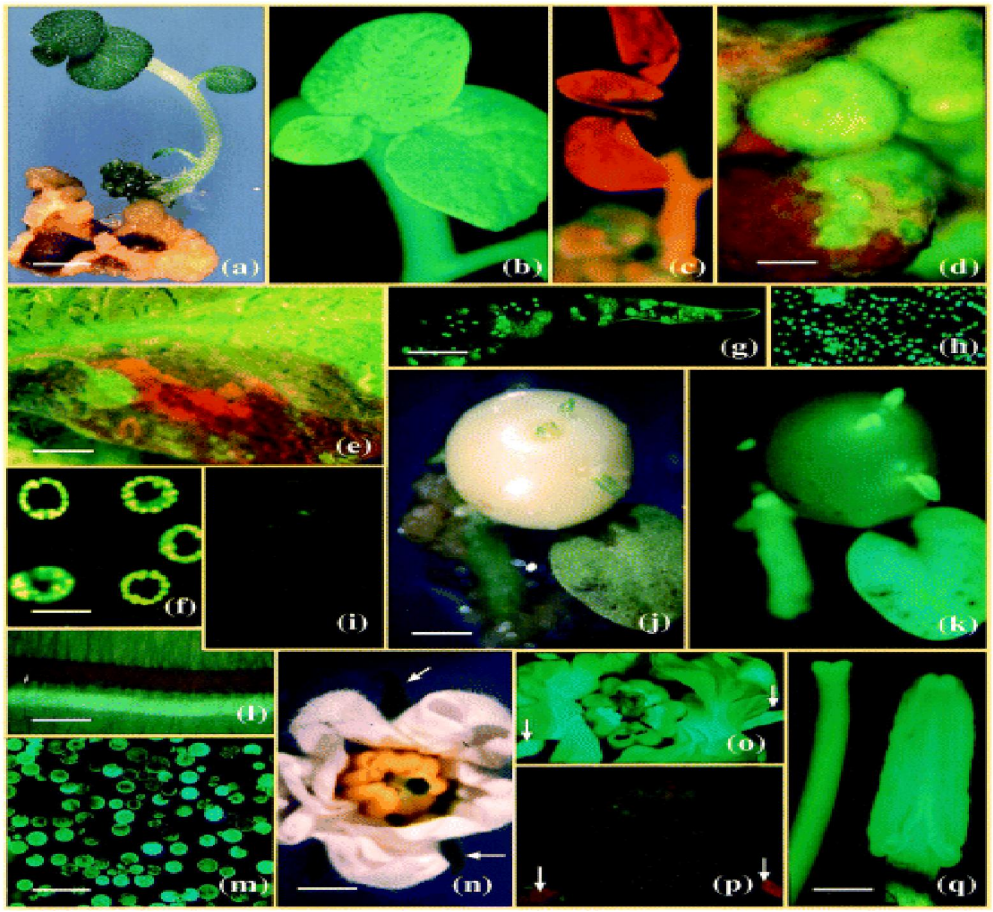


Fig. 1. GFP in plastid transformants of potato (Sidorov *et al*., 1999).
(a) Appearance of spectinomycin resistant regenerate under visible day light condition (bar = 8 mm). (b) GFP fluorescence of transformed and (c) autofluorescence of non-transformed regenerants excited by blue light. (d) Chimeric callus (bar = 0.3 mm) and (e) leaf segment (bar = 0.8 mm) illuminated with blue light. (f) GFP expression in chloroplasts of stomatal guard cells of leaf epidermis (bar = 38 µm). (g) Expression of GFP in plastids of trichome cells (bar = 60 µm). (h) Fluorescence of freshly isolated mesophyll chloroplasts under blue light. (i) Appearance of wild-type microtuber under blue light. (j, k) Microtubers of transformed plants under visible and blue light, respectively (bar = 3 mm). (l) Red autofluorescence (top) and green GFP fluorescence (bottom) of roots from non-transformed and transformed plants (bar = 0.4 mm). (m) Isolated mesophyll protoplasts in blue light (bar = 220 µm). (n) Transplastomic potato flower under visible (bar = 3.6 mm) and (o) blue light. (p) Appearance of wild-type flower under blue light. Arrows in (n, o, p) indicate the sepals. (q) Detection of GFP fluorescence in pistil and anther, excited by blue light (bar = 1.6 mm).

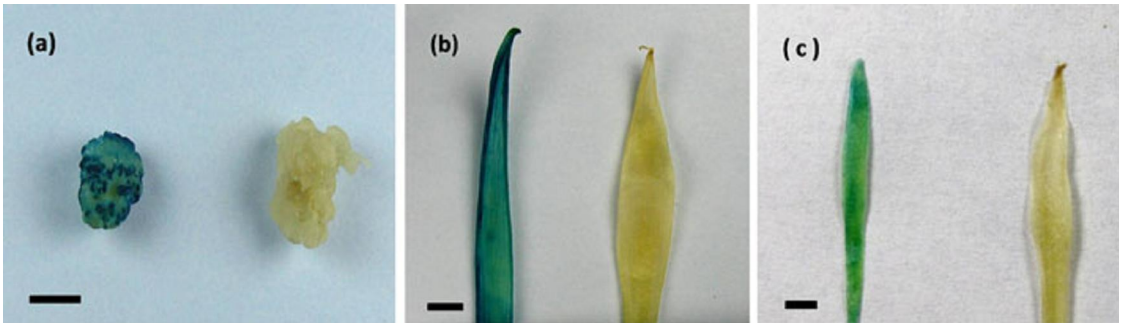


Fig.2. Histochemical β-glucuronidase (GUS) assay of transgenic plants. Stable GUS expression on calli (a), leaf of *Lilium* x *formolongi* 'Akasu' plants (b), and leaf of 'Acapulco' (c). Transgenic plant (left) and non-transformed plant (right), respectively (Azadi *et al.,* 2010).

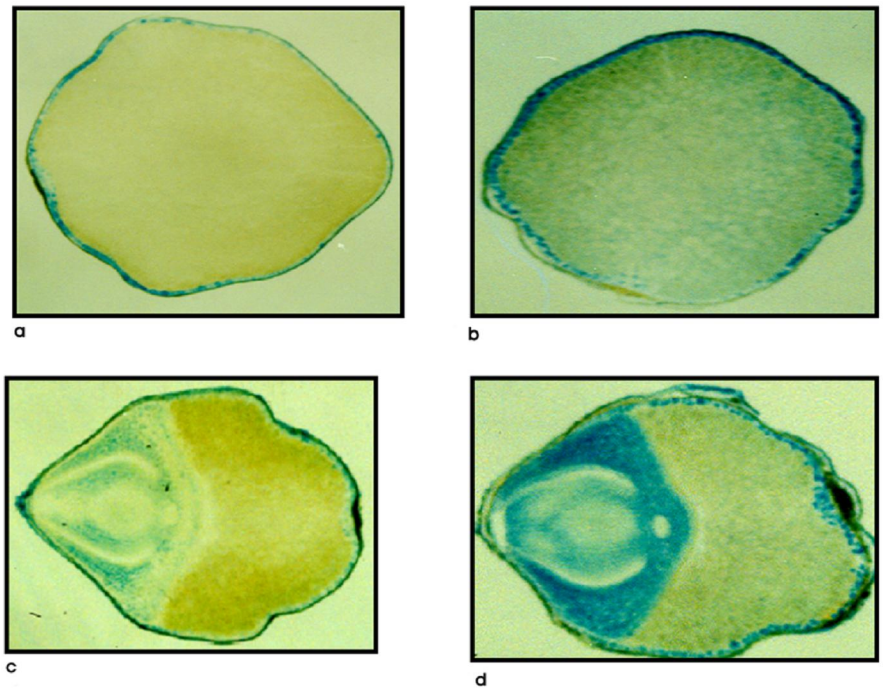


Fig. 3. Transverse section of mature nontransformed (a, c) and transgenic (b, d) rice grains. Blue colour indicates the presence of iron. In control, iron is the restricted to aleurone and embryo, and is not found in the endosperm. In transgenic rice grain, in addition to aleurone and embryo, iron is also strongly present in the endosperm cells. Higher accumulation of iron in embryo of transgenic rice, is also shown by intensity of color compared to control (Sivaprakash *et al*., 2006)**.**

2.In detection and localization of lignin, phenolics, lipids, suberin, alkaloids and tannins in certain plants.

In a study of leaf structure and secretory activity of *Ecballium elaterium* (Fig. 4)**;** a hairy pharmaceutical perennial plant; histochemical method was applied. The amphistomatic leaf showed a characteristic structure due to special cells supporting the conductive bundles, a remarkable shortage of mechanical tissue, and the existence of pectin strands between mesophyll cells. The secreting activity is limited mostly to secretary hairs. Such a structural character points to a remarkable strategy of such a species coping with stress conditions of its habitat (Nikolaos *et al*., 2011).

In such a study, unsaturated lipids, sesquiterpenes, flavonoids, terpene containing steroid, alkaloids, tannins, catechol tannins, polyphenols and other phenolic compounds, monoterpene phenols and phenolic tannin precursors were identified and localized within the cells.

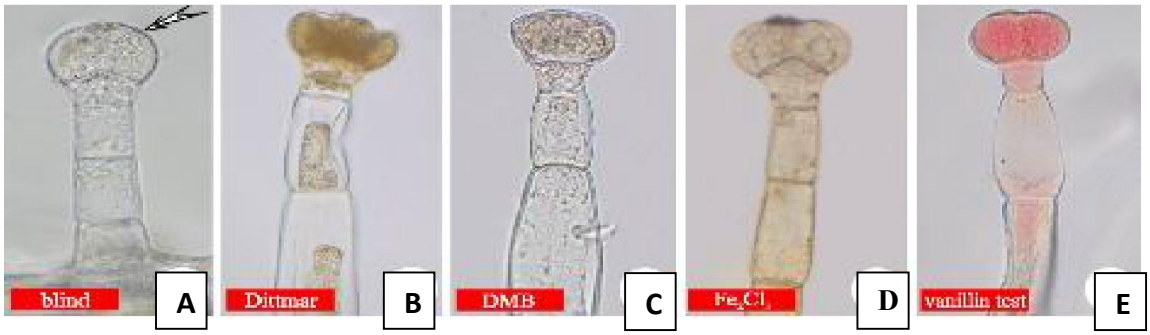
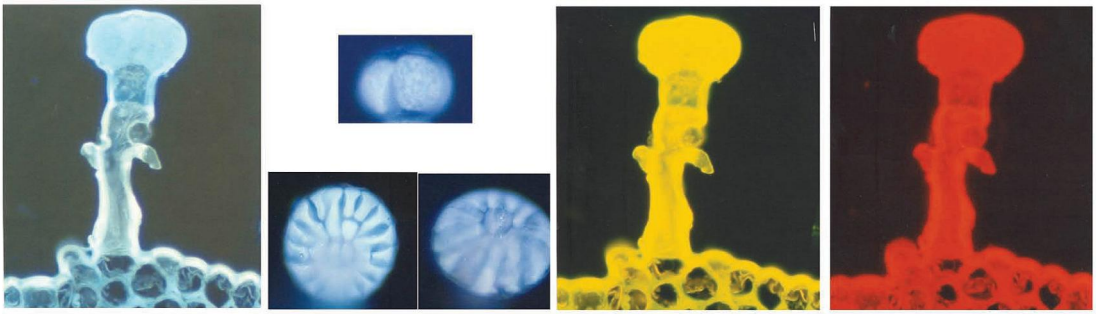


Fig. 4. Histochemical investigation of *Ecballium elaterium* secretive hairs (Nikolaos *et al*., 2011).
(A) Blind, no treatment. The arrow points at the head-cells, (B)Dittmar reagent for alkaloids; (C) DMB (DiMethoxy Benzaldehyde or veratralaldehyde) for phenolic tannin precursors; (D) Ferric chloride for polyphenols; (E)Alcoholic vanillin/ HCl (vanillin test) for phenolic compounds.

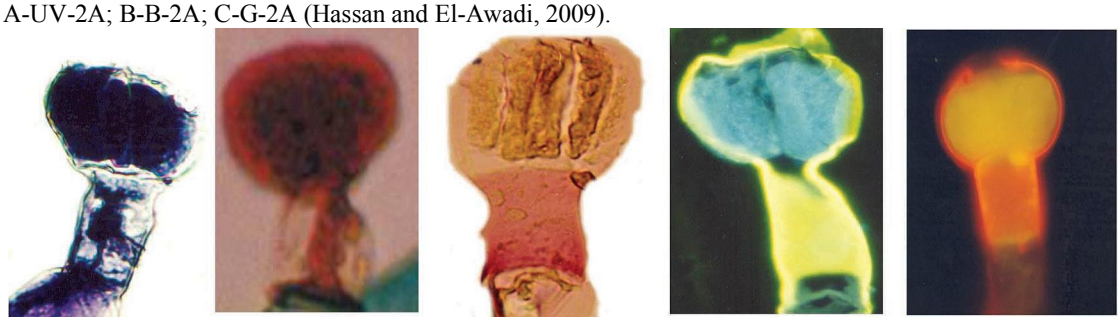
Corresponding results indicated the existence of lignin, phenolics, lipid and suberin materials in the glandular trichomes of the parasitic plant broomrape. The phenolic substances were detected in the neck cell and gland secreted cells by autofluorescence and histochemical tests (Hassan and El-Awadi, 2009). However, secreting cells inside the gland had emitted the whitish autofluorescence (UV-2A) of lignin and phenolic substances as shown in Fig. (5-a).

The histochemical light - stain reaction tests revealed the presence of lignin (red, double stain), phenolics (blue, toludine blue O), lipid (yellow, fluoroll yellow 088 or red, fat red 7B) and suberin (red, mixed Sudan III&IV). These substances were located in the outer layer of trichomes and in the neck cell. In secreting cells of the examined gland, the phenolic substances were detected by the toludine blue O and the double stains (Fig. 5-b)



UV-2A B-B-2A G-2A

Fig. 5-a. Autofluorescence of non-stained sections showing the light-colour reaction of broomrape glandular trichome under A-UV-2A; B-B-2A; C-G-2A (Hassan and El-Awadi, 2009).



A **B** **C** **D** **E**

Fig. 5-b. Histochemical tests showing the chemical composition of broomrape plant glandular trichomes. A, stained with toludine blue O under visible light; B, Stained with double stain under visible light; C, stained with fat red 7B under B-2A light; D, stained with fluorol yellow 088 under UV-2A light; E, stained with Sudan III & IV under B-2A light (Hassan and El-Awadi 2009).

Histochemical color-stain reaction methods applied in weedy *Amaranthus viridus* L. plant indicated highly subersized cuticle using sudan *III* & *TV* mixed stain under blue light illumination (red colour). Strong lignifications in outer layer appeared as red color and in between cortical cell (torques color) was detected by toludine blue o stain (Hassan *et al.,* 2003) as shown in Fig. (6).

3- **In the supposed defense mechanism actions:**

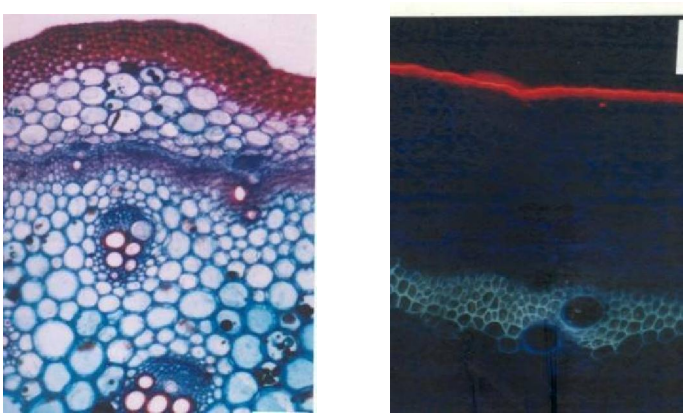
a-Parasitic plant host - root interaction

Wound responses in broomrape host root tissues` and in a host-pathogen interaction

Roots of different hosts of the holophrastic weed known as broomrape (*Orobanche spp.*) were examined histochemicaly for the occurrence of structural cellular barrier formation following wounding / penetration (Tables 3 and 4). Such barrier might function to impede the successful development of parasite haustorium interaction, i.e. as a self-defense mechanism.

In faba bean and white bean, brown deposits occurred in walls adjacent to the damaged cells of the epidermis, cortex and stele. Via stain reactions and colorations these deposits were detected as melanin. Additionally, walls bordering damaged site at the level of the endodermis and within the stele become suberized and lignified. In peas, which possesses a lignified hypodermis, the response was similar but lignin was also deposited in the walls of the endodermis and hypodermis adjacent to the wound. In sunflower, which possesses a suberized hypodermis, melanin was deposited in the hypodermis and lignin and suberin occurred within the stele. In all these broomrape host species

melanization conferred the modified cell wall many of the properties associated with lignified and suberized structures such as impermeability and resistance to chemical degradation (Brammall and Hassan, 1995).



A B

Fig. 6. *Amaranthus viridis* stem (Hassan *et al.,* 2003)
A: Toludine blue o stain under visible light and B: Sudan *III* & *TV* under blue light

Table 3. The distribution of lignin, suberin and melanin in uninjured tissues of faba bean, peas, white bean and sunflower (Brammall and Hassan, 1995)

Defense materials	**Faba bean**	**Peas**	**White bean**	**Sunflower**
Lignin	Xylem Phloem Endodermis Casparian band	Xylem Phloem Epidermis Outer cortical layer (Hypodermis) Endodermis Casparian band	Xylem Phloem	Xylem Phloem Casparian band
Suberin	Endodermal cells	Outer cortical layer (Hydridermis) Endodermis Casparian band	Endodermal cells	
Melanin	Epidermis Outer cortical layer	Epidermis Outer cortical layer	Epidermis Outer cortical layer	Epidermis

Table 4. The distribution of lignin, suberin and melanin in wound healing root tissues of faba bean, peas, white bean and sunflower (Brammall and Hassan, 1995)

	Faba bean	Peas	White bean	Sunflower
Lignin	Interior to endodermis	Interior to endodermis Epidermis Hypodermis	Interior to endodermis	Stele
Suberin	Endodermal cells	Hydridermis Endodermis Casparian band	Endodermal cells stele	Hydridermis (Outer cortical layer) Stele
Melanin	Epidermis cortical parenchyma stele	Epidermis Cortical parenchyma stele	Epidermis Cortical parenchyma stele	Epidermis Cortical parenchyma Stele

b-Host-pathogen interaction

In the Japanese birch (*Betula platyphylla* var. japonica, Tohoku), infection with a canker-rot fungus; the *Inonotus obliquus* IO-U1 strain. In intact wounded, and infected plantlets changes were observed morphologically in the treated portion. Phenolics first deposited at the cut margin. Subsequently the phenolic was detected in the vessels after 4h infection. Their deposition then extended to the xylem elements, the cortex, and to the pith with an increase in the infection period. The deposition of phenolics was extensive at 10 days post inoculation (dpi), when most of the cells were entirely filled with phenolics.

A necrophylactic periderm (NP) was formed at the junction of the original periderm with a layer of 2–4 new phellen cells at 30 dpi. Based on the results obtained, phenolics deposition and NP formation are considered to occur as infection - induced responses in Tohoku birch plantlets under infection with *Inonotus obliquus*, strain IO-U1 (Rahman *et al*., 2008).

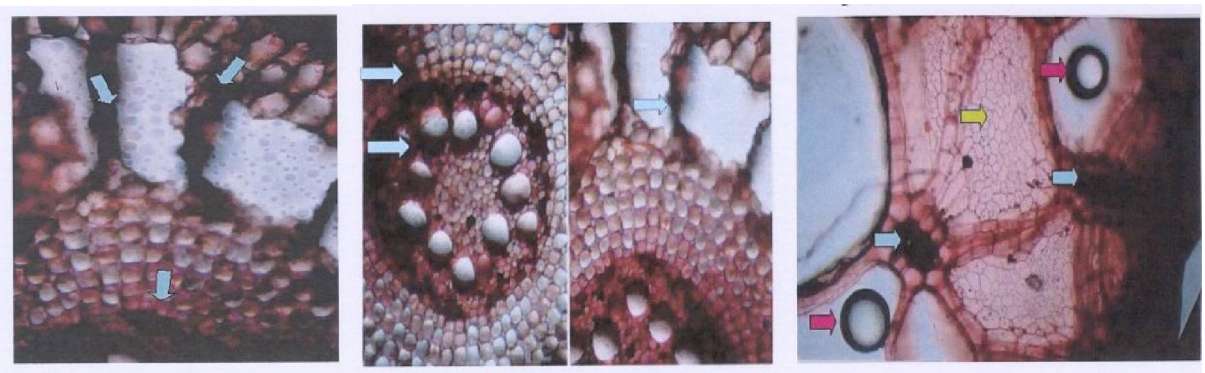
4-In detection of heavy metals,i.e. pollution and contamination:

a-Heavy metals` accumulation

Histochemical methods were employed in the detection of the heavy metals (Cd, Pb, Ni, Zn) and strontium, their distribution, accumulation, and translocation within the tissues of higher plants (Table 5). In this respect, detailed protocols of metal detection with metallochrome indicators dithizone (Cd, Pb), dimethylglyoxime (Ni), sodium rhodizonate (Sr), zincon (Zn), and fluorescent indicator Zinpyr_1 (Zn) by light and fluorescence microscopy were described (Seregin and Kozhevnikova, 2011).

In their study Warrier and Saroja (2008) investigated the occurrence of heavy metals and their accumulation in water hyacinth [*Eichhornia crassipes* (Mart.) Solms].

The histochemical staining examinations indicated the accumulation in the epidermis and vascular bundles of the roots and petiole. In the leaf sections the palisade tissues were deeply stained, showing the high accumulation of the metals within the leaves (Fig. 7).



A B C

Fig. 7. Histochemical investigation showing accumulation of heavy metals in different tissues of water hyacinth (Warrier and Saroja, 2008):
A: Cs of root showing translocation of heavy metals to the vascular bundles from the epidermis
B: Cs of root showing accumulation of heavy metals in the epidermal regions and translocation on the vascular bundles.
C: Cs of petiole of hyacinth
Absence of heavy metals accumulation with the petiole
Translocation of heavy metals through the vascular bundles of the petiole
Air bubbles

Table 5. Histochemical analysis for detecting distribution of Cd, Pb, Ni, Sr, and Zn in plant tissues

Reagent	Metal	Protocol of reagent preparation and color of produced complex/ fluorescence
Dithizone $C_{13}H_{12}N_4S$ (M*r* = 256.3) (metallochrome indicator)	Cd, Pb	Dithizone is dissolved (0.5 mg/ml) in a mixture of acetone and distilled water (3:1); 1–2 drops of glacial acetic acid are added to 6 ml of this solution in order to improve the sensitivity of reaction. Solution should be used fresh. The complex of dithizone with Cd and Pb is red (Seregin and Ivanov, 1997).
Dimethylglyoxime $C_4H_8N_2O_2$ (M*r* = 116.1) (metallochrome indicator)	Ni	1% solution of dimethylglyoxime in 1.5% solution of NaOH in 0.05 M borax (Na2B4O7 · 10 H2O) (pH 9.8–10.4). Solution may be stored for a week. The complex of dimethylglyoxime with Ni is crimson (Seregin *et al.*, 2003).
Sodium rhodizonate $C_6Na_2O_6$ (M*r* = 214.0) (metallochrome indicator)	Sr	0.2% solution of sodium rhodizonate in distilled water. Solution may be stored for a week. The complex of sodium rhodizonate with Sr is grayish brown (Seregin and Kozhevnikova, 2004).
Zincon $C_{20}H_{15}N_4NaO_6S$ (M*r* = 462.4) (metallochrome indicator)	Zn	0.0065 g of zincon is dissolved in 0.2 ml of 1 M NaOH in borax (Na_2B_4O7 10 H_2O) solution (pH 9.8–10.4) and adjusted with distilled water to 10 ml so that the concentration of borax in the solution becomes 0.05 M; the obtained solution is heated to 80°C and cooled to room temperature. The solution may be stored for a week. The complex of zincon with Zn is blue (Seregin *et al.*, 2011).
Zinpyr_1 $C_{46}H_{36}C_{l2}N_6O_5$ (M*r* = 823.2) (fluorescent indicator). Excitation and emission maximums of Zinpyr_1 are located in the visible region: at 490 and 525 nm, respectively	Zn	In order to prepare 5 mM stock solution, a sample of reagent is dissolved in dimethyl sulfoxide. Stock solution is stored at –20°C in darkness. Directly before the analysis, reagent is thawed and diluted to 10 μM with super deionized water. In the presence of Zn, Zinpyr_1 produces green fluorescence (Sinclair *et al.*, 2007).

b- In detection of H_2O_2 and $O^{2\bullet-}$ a result of cadmium contamination

Romero-Puertas *et al.* (2004) studied the effect of cadmium on H_2O_2 and $O_2^{\cdot-}$ production in leaves from pea plants grown for 2 weeks with 50 μm Cd, by histochemistry with diaminobenzidine (DAB) and nitroblue tetrazolium (NBT), respectively. The subcellular localization of the reactive oxygen species (ROS) was studied by using $CeCl_3$ and Mn/ DAB staining for H_2O_2 and $O_2^{\cdot-}$, respectively, followed by electron microscopy observation. In leaves from pea plants grown with 50 μm $CdCl_2$, a rise of six times in the H_2O_2 content took place in comparison to control plants. The accumulation of H_2O_2 was localized mainly in the plasma membrane, mesophyll and epidermal cells, as well as in the tonoplast of bundle sheath cells. In mesophyll cells, the accumulation of H_2O_2 was observed in mitochondria and peroxisomes. Localization of $O_2^{\cdot-}$ production was demonstrated in the tonoplast of bundle sheath cells, and plasma membrane from mesophyll cells. The Cd-induced production of the ROS, H_2O_2 and $O_2^{\cdot-}$, could be attributed to the phytotoxic effect of Cd. In this connection, lower levels of ROS were assumed to function as signal molecules for the induction of defense genes against the injurious effects of the heavy metal (Fig. 8).

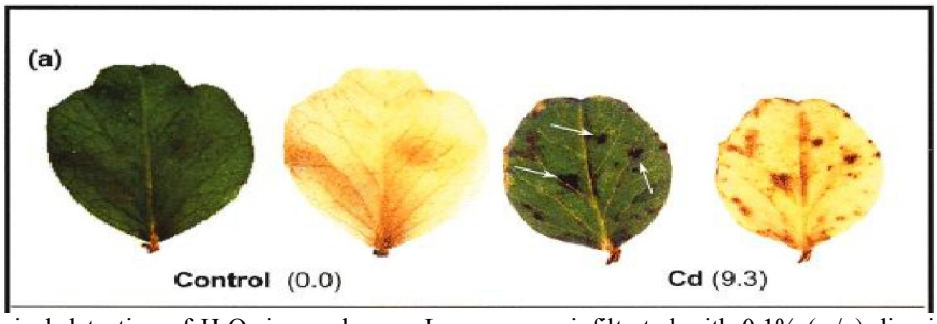


Fig. 8. Histochemical detection of H_2O_2 in pea leaves. Leaves were infiltrated with 0.1% (w/v) diaminobenzidine (DAB) for 8 h in the dark. (a) Leaves from control and Cd-treated plants. To visualize DAB deposits, leaves were decoloured in boiling ethanol (right leaves). Arrows indicate brown deposits of H_2O_2 (Romero-Puertas *et al.,* 2004).

However, Jin *et al*. (2008) found that exposure to cadmium resulted in significant ultrastructure changes in the root meristems and leaf mesophyll cells of *S. alfredii*. Damages were more pronounced in NHE even when Cd concentrations were one-tenth of those applied to HE. In the cadmium stress damaged chloroplasts resulted in imbalanced lamellae formation which is coupled with early leaf senescence. Histochemical results revealed that glutathione (GSH) biosynthesis inhibition led to overproduction of hydrogen peroxide (H_2O_2) and superoxide radical ($O_2\cdot^-$) in HE but not in NHE. The GSH biosynthesis induction in root and shoot exposed to elevated Cd conditions, however, might be involved in Cd tolerance and hyper accumulation in HE of *S. alfredii* H (Fig. 9).

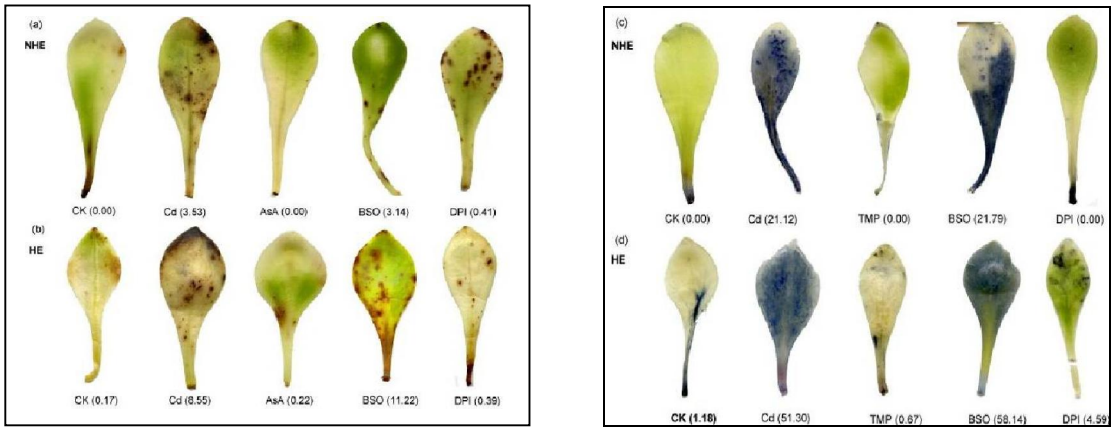


Fig. 9. Histochemical detection of H_2O_2 (a: NHE; b: HE) and $O^2\cdot^-$ (c: NHE; d: HE) in leaves of *Sedum alfredii* H. Excised leaves from both control and Cd-treated plants (NHE and HE) were grown in 10 μM and 100 μM Cd for 7d, respectively immersed in diaminobenzidine (DAB) or nitroblue tetrazolium (NBT) solution to visualize brown or blue spots characteristic of DAB or NBT reaction with H_2O_2 and $O_2\cdot^-$, respectively. Then, leaves were bleached by immersing in boiling ethanol to visualize the spots.
Treatment symbols: AsA-H_2O_2 scavenger, TMP-$O_2\cdot^-$ scavenger, BSO- glutathione synthesis inhibitor, DPI-oxidase inhibitor. The results were expressed as percentage of spot area in pixels, vs. total leaf area [(spot area/ total leaf area) × 100]. Leaves were taken from control and Cd exposed plants, plus from Cd exposed plants treated with other compounds. The effect of AsA, TMP, BSO, and DPI on control plants is excluded; the percentage effect in treated plants is indicated in brackets. Results are reported from five representative individual experiments (Jin *et al*., 2008).

c-In the detection of aluminum accumulation, lipid peroxidation, callose production, and plasma membrane integrity

In peas (*Pisum sativum* L.) roots, staining were observed to distribute similarly on the entire surface of the root apex regarding aluminum accumulation, lipid peroxidation, and callose production). Meanwhile, the loss of plasma membrane integrity (detected by Evans blue uptake) was localized exclusively at the periphery of the cracks on the surface of root apex (Yamamoto *et al*., 2001).

They added that the enhancement of four phenomena, i.e. aluminum accumulation, lipid peroxidation, callose production and root elongation inhibition displayed similar aluminum dose dependencies which occurred at 4hs exposure. The loss of membrane integrity, however, was enhanced at lower aluminum concentrations and after a longer aluminum exposure of 8h. The addition of butylated hydroxyanisole (a lipophilic antioxidant) during aluminum treatment was found to completely prevent only the lipid peroxidation and callose production by 40%. Thus, lipid peroxidation was suggested to represent relatively early symptom induced by the accumulation of aluminum and appear to cause, in part, callose production. Whereas, the loss of plasma membrane integrity represented a relatively late symptom caused by cracks in the root due to the inhibition of root elongation (Figs. 10 & 11).

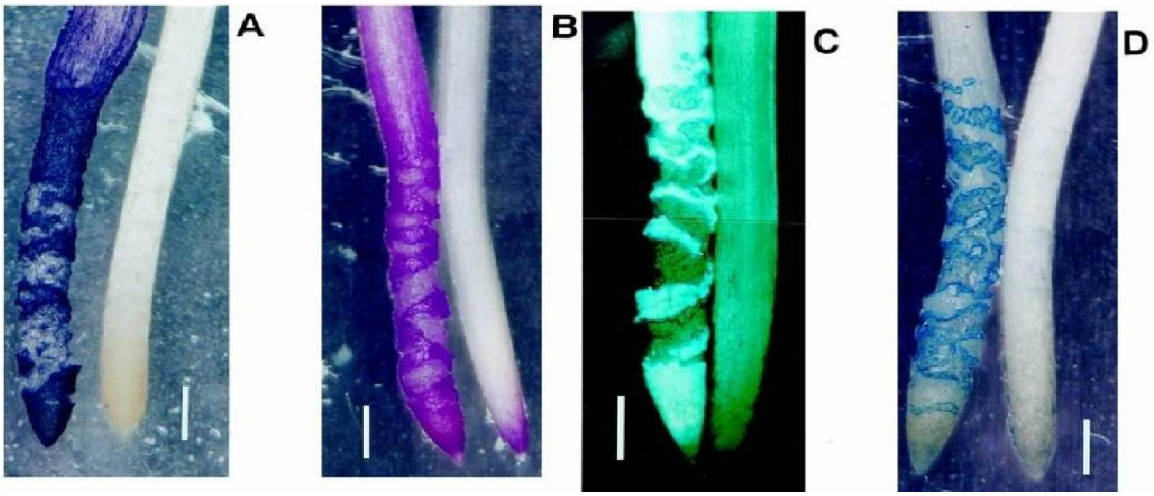


Fig. 10. Histochemical detection of lipid peroxidation and other events caused by aluminum in pea roots. Pea seedlings were treated with (left) or without (right) 10 µm aluminum in 100 µm $CaCl_2$ (pH 4.75) for 24h. The roots were stained with hematoxylin (A, aluminum accumulation), Schiff's reagent (B, lipid peroxidation), aniline blue (C, callose production), or Evans blue (D, the loss of plasma membrane integrity). The positive staining of each technique in the photomicrographs appears as bright images in panels A, B, and D and as a fluorescent image in panel C. The bar in each graph indicates 1 mm (Yamamoto *et al*., 2001).

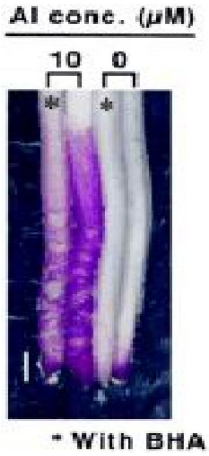


Fig. 11. Histochemical detection of Butylated hydroxyanisole (BHA) effect on the aluminum-enhanced lipid peroxidation in pea roots. Pea seedlings were treated with or without 10 µm aluminum in the presence (*) or absence of 20 µm BHA in 100 µmCaCl2 (pH 4.75) for 24 h. The roots were stained with Schiff's reagent for the detection of lipid peroxidation. The positive staining shows a bright image in the photomicrograph. Bar indicates 1 mm (Yamamoto *et al*., 2001).

5-In detection of active substances in aromatic plant; citral accumulation in lemongrass

Luthra *et al*. (2007) located the sites of citral accumulation in lemongrass (*Cymbopogon flexuosus* Nees ex Steud) wats (cultivar OD-19) by Schiff's reagent, which upon its reaction with aldehydes (citral) gives a purple-red colouration. Using this technique, single oil-accumulating cells were detected in the adaxial side of leaf mesophyll commonly adjacent to non-photosynthetic tissue and between vascular bundles. In this respect, however, the citral lacking cultivar GRL-1 (geraniol rich) leaf sections, which also was subjected to Schiff's reagent could be compared to the cultivar OD-19 leaf sections. In lemongrass mutant GRL-1, those specialized cells, however, are not be stained due to lack of citral. Hence, it could be confirmed that the observed schiff's staining reaction is associated with the accumulation of citral substance in a given cell (Fig. 12).

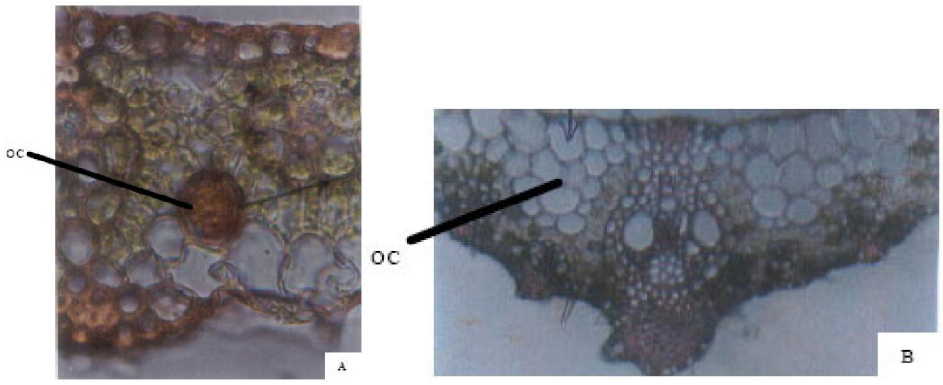


Fig. 12 (Luthra *et al*., 2007)
(A) Cross section of *Cymbopogon flexuosus* cv. OD-19 leaf showing the red colored oil cells (OC) as the citral accumulating site, (B) Cross section of mutant chemotype GRL-1 leaf showing colorless oil cells (OC) indicating the absence of citral

6. In localization of the site of monoterpene phenols` accumulation in plant secretory structures

Gersbach *et al.* (2001) reported a new histochemical method aimed to localize monoterpene phenols in essential oil secretory plant structures. The method was adapted from a spot test originally devised for the *in vitro* detection of phenolic compounds in organic analyses. Plants subjected to the test were the Lamiaceae species, i.e. *Thymus vulgaris* L., *Oreganum vulgare* L. and *Mentha x piperita* L. which are known to accumulate essential oils in their glandular trichomes.

A reagent consisting of 4-nitrosophenol in conc. H_2SO_4 was applied to leaf sample of each species. The positive reaction revealed the existence of phenol was indicated by the production of colored indophenols. Same method was employed in detection of monoterpene phenols in the trichomes of *T. vulgaris* (thymol) and *O. vulgare* (carvacrol), appeared as changes to red and green colors, respectively. Whereas, negative color reaction proved the absent of phenol in the trichomes of *M. x piperita*. The results were confirmed by GC-MS analysis of leaf volatile extracts from each species, and via an *in vitro* tests with thymol and carvacrol (Fig. 13).

It is suggested that this method could be used in a rapid field survey to identify the existence of bioactive compounds in certain plants.

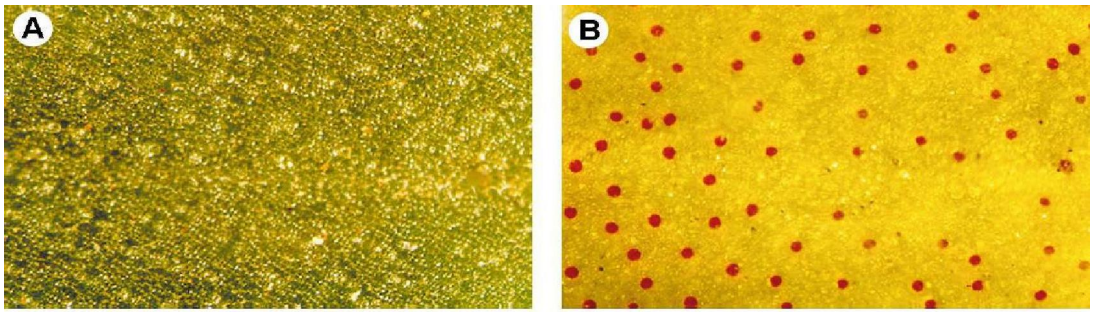


Fig. 13: A&B: Histochemical tests; A and B, Leaf of *Thymus vulgaris* before and after treatment with nitrosophenol reagent respectively; peltate glandular trichomes in B are stained red as a result of thymol condensing with nitrosophenol to produce a red indophenols (Gersbach *et al.* (2001).

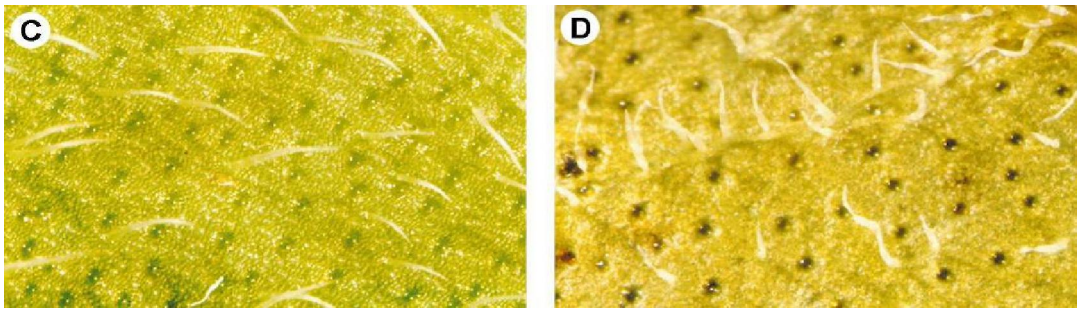


Fig.13: C & D. Leaf of *Oreganum vulgare* before and after treatment with nitrosophenol reagent respectively; peltate glandular trichomes in D are stained green as a result of carvacrol condensing with nitrosophenol to produce a green indophenols (Gersbach *et al.* (2001).

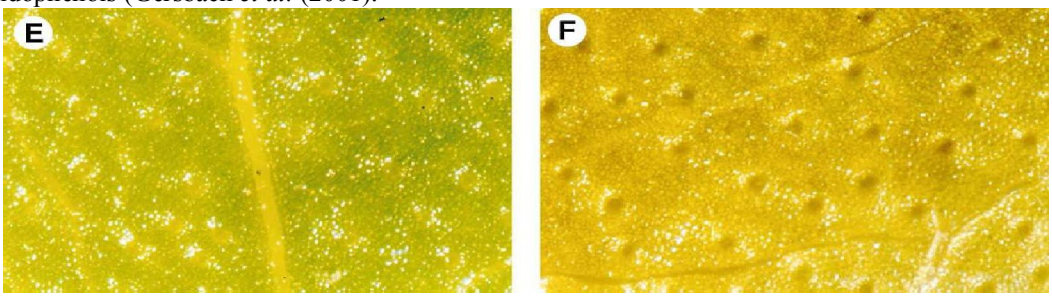


Fig. 13: E & F. Leaf of *Mentha piperita* before and after treatment with nitrosophenol reagent respectively; the glandular trichomes in F are not stained, indicating no reaction; therefore no phenol is present (Gersbach *et al.* (2001).

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Climate Change Adaptation Needs for Food Security in Egypt

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Abstract: Climate change will affect all four dimensions of food security, namely food availability (i.e., production and trade), access to food, stability of food supplies, and food utilization. The vulnerability of the agriculture in Egypt to climate change is mainly attributed to both biophysical and socioeconomical parameters. This study is to investigate climate change impacts based on field study results and projects activity outputs during last decade in Egypt. An analysis of climate risks for crops in food-insecure regions in Egypt was conducted to identify adaptation priorities, based on statistical crop models and climate projections for 2030, from different general circulation models. Results indicate that Egypt, without sufficient adaptation measures, will likely suffer negative impacts on several crops that are important to large food-insecure human populations. Adaptation planning in agriculture is designing and applying of national adaptation strategy for the agriculture sector. The strategy is facing a group of barriers and limitations (eg. existing scientific, information and policy perceptions, poor adaptive capacity of the rural community, lack to financial support, and absence of the appropriate institutional framework).

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Key words: Food security, social stability, net income, vulnerability, national adaptation strategy.

1. Introduction

Egypt appears to be particularly vulnerable to climate change, because of its dependence on the Nile River as the primary water source, its large traditional agricultural base, and its long coastline, which is already undergoing both intensifying development and erosion (**Rosenzweig et al 2002; World Bank Report 2007**).

The first and second Egyptian communications reports (**EEAA 1997**), which were prepared by the Egyptian Environmental Affairs Agency (**EEAA 2006**) submit to the United Nations Framework Convention on Climate Change (UNFCCC), reported that Egypt is one of the most vulnerable countries to the potential impacts and risks of climate change, even though it produces less than 1 % of the world total emissions of greenhouse gases. More than 95 % of the water budget of Egypt is received from the River Nile which is generated outside Egypt's territory. Numerous studies showed that River Nile is very sensitive to temperature and precipitation changes **(Riebsame 1995)**. Agriculture in Egypt is expected to be especially vulnerable because of hot climate. Further warming is consequently expected to reduce crop productivity. These effects are exacerbated by the fact that agriculture and agro-ecological systems are especially prominent in the economics of Egypt as one of the African countries **(Riebsame 1995)**.

More studies were made to assessment the potential impacts of climate change on crop productivity and crop water use under different agro-climatological zones in Egypt **(Abou Hadid 2006 and El-Marsafawy et al 2007)**. Table 1 summarized the studies result of impact of climate change on the productivity of some major crops in Egypt up to 2050's. From these results it can be concluded that, climate change could decrease national food production from 11 % to 19 %.

Adaptation is a key factor that will shape the future severity of climate change impacts on food production **(Easterling 2007)**. Although relatively inexpensive changes, such as shifting planting dates or switching to an existing crop variety, may moderate negative impacts, the biggest benefits will likely result from more costly measures including the development of new crop varieties and expansion of irrigation **(Abou Hadid 2006)**. These adaptations will require substantial investments by farmers, governments, scientists, and development organizations, all of whom face many other demands on their resources. Prioritization of investment needs, such as through the identification of "climate risk hot spots" **(Burton and van Aalst 2004)**, is therefore a critical issue but has received limited attention to date.

2. Study Area and Methodology

2.1 Time scale

Several different criteria were considered for the study time scale. First is the importance of the crop to a region's food-insecure human population

[hunger importance (HI)]. Second is the median projected impact of climate change on a crop's production by 2030, assuming no adaptation. For this analysis, the study generates multiple (i.e., 100) projections of impacts based on different models of climate change and crop response, in order to capture relevant uncertainties.

2.2 Major food-insecure crops

Three major food-insecure region identified (e.g. wheat, maize and milled rice), each of which (i) comprise groups of country with broadly similar diets and agricultural production systems and (ii) contain a notable share of the country's malnourished individuals as estimated by the Food and Agriculture Organization **(FAO 2012)**. The use of projected malnourished populations in 2030 rather than current population values had a very small influence on the rankings.

2.3 Climate change to 2030

Global average temperatures are projected to rise by about 1°C by 2030 (i.e. well outside the natural range). Higher latitudes will warm more rapidly than lower ones and land areas will warm more rapidly than the oceans. Consequently, average temperatures in the higher latitudes may rise by 2°C, possibly double the increase in the tropics **(IPCC 2001)**. Parts of Central America, South Asia, northern and southern Africa and Europe could suffer appreciable falls in available water resources.

To project climate changes for the crop region, along with their uncertainties, general circulation models (GCMs) output were used (Model simulations under SRES (Special Report on Emissions Scenarios) emission scenarios corresponding in MAGICC/SCENGEN the emissions scenarios are referred to as a reference and policy **scenarios (Wigley and Raper 2011 and Tubiello 2005)**. Data which generated are represented in one scenario A1. These scenarios are described by **IPCC 2001** as follows: The A1 scenario describes future regions of very rapid economic growth, global population that peaks in mid-century and declines thereafter, and the rapid introduction of new and more efficient technologies.

2.4 Evaluating climate change impacts

Depend on the obtained results from most of studies a several options exist for evaluating climate change impacts across a suite of crops. The data sets on historical crop harvests were used. The model's strength came primarily from a (typically negative) temperature effect on yield.

The probability distribution of production changes for 2030 were estimated (the average from 2020 to 2039 relative to that from 1999 to 2009) for each crop using a Monte Carlo procedure that propagated both climate and crop uncertainties. To facilitate comparison between crops and regions, we expressed production changes for all crops as a percentage of average values for 1999 to 2009.

3. Results and Discussion

3.1 Impact of climate change on some strategic crops in Egypt

Climate change impact studies that were based on field studies, predicted a reduction in the productivity of the major crops in Egypt. Tables (1 & 2) show the impact of climate change on some major crops in Egypt as a summary of several local studies. These changes in crop productivity are mainly attributed to the projected temperature increase, which affect the grain filling periods and have detrimental effects on sensitive development stages such as flowering, thereby reducing grain yield and quality. Crop-water stress is the other factor causing productivity reduction under climate change.

Impacts of climate change on wheat showed that increasing in temperature will reduce length of growing cycle and the time needed to full tillering in addition to the final yield. This subsequently will reduce the amount of grain yield; accelerate time for maturity and harvesting. For +1.5°C scenario, reduction in grain yield, as predicted by the model, will be in average among cultivars of 12% at Sakha location, 9% at Sids location and 11% at Shandaweel location. Scenario of +3.5°C will reduce grain yield within an average of 27% at both Sakha, Sids locations, and 31% at Shandaweel location. We can conclude that reduction in wheat grain yield at the three locations has high probability in the future with accelerating growing cycle, especially at +3.5°C, which needs to define earlier sowing suitable dates and adaptive agronomical practices **(Hassanein et al 2012)**.

Pests and disease remain important factors affecting negatively the crops productivity. The severities of pests and disease impact on the productivity are projected to increase under climate change conditions. The recent scientific observations concluded that the severity of some pests and disease affecting the strategic crops have increased in the last few decades **(Abolmaaty et al 2010 and Yones et al 2011)**. This increase in severity is mainly attributed to both climatic and socioeconomical reasons.

The impact of climate change on the pests and disease in relation to crops productivity, is studied in limited scientific trials, but not yet well studied at the national level under Egyptian conditions. For example, severe epidemics of tomato late blight (*Phytophthora infestans*) emerged in the last few years. In practice, an epidemic onset is expected to lead to 2-4 additional sprays to be applied at the coming decades of the 2025-2100's **(Fahim et al 2007**

and Fahim et al 2010). Furthermore, it is a challenge for potato late-blight researches in the future to find a balance between reduction use of pesticides usage and the pressure to increase pesticide utilize due to changes in climate and challenging the pathogen populations. Another study indicated that, the severities of current cultivars of wheat to leaf rust caused by *Puccinia triticina* and stripe rust disease caused by *Puccinia striiformis* increase with increasing temperature, which is projected under climate change conditions **(Abolmaaty 2006)**. Some studies found that, generation numbers of *Tuta absoluta* under climate change conditions increased especially in Qena governorates (south Egypt). However, the expected generation numbers of the pest at 2050 and 2100 are be 12-14 and 13-15 generations per year, respectively (**Abolmaaty et al 2011**).

The limited investigations in pests and disease concluded that it is a challenge for the agricultural sector in the future to find a balance between the environmental protection demands for reducing use of pesticides and the pressure to increase pesticide use due to climate change. Furthermore, the possibility of emergence of foreign species endeavouring the local environment species of pest and diseases under climate change conditions is one of the high risks that may face the agriculture production in the future.

Despite the effects of long term projected changes in temperature, the agriculture in Egypt is less sensitive to climate variability, due to the reliance on irrigated agriculture system. Yet, heat and cold waves cause several harmful impacts in crops productivity, especially for fruits and vegetables. A recent study found that the intensity of the heat and cold waves increased in the past 20 years, this represents more risks for the growers [8].

Table 1: Change in major crop production (excess or deficit) in Egypt by the year 2050 due to climate change.

Crop	Current conditions			Climate change conditions	
	Base Yield (t/ fed)	Area (Mfed)	Total Yield (Mt)	Change %	Deficit or excess (Mt)
Wheat	2.732	2.920384	7.977051	-18	-1.435869
Maize	3.39	1.860363	6.306052	-19	-1.198150
Rice	4.091	1.769782	7.240519	-11	-0.796457

Source: **Hassanein M. K., 2010**. Climate change risk management in Egypt, Food Security FAO project UNJP/EGY022 report number 6.1.2.1. pp 92.

Table 2: Projected changes in crop production of some major crops in Egypt under climate change conditions

Crop	Change %		Reference
	2050s	2100s	
Wheat	-15%*	-36%**	**Medany and Hassanein 2006**
Rice	-11%		**Eid and El-Marsafawy 2002**
Maize	-19%		**Eid et al 1997**
	-14%	-20%	**Hassanien and Medany 2007**
Soybeans	-28%		**Eid and El-Marsafawy 2002**
Cotton	+17%*	+31%**	**Eid et al 1997**
Potato	-0.9 to -2.3%	+0.2 to +2.3 %	**Medany and Hassanein 2006**

* Temperature increase by 2°C; ** Temperature increase by 4°C

3.2 Adaptation to climate change impacts on main crops in Egypt

Crop production and cropping systems studies concluded that changing sowing dates and management practices were among the important adaptation measures oriented to ameliorate the harmful impact of the climate change on the crop yield **(Abou Hadid 2006)**.

Investigation of impact of climate change on the national cropping pattern**, (Hegazy et al 2008)** tried a range of temperatures over a range of cultivars of major crop species indicated that sowing dates could be managed in order to allow maximum predicted planting area in a given region in Egypt. For instance, the current maximum area suitable for cotton planting may show few variations over the coming hundred years. In this case, the sowing dates should be changed from the hotter months (February to April) to the cooler months (January to February).

Changing sowing dates could increase the flexibility of the farming system to face temperature and water requirements increase due to climate change, as a single factor effect. This adaptation option is facing some implementation difficulties related to the overall crop calendar arrangements, and it may be limited by the marketing opportunities,

which may not match the new harvesting dates, especially for cash crops. The acceptability of changing planting date option needs further studies regarding the conflict with other existing crops as the Egyptian cropping system is based on 12-month cycle **(El-Marsafawy 2007**).

The study of **Medany et al 2009** Concluded that changing cultivars and changing crop pattern are the most promising adaptation measures that should be applied at the national level, to overcome the harmful impacts of climate change in crop production.

Furthermore, in order to adapt to the expected disease severity in major crops, breeding of disease tolerant cultivars is urgently needed. At the same time, monitoring system for the current and new races of plant pests and diseases in the country is highly required. Furthermore, application of deficit irrigation measures is more acceptable during water shortage circumstances, compared to reduction of irrigated area **(Medany et al 2009)**.

3.3 Chaining in the main crop commodities self-sufficient in Egypt towards to 2030 under different scenarios

Self-sufficiency in strategic crops shown in Table 3 and Figure 1 indicated that, under future climatic changes conditions towards to 2030 (with no action scenario), it can be predicted that if the agricultural area and production as the same current, self-sufficient with wheat, maize and milled rice could reach 33.6, 24.8, 86.9 % compared to 57.4, 53.9, and 160.0 % under current conditions. In addition, increasing in population growth rate and the shortage in water supply as well as the rising in sea level with its effect on salinaization of North Nile Delta which could decrease the total agriculture area, the situation in food security will more and more seriously in the future.

Yield declines for the most important crops. Climate change will have varying effects on irrigated yields across regions, but irrigated yields for all crops in Egypt will experience large declines. Climate change will result in additional price increases for the most important agricultural crops–rice, wheat, and maize.

On the other hand, with positive action against risks of climate change on self-sufficiency in strategic crops Table 3 shown that under increase of agricultural area and production with 10% (depend on **SADS 2030**), self-sufficient with wheat, maize and milled rice could reach 49.4, 36.7, and 133.7 %, respectively.

Therefore, it is vital that action be taken now to counter this threat. Actions should include measures to reduce agriculture's role as a driving force for climate change, through the reduction of GHG emissions, as well as measures to mitigate and adapt to climate change.

Table 3: Estimated rates of self-reliance and self-sufficiency in the main food commodities, under climate change with action and no action of adaptation

Main food commodities	Current production of 2011			2030 estimates*** with no climate change impact		
	Prod.* (1,000 tons)	Requirements (1,000 tons)	Self-suf.(%)	Prod. (1,000 tons)	Requirements (1,000 tons)	Self-Suf. (%)
Wheat	8407	14650	57.4	8407	18709	44.9
Milled rice	5675	3528	160.9	5675	4664	121.7
Maize	6876	12827	53.6	6876	20600	33.4
People population	83 millions			106 millions		
	2030 estimates*** with climate change + no adaptation action			2030 estimates *** with climate change + adaptation action		
	Prod. (1,000 tons)	Requirements (1,000 tons)	Self-suf(%)	Prod. (1,000 tons)	Requirements (1,000 tons)	Self-Suf (%)
Wheat	6279.8	18709	33.6	9247.7	18709	49.4
Milled rice	4052.17	4664	86.9	6242.5	4664	133.8
Maize	5103	20600	24.8	7563.6	20600	36.7
People population	106 millions			106 millions		

Sources of row data: * FAOSTAT | © FAO Statistics Division 2013
** CAPMS| Central Agency for Public Mobilization and Statistics 2013 *** SADS 2030 | Sustainable Agriculture Development Strategy Towards 2030

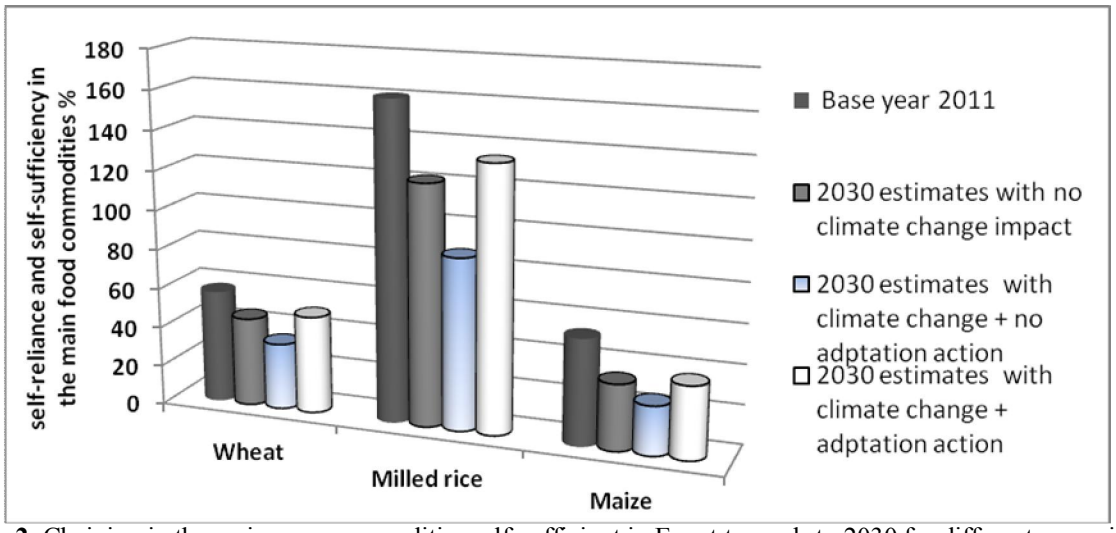

Figure 2: Chaining in the main crop commodities self-sufficient in Egypt towards to 2030 for different scenarios.

3.4 Recommendation for research topics & needs of climate change to food security

Depend on most of studies results and projects activity outputs conducted in Egypt, significant attention needs to be given to the following topics:

- Assess the impact of climate change on the productivity of the major crops, with wide scale assessments covering the Egyptian agricultural map.
- Study the impact of climate change in agriculture water-use.
- Study the impact of climate change on soil degradation and land use change, with special attention to the agricultural hotspots in the Nile Delta region.
- Study the impact of climate changes on the pest and disease cycles and severities, host plants disease resistance, epidemics evolution, and native pests and diseases migration.
- Study the impacts of climate change on livestock, aquaculture and fishing.
- Study the impact of extreme weather events, on terms of heat and cold waves and sand storms, in the productivity of plant and livestock production.
- Developments of risk assessment tools and identify agricultural hotspots in Egypt to assess the risks of agriculture sector under the projected climatic changes and extreme weather events.
- Encourage academic and on the job training of the subject and provide the necessary professional assistance through technical, local and foreign consultants, short courses, web applications, seminars and workshops.
- Develop national emission factors and methodologies, in order to conduct more accurate determinations of GHGs emissions from agriculture sector.
- Conduct mitigation studies of GHGs emissions from different agricultural sources, with special attention to paddy rice, soil management, livestock, animal waste management systems, and agricultural waste burning.
- Study the economics of mitigation from the different GHGs sources from agriculture sector.
- Study the vulnerability and the adaptation of the cropping pattern and systems at farm, regional and national levels.
- Conduct wide scale assessment of field crops stress- tolerant varieties development, in terms of heat, water shortage and salinity stresses.
- Study the vulnerability and the adaptation of the on-farm irrigation system at farm, regional, and national levels.
- Study the possible adaptation measures to face the agricultural land loss due to SLR effect.
- Develop adaptation measures of soil maintenance, under different agricultural systems, with special attention to the hotspots agricultural locations in Egypt.
- Develop integrated adaptation scenarios of plant protection, based on integrated crop management (ICM) and biological control concepts.
- Develop integrated adaptation scenarios for livestock, fishing and aquaculture.
- Conduct comprehensive studies of the adaptation requirements, costs, inter-sectors

relationships and feedbacks of the agriculture sector and rural communities.

4. Conclusion

The potential decreases in food production up to 2030 are relatively small and most countries should be able to compensate for climate change impacts by improving agricultural practices. Priority should be given to raising the resilience of agricultural ecosystems, increasing the cropped area, and raising and diversifying yields through improved access to genetic resources and technologies. Moreover, the growing income should make it possible for many of them to choose between greater food imports and greater mitigation and adaptation by agricultural sector to overcome climate change impacts.

Up to 2030, the most serious and widespread agricultural and food security problems related to climate change are likely to arise from the impact on climate variation, and not from progressive climate change, although the latter will be important where it compounds existing agro-climatic constraints. However, the more frequent extreme events will not necessarily increase food insecurity in all situations, given the other economic and social changes taking place. Institutional changes are going to be as important as or more important than technological ones. Institutional actions will be needed to raise national preparedness and reduce rural and urban poverty to enable vulnerable low-income groups to purchase all of their basic food requirements. Policies for agricultural development will need to emphasize the importance of improving not just the production capacity of agricultural ecosystems but also their diversity and resilience. It is vitally important to initiate the institutional and technological changes now, because of the long lead times for the development of new technologies and for the improvement of road and rail links between food-deficit and surplus areas, and between ports or railheads and isolated rural areas.

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11/19/2013

Assessment of Drought Impact in Africa Using Standard Precipitation Evapotranspiration Index

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Abstract: This paper assesses the impact of drought in Africa and selects the vulnerable areas to drought by using Standard Precipitation Evapotranspiration Index (SPEI) as a new index for drought monitoring during the period from 1960 to 2008 at time scales 12. Results of this study refer to the characteristic of drought over Africa using the Stander Precipitation Evapotranspiration Index (SPEI) at time scale 12 for month of Dec. during the period from 1960 up to 2008 was analyzed. Results concluded that the first decades were less drought area and the drought increased with time. Frequency of drought (SPEI values ≤ -1) increased in last decades. There are most difference between extreme drought and wet events while the severe and moderate classes were closer. The assessment of the drought impact in Africa needs to determine several systems (water resource, natural vegetation and crops) to quantify the impact of drought in terms of both system's resistance and resilience, to produce drought impact curve for each system and region.

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Key words: Drought, Drought index Assessment, Impact and Standard Precipitation Evapotranspiration Index (SPEI)

1. Introduction

Droughts are recognized as an environmental disaster and have attracted the attention of environmentalists, ecologists, hydrologists, meteorologists, geologists and agricultural scientists. Droughts occur in virtually all climatic zones, such as high as well as low rainfall areas and are mostly related to the reduction in the amount of precipitation received over an extended period of time, such as a season or a year. Temperatures; high winds; low relative humidity; timing and characteristics of rains, including distribution of rainy days during crop growing seasons, intensity and duration of rain, and onset and termination, play a significant role in the occurrence of droughts. In contrast to aridity, which is a permanent feature of climate and is restricted to low rainfall areas **(Wilhite, 1992),** a drought is a temporary aberration. In Africa drought is the natural hazard that affects a large number of people with the most negative consequences, being responsible for famine **(Scrimshaw, 1987)**, epidemics and land degradation **(Bandyopadhyay, *et al*., 2012; UN, 2008).** Among the most important natural disasters affecting the world, drought record the two highest number killed between years 1974 and 2007, where it killed 450,000 and 325,000 persons in 1984 and 1974 in Ethiopia/Sudan and the Sahel region, respectively **(UN, 2008).** Drought increases the structural problems of the African continent and in the last decades has caused a decrease of crop yields, unemployment, impoverishment and even forced migrations **(Scheffran *et al*., 2012d; UN, 2008)**. The problem may increase in the future since the current population projections predict a demographic increase in the regions affected by chronic water deficits in Africa, and climate change models also indicate the likely increase of drought severity during the 21st century **(Dai, 2011)**, which are likely to increase famine **(Marta M. Jankowska et *al*., 2012)** social conflicts and the risk of civil wars in African countries **(Burke *et al*., 2009).** Understanding, monitoring and mitigating drought is a very difficult task as a consequence of the intrinsic nature of the phenomenon. In addition, assessing the impact of drought on ecosystems and societies is also a complex task, because the same drought severity may have different consequences in different regions and systems due to the underlying vulnerabilities. New technologies based on geospatial information are available to determine the risk and vulnerability of a system to a drought and to develop monitoring and early warning systems based on real-time information to support decision making (**Sergio M. *et al* 2012).** The objective of this study is to monitoring, assessing drought impact in Africa, and selected the vulnerable areas to drought by using Standard Precipitation Evapotranspiration Index (SPEI).

2. Material and method:

Monitoring and assessing drought in Africa has been done using the Standard Precipitation Evapotranspiration index (SPEI) **(Vicente-Serrano *et al*. 2010b),** which takes into account both precipitation and potential Evapotranspiration (PET) demand of the atmosphere. This index is calculated as the difference between monthly precipitation (P) and the potential evapotranspiration (PET),

D = P – PET

The probability distribution of cumulative D series is aggregated at selected time scales. The 3-parameter Log-logistic distribution adopted for standardizing the *D* series for all time scales. And then transform it to Z –score by converting F(x) values obtained to z-standardized values. For example, following the classical approximation of **Abramowitz and Stegun (1965):**

$$SPEI = W - \frac{(C_0+C_1W+C_2W^2)}{(1+d_1W+d_2W^2+d_3W^3)}$$

Where $W = \sqrt{-\square * \square\square(\square)}$ *for* $P \leq 0.5$, P is the probability of exceeding a determined *D* value, $P = 1-F(x)$. If $P > 0.5$, P is replaced by 1−P, and the sign of the resultant SPEI value is reversed. The constants are: $C_0 = 2.515517$, $C_1 = 0.802853$, $C_2 = 0.010328$, $d_1 = 1.432788$, $d_2 = 0.189269$ and $d_3 = 0.001308$.

The average value is 0, and the standard deviation is 1.

Global gridded dataset of the Standard Precipitation - Evapotranspiration Index (SPEI) at time scale 12 months is downloaded from http://digital.csic.es/handle/10261/48169 with spatial resolution of 0.5º lat/lon. Temporal coverage between January 1901 and December 2009. The FAO-56 Penman - Monteith's method has been used for computing PET. Unbiased probability weighted moments (ub-pwm) method has been used for fitting the log-Logistic distribution. The whole world is put in one single netCDF file.

3. Result and Disscusion

The Standard Precipitation Evapotranspiration index (SPEI) as drought index has been showed in figures (1 and 2) from 1960 upto 2008 for month of Dec. at time scale 12 to include all months of each year for decades 1960, 1970, 1980, 1990, and 2000 respectively. It's observed that the older decades have less area of drought events, where the decade 1960 has most wet categories percentage area and decade 2000 has most drought categories percentage area compared with other studied decades. Year of 1961 has the most extreme wet area percentage while year of 1996 has the most extreme drought area percentage. Years of 1976, 1991, 1999, 2001, and 2006 haven't exposed to extreme drought.

The frequency distribution of SPEI values in 7 classes of drought category (%) over Africa at time scale 12 for month of Dec. during studied decades showed in table (1). As it observed all drought categories are increasing with time and all wet categories are decreasing with time, while the normal condition was nearly close during study period.

Figure (3) represents the average distribution of SPEI categories over Africa during study period, and it shows the normal condition around 79 % out of total values, moderate drought and wet were around 10.3 % and 5.1%, severe drought and wet were around 1.7% and 2.5%, and extreme drought and wet were around 0.2% and 2.3%.

Table (1): The frequency distribution of SPEI values in 7 classes of drought category (%) in Africa at time scale 12.

Classification	decade 60	decade 70	decade 80	decade 90	decade 2000
Extreme Drought	0.1	0.1	0.2	0.3	0.4
Severe Drought	0.6	1.2	2.2	2.1	2.3
Moderate Drought	5.2	8.0	12.5	12.8	13.1
Normal	75.7	81.0	76.4	80.5	80.5
Moderate Wet	7.8	5.3	6.1	3.2	3.0
Severe Wet	5.2	2.9	2.1	1.3	1.2
Extreme Wet	5.4	2.6	1.6	1.0	0.9

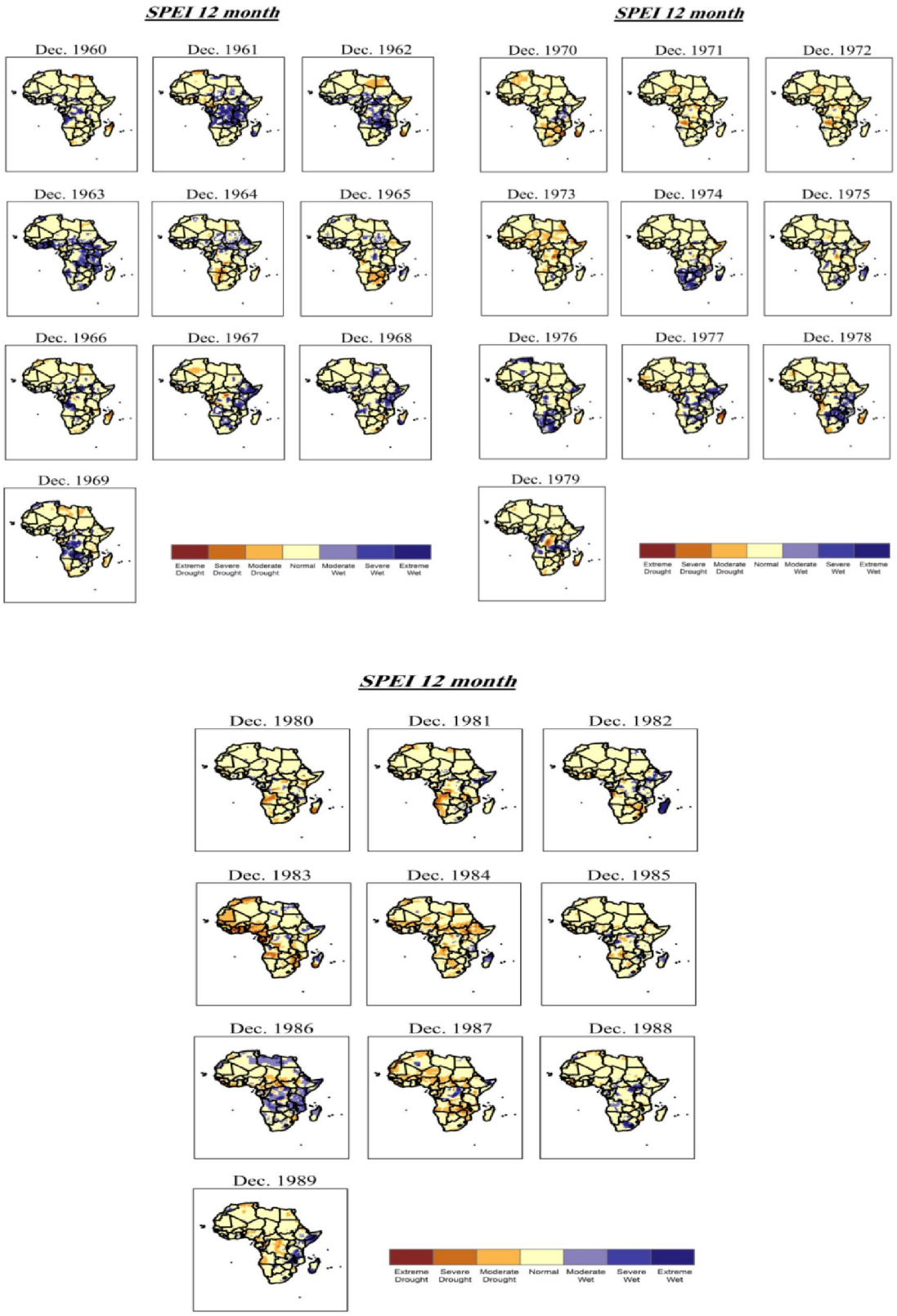

Figure (1): SPEI at time scale 12 for month of Dec. during the period from 1960 to 1989.

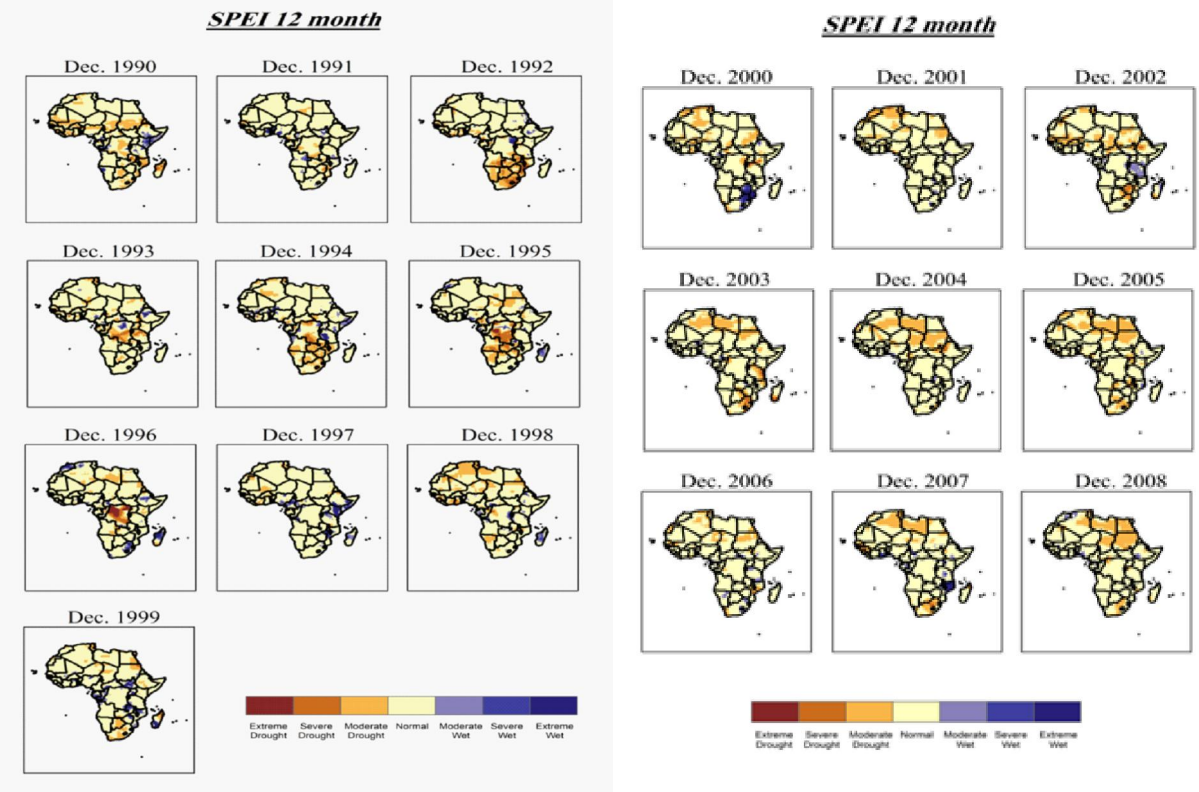

Figure (2): SPEI at time scale 12 for month of Dec. during the period from 1990 to 2008.

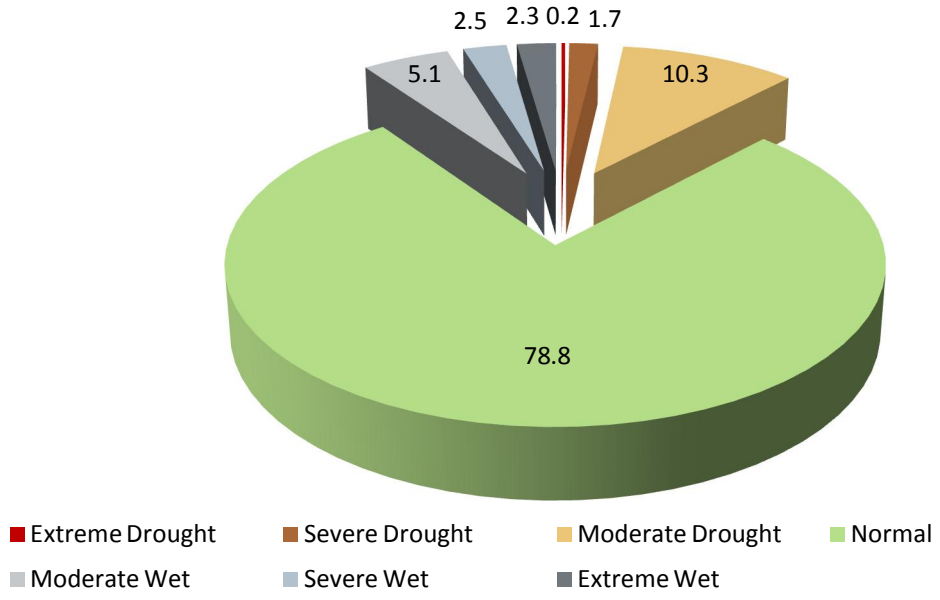

Figure (3): Average distribution of SPEI categories in Africa.

Figure (4) show the comparison between the average percentage of total drought (values ≤ -1) and wet categories (values ≥1) from 1960 up to 2008, where it was noted the predominance of wet event during decades 1960, then it approached a lot of drought event at 1970 decade, then followed by decreasing, and increasing of drought event.

By separating each drought and wet classification for studied decade as shown in figure (5) it was noted that, the biggest difference was between the extremes drought and wet events and this difference was less in sever and moderate classes. Also the extreme wet event greater than extreme drought in all studied decades.

In recent years, large scale intensive droughts have been observed on all continents affecting large areas in Europe, Africa, Asia, Australia, South America, Central America and North America **(Le Comte, 1995)** and high economic and social costs have led to increasing attention to drought **(Downing and Bakker, 2000).** Since the late 1960s, the Sahel (a semiarid region in West Africa between the Sahara desert and the Guinea coast rainforest) has experienced a drought of unprecedented severity in recorded history. Drought has a devastating impact on this ecologically vulnerable region and was a major impetus for the establishment of the United Nations Convention on Combating Desertification and Drought **(Zeng, 2003)**. While the frequency of droughts in the region is thought to have increased from the end of the 19th century, three long droughts have dramatic environmental and societal effects upon the Sahel nations. Famine followed severe droughts in the 1910s, 1940s, 1960s, 1970s and 1980s, although a partial recovery occurred from 1975–1980. While at least one particularly severe drought has been confirmed in each century since the 1600s, the frequency and severity of the recent Sahelian drought stands out the famines and dislocation on a massive scale from 1968 to 1974 and again in the early and mid-1980s was blamed on two spikes in the severity of the 1960– 1980s drought period **(Batterbury and Warren, 2001). Bordi and Sutera (2001)** found dry conditions over Europe, Eastern Asia, Central Africa and the Caribbean region to be interconnected and affected by the tropical climatic variability.

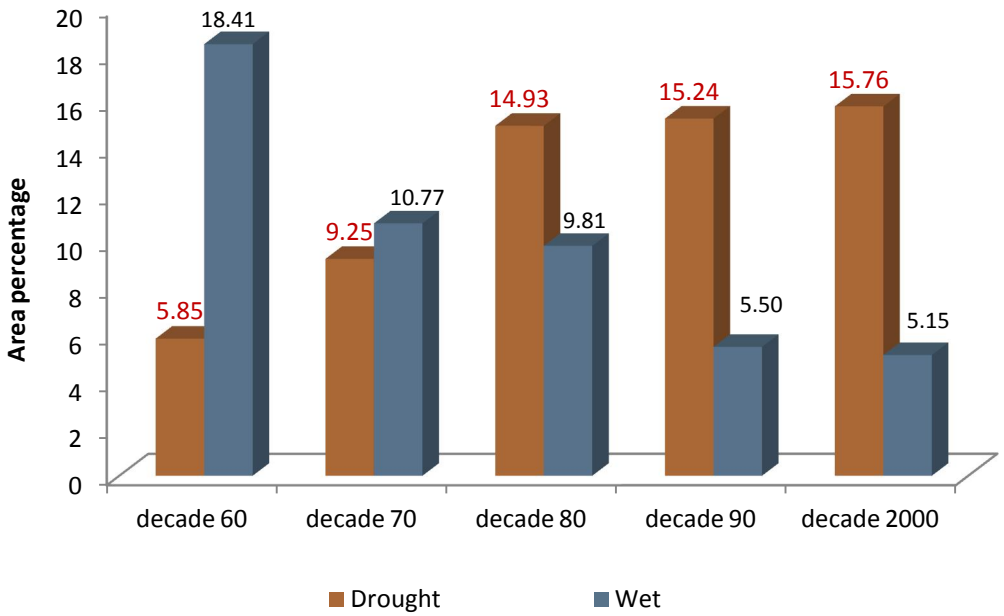

Figure (4): Comparison between the average percentage of total drought and wet categories.

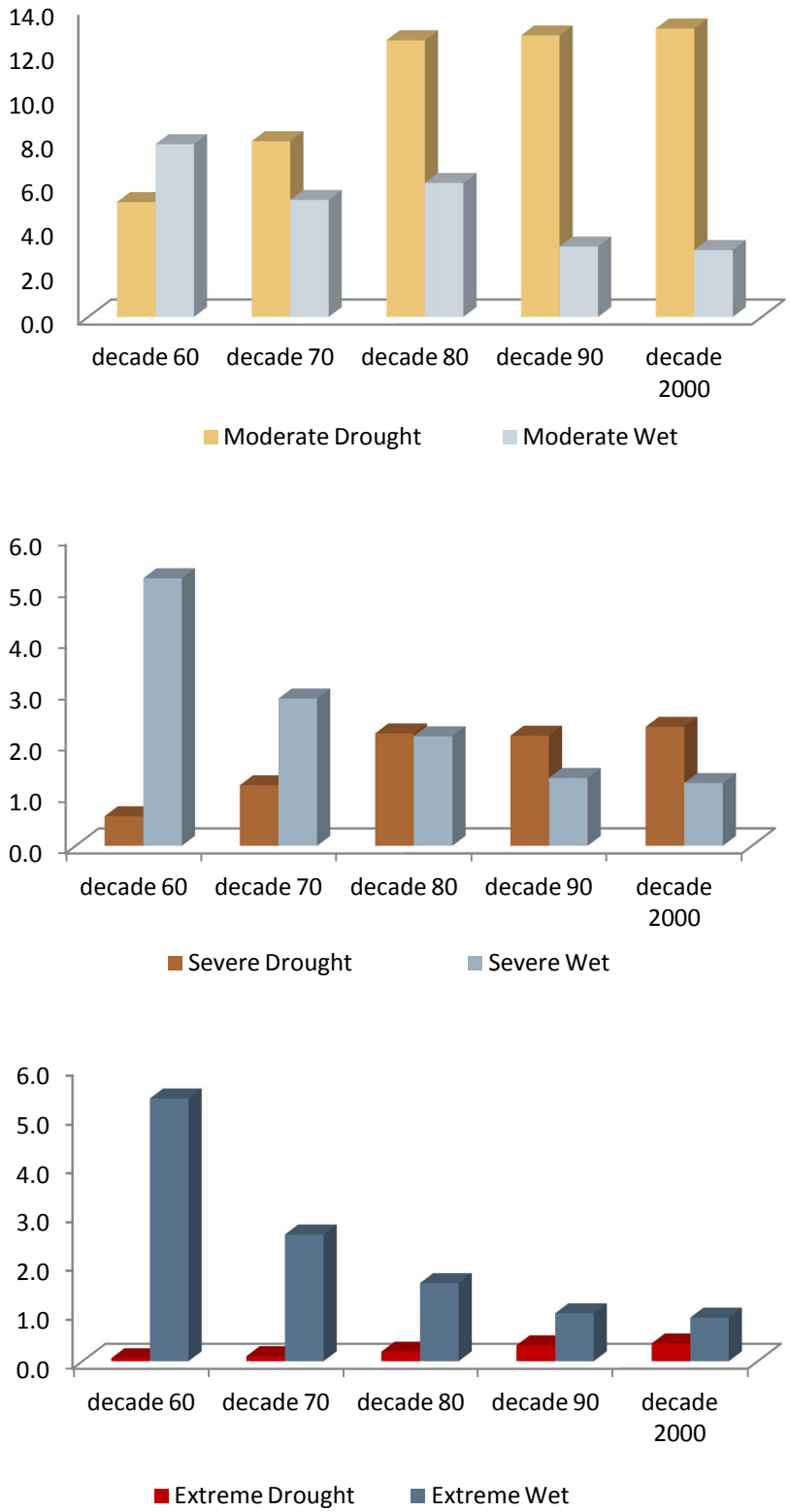

Figure (5): Comparison between drought and wet classification in each decade.

Conclusion

The characteristic of drought over Africa using the Stander Precipitation Evapotranspiration Index (SPEI) at time scale 12 for month of Dec. during the period from 1960 up to 2008 was analyzed. Results concluded that the first decades were less drought area and the drought increased with time. The frequency of drought (SPEI values $\leq$ -1) increased in last decades. There are big difference between extreme drought and wet events while the severe and moderate classes were closer. Assessment of the drought impact in Africa needs to determine of several systems (water resource, natural vegetation and crops) to quantify the impact of drought in terms of both system's resistance and resilience, and to produce drought impact curve to each system and region.

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11/22/2013

The Impact of Autocorrelation on the Performance of the MEWMA Control Chart with Mild Correlation

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Abstract: As it is indisputable any time conventional control charts are used you have the suggested assumption that observations are usually independently in addition to identically distributed as time passes. However, in reality, this sort of findings generated through continuous along with discrete production procedures are usually serially correlated, which violates the independence assumption of conventional control charts as well as modify the performance of control charts adversely. In this paper, we investigate the performance of MEWMA control chart with autocorrelated data with mild correlation being controlled. The generated data were applied to MEWMA control chart procedure and showed an in-control state, as the generated observations had been put through normality tests with the assumptions and also sensitivities for departure to normality, and ended up being normal by all standard. Therefore, this provides an alternate for the quality practitioners to consider for the continuous and discrete production processes even the autocorrelation doesn't have impact on the performance of MEWMA control limits once the mild correlation continues to be controlled.

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Keywords: Autocorrelation, MEWMA control chart, Mild correlation, Normality test, Statistical process control.

1. Introduction

Statistical process control methods tend to be traditionally used in industry to monitor procedures as well as enhance the quality of products, traditional statistical process control methodology guarantees which process data are statistically independent.

This particular assumption retains within continuous as well as discrete processes, as stated by (D.H., Pignatiello et al. 2000) that many processes such as chemical manufacturing, electricity generation, water quality processing, waste water etc. generate autocorrelated data which violates the assumption of traditional control charts, results in unnecessary large ARL values. Johnson and Bagshaw and Johnson (Bagshaw and Johnson 1975), discussed the effects of autocorrelation on the performance of cumulative sum (CUSUM) control charts. (Harris and Ross 1991) discussed the impact of autocorrelation on CUSUM and exponentially weighted moving average (EWMA) control charts and showed that positive autocorrelation could also adversely impact the performance of these charts. (Woodall and Faltin 1993) discussed the effects of autocorrelation on the performance of control charts and made recommendations on how to deal with autocorrelation. (Zhang 1997) proposed a statistical control chart for stationary processes and compared its performances to some of the charts recommended for autocorrelated data.

The idea associated with independence is not even approximately satisfied in certain manufacturing processes, because the characteristics tend to be measured over time order from the production, which might expose autocorrelation, that may possess a significant effect on the performance of control chart procedure.

In the univariate case, when significant autocorrelation is observed, the overall approach of process monitoring methods would be to fit a time series model to the process data. The residuals, which are independent, are then used to construct the control chart (Alwan and Roberts 1988; Lu and Renolds 1999) have extensively discussed it. Much more, when the model is not sufficient, the residuals might not be independent, consequently, there will be alarms.

Lots of approaches can be found in the literature for dealing with autocorrelation. (Montgomery and Mastrangelo 1991) discussed different model-based approaches, a model- free approach, and an engineering controller, and recommends model-based approaches for eliminating autocorrelated structures.

There are plenty of literatures that talks on the performance of MEWMA control chart, for example, (Lowry, Woodall et al. 1992; Borror, Montgomery et al. 1999; Testik, Runger et al. 2003) and so many other researchers but none yet have discuss the effect of autocorrelation on the performance of MEWMA using the mild level of correlation.

In this article, we are going to investigate the impact of the performance on the control limits for the

multivariate exponentially weighted moving average (MEWMA) control procedure when observations are autocorrelated with mild level of correlation being controlled.

The outline of the rest of this article is as follows;

In the next section, we described the Multivariate exponentially weighted moving average and in section 3, we have talked on the materials and methods used in the analysis of data to explain our findings.

Finally, we summarise our findings in section 4.

2.Multivariate Exponentially Weighted Moving Average Control Procedure

The univariate EWMA chart is based on the values

$$Z_t = rX_i + (1-r)Z_{i-1} \quad (2.1)$$

i= 1,2,..., where $Z_0 = \mu_0 = 0$ and

$0 < r \leq 1$

(Roberts 1959) showed that if $X_1, X_2,...$ are iid $N(0,\sigma^2)$ random variables, then the mean of Z_i is 0 and the variance is

$\sigma_{Zi}^2 = \{r[1-(1-r)^{2i}]/(2-r)\sigma^2$, i=1,2,...,

Thus, when the in-control value of the mean is 0, the control limits of the EWMA chart are often set at $\mp L\sigma_{Zi}$, where L and r are the parameters of the chart. (Lucas and Saccucci 1990) discussed the choice of r and L from the univariate EWMA chart in details.

In the case of multivariate, a natural extension is to define the vectors of EWMA's

$$Z_i = RX_i + (1-R)Z_{i-1} \quad (2.2)$$

$i=1,2,...$, where $Z_0 = 0$ and $R = diag(r_1, r_2, ..., r_p)$ $0 < r_j \leq 1, j = 1,2,...,p$.

The MEWMA chart gives an out of control signal as soon as

$$T_i^2 = Z_i' \Sigma_{Zi}^{-1} Z_i > h_4 \quad (2.3)$$

when

h_4 (>0) is chosen to achieve a specified in control ARL and Σ_{zi} is the covariance matrix of Z_i.

The ARL performance of the MEWMA chart depends only on the noncentrality parameter λ

In

$$\lambda = (\mu' \Sigma^{-1} \mu)^{1/2} \quad (2.4)$$

It is then much easier to make ARL comparisons among several multivariate control charts if all of the charts have this property (Lowry, Woodall et al. 1992).

However, as (MacGregor and Harris 1990) suggested for the univariate case, using the exact variance of the EWMA statistic leads to a natural fast initial response (FIR) for the EWMA charts, which is also true with the MEWMA control chart.

That leads to the assumption that for the chart design and the ARL comparisons the asymptotic (as $i \to \infty$) covariance matrix, then

$$\Sigma_{Zi} = \{r/(2-r)\}\Sigma \quad (2.5)$$

is used to calculate the MEWMA statistic.

(Lowry, Woodall et al. 1992) gave a table that contains ARL profiles of general MEWMA charts for various values of r, smaller values of r are more effective in detecting small shifts in the mean vector which is analogous to the univariate case.

This article talks on the application of MEWMA to the autocorrelated with mild level of correlation.

We generated a set of data from a multivariate random process for the 3-quality characteristics of interest by developing a (Mathworks. 2011) Mat lab source codes. As shown in Table 1 below:

3. Materials and Methods

Table 1: The MEWMA scheme

Observations				MEWMA vector			MEWMA Statistic
i	X_1	X_2	X_3	Z_1	Z_2	Z_3	T^2_i
1	0.61	1.12	3.91	0.06	0.11	0.32	1.24
2	1.57	-1.69	3,95	0.21	-0.07	0.68	2.68
3	0.56	1.92	-3.33	0.25	0.13	0.28	0.61
4	0.40	3.64	-2.38	0.26	0.48	0.02	1.01
5	-0.33	-1.19	0.82	0.20	0.31	0.10	0.38
6	-0.30	1.25	-4.21	0.15	0.41	-0.34	1.54
7	0.79	2.71	2.31	0.22	0.64	-0.08	1.33
8	0.92	-1.93	-2.97	0.29	-1.35	-0.36	0.57
9	0.81	0.35	4.64	0.34	-1.18	0.14	0.28
10	1.39	0.31	-1.08	0.44	-1.03	0.01	0.07
Control Limit						$h_4 = 8.66$	

Table 1 present the generated autocorrelated data for the three characteristics (X1,X2, X3) which was used to determine the MEWMA vector as well as the MEWMA statistic using (2.2) and (2.3) respectively.

The multivariate normal distribution is considered with unit variances and a correlation of 0.1, the process mean is on target (0,0,0) for the first 5 observations and then shifts to (1,2,3) for the last 5 observations. (X_1, X_2, X_3) are the observations in the table while Z_1, Z_2, Z_3 are the MEWMA vectors with r = 0.1 also the values of T_i^2 were obtained using equation (2.3) with covariance matrix using equation (2.5) which provides the natural (HS) feature for the MEWMA chart. The value of h_4 was obtained using the simulation to provide in-control ARL's of 200. Table 1shows the data used to determined the MEWMA vector as well as the MEWMA statistic. A mat lab codes was developed to generate the desired

data for the 3 characteristics of interest. The codes can be obtained from the authors based on request.

As observed by Testik, *et al* that quality practitioners should check the assumptions and the sensitivities to departures from normality before operational use of the multivariate control chart for the individual observations, if a process shows evidence of even moderate departure from the normality, the control limit may be entirely inappropriate. In view of their suggestion that we subject the generated autocorrelated for test of normality using the graphical and statistical methods, since there is not a direct test for multivariate normality, we generally test each variable individually and assume that they are multivariate normal if they are individually normal.

The 3- variables were subjected to normality test so that the data can be fit for the analysis, from the outcome of the test, it was found that the variables are normally distributed as shown in Table 2, the *Shapiro-Wilks's* significance values are all greater than 0.05. Also to support the S-W, the normality plot shows that all the 3- variables are normal as shown in Figures 2-4.

The autocorrelated values were now used to determine the MEWMA control chart with the usual procedures as spelt out by Lowry et al (1991), the control chart and T^2 Statistic were generated as shown in Figure1, which indicates that the control chart has the UCL of 10.81 and all the 10 observations were within the control limit, none is outside or showing an alarm.

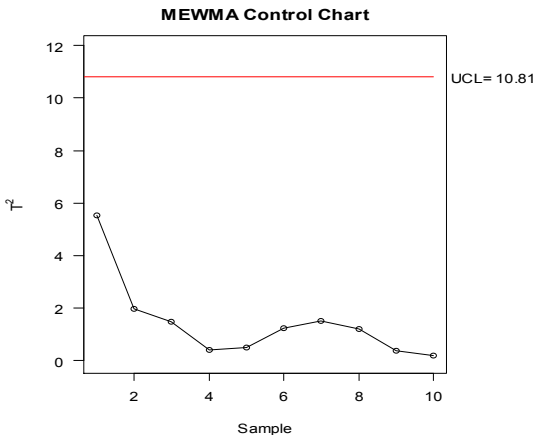

Figure 1: The MEWMA control chart of the data

Figure 1 present the MEWMA control with the points/values lying within the control limits, with the upper control limit of 10.81 while the lower control limit being 0.

Table 2: Showing the Results of Normality Test

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
X1	.150	10	.200*	.934	10	.493
X2	.138	10	.200*	.957	10	.753
X3	.174	10	.200*	.913	10	.302

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

Table 2 present the results of the test of normality showing the Kolmogorov-Smirnov and Shapiro-Wilk values, here since our samples is less than 50 we shall consider the Shapiro-Wilk's values instead of K-S value which is for sample size 50 and above. From S-W table all the values for the 3-characteristics on Significance column shows its values greater than 0.05, which is the rule of thumb for a variable to be normally distributed otherwise it is not normal.

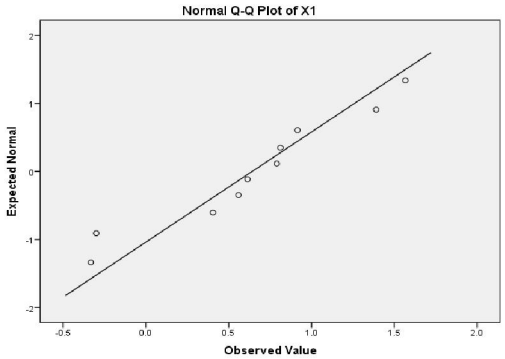

Figure 2: The Q-Q Plot for X1

Figure 2 present the Q-Q plot for the first characteristic (X1), as we can see that the almost all points are attached to the fit line, which indicates the normality of characteristic under consideration.

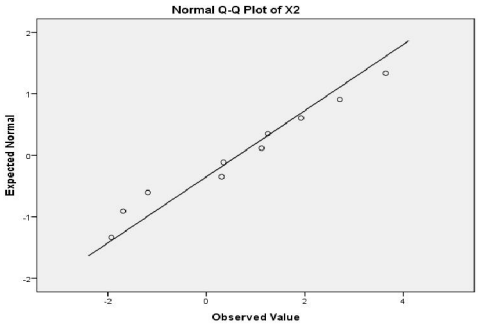

Figure 3: The Q-Q Plot for X2

Figure 3 present the Q-Q plot for (X2), here also the points are almost attached to the fit line, that's indicates the normality of the variable under consideration.

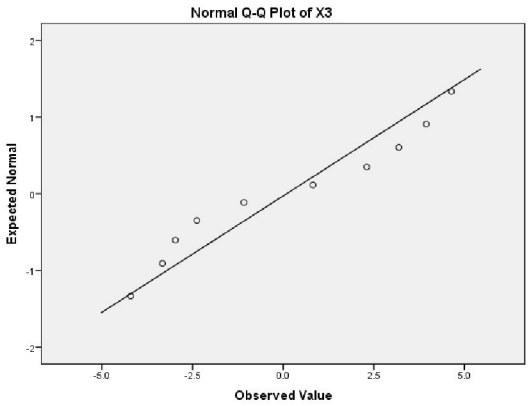

Figure 4: The Q-Q Plot for X3

Figure 4 present also the Q-Q plot for the last characteristics (X3), the points are almost clustered to the fitted line an indication of the normality.

Based on these data generated and plotted, it is observed that the use of autocorrelated data to the MEWMA when the correlated values are being controlled can lead to an in-control and it is a good alternative for the practitioners to use for the continuous data usage.

4. Conclusion

From Figure 1, we can see that the generated autocorrelated data with the mild correlation applied on the MEWMA control chart has produced an in-control chart with all its values/points lying within the control limits with no point raising an alarm.

For the quality practitioner to operationally use the multivariate control charts, it has to be check for the assumptions and sensitivities to departures from the normality.

The generated data were subjected to normality test which proves to be normal by all standard. With these results of this article it can be an alternative to other techniques for the quality practitioners to adopt for use in continuous data as well as the discrete data. With this findings its eminent to conclude that the autocorrelation has no effect on the performance of the MEWMA control limits when mild correlation is controlled.

Finally, we conclude the discussion that the autocorrelated data with mild correlation controlled can result into the in-control process on multivariate exponentially weighted moving average, the above method was tested using 3 characteristics of interest but can be extended to higher quality characteristics desired.

We are recommending that the autocorrelated data with mild level of correlation being controlled should be applied to other statistical process control techniques.

APPENDIX A: Derivation of the Covariance Matrix for Z_i

By repeated substitution of (2.2), it can be shown that

$$Z_i = \sum_{j=1}^{i} R(I-R)^{i-j} X_j.$$

Thus

$$\Sigma_{Zi} = \sum_{j=1}^{i} var[R(I-R)^{i-j} X_j]$$

$$=\sum_{j=1}^{i}[R(I-R)^{i-j} \Sigma(I-R)^{i-j} R].$$

Because R and $(I\text{-}R)$ are diagonal matrices, the (k,l)th element of Σ_{Zi} is

$$r_k r_l [1-(1-r_k)^i(1-r_l)^i]/[r_k + r_l - r_k r_l]\sigma_{k,l}, \quad (A.1)$$

where $\sigma_{k,l}$ is the (k,l)th element of Σ. If $r_1=r_2=\ldots=r_p=r$, then the expression in (A.1) simplifies to $\{r[1-(1-r)^{2i}] /(2-r)\} \sigma_{k,l}$, so that

$$\Sigma_{Zi} \{r[1-(1-r)2i] /(2-r)\} \Sigma. \quad (A.2)$$

The covariance is derived here under the assumption that the control rule is ignored, but it can offers some guidance on the type of control rule to be used. {Lowry, 1992 }

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11/22/2013

Impact of driving style on fuel consumption

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Abstract: The aim of this paper has studied the effects of changes in the speed and acceleration of the driving style on fuel consumption cars. First, it is indicated cycles with constant acceleration in three ranges: Local driving; a slower pace than km/h32, City driving; between 32 km/ to72 km/ h, The highway driving; With faster than72 km/h. then they calculate the fuel consumption rate of cars in each group. In this way, we can evaluate the effect of acceleration on fuel consumption in each of them. Also, the cycle with the same acceleration can be estimated by comparing the fuel consumption. In the end, we can see that at low driving speeds, fuel consumption will increase compared to driving at high speed. It is important that in city driving, acceleration is the fuel consumption of determining factor.
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Keywords: Average speed, acceleration, fuel consumption, Local driving (Low speed driving), Average speed driving (City driving), the highway driving

1. Introduction

The main purpose of this paper in order to reduce fuel consumption includes: Increase in fuel prices in recent years and Air pollution caused by emissions greenhouse gases. Despite advances in automotive technology and high technology cars entering into the transportation system, it is still necessary to create new techniques to reduce fuel consumption in the transportation fleet. To reduce oil consumption and greenhouse gases released by them. There are many ways to reduce fuel consumption and greenhouse gas emissions; For example, the replacement of vehicles with more advanced technology, to reduce the distance, to reduce the weight and size of cars, Use public transport for transportation.

One of the main obstacles to achieving a significant reduction in fuel consumption replaces them very late. It takes more than 30 years; all the cars can be replaced. Due to the slow movement of the fleet, the fuel consumption is about 10 years late. Therefore, the special methods are needed to fuel cars (vehicles especially common) to decrease. Some studies indicate that personal behavior of drivers is one of the factors on fuel consumption and greenhouse gas emissions. Bin 2005 has shown that personal behaviors have been allocated 40% of total CO2 emissions in the United States. Driving style changes include high-speed driving and the lack of appropriate driving speed. Some studies have shown that drivers can reduce their fuel consumption by up to 15% with the change in driving style. West and et al (1998) have estimated a reduced speed from 70 mph to 65 mph reduces fuel consumption by as much as 8%. Shavarby et al (2005) found the lowest fuel consumption is between60 km/h to 90km/h. Wang et al (2008) found the lowest fuel consumption is between 50km/h to 70km/h. Tests done by Lisa Bard in 2009 as has been summarized in Table 1.

Table 1: The Fuel Consumption Test on cycles at a constant speed for several cars

	Vehicle Class and Engine	HP/Lb	Fuel Consumption			Sensitivity of Fuel Cons. to Speed (L/hr)	Fuel Saving of slowing from 75 to 65 mph
			55 mph	65 mph	75 mph		
Toyota Yaris	Subcompact 1.5-liter 4-cyl.	0.041	5.5	6.2	6.9	4.3	10%
Acura TSX	Compact 2.4-liter 4-cyl.	0.054	5.9	6.6	7.7	5.5	14%
Toyota Camry	Mid-Size 2.5-liter 4-cyl.	0.047	5.8	6.7	7.9	6.4	15%
Toyota RAV4	Small SUV 2.5 liter 4-cyl.	0.049	6.8	8.0	9.1	7.1	12%
Lexus RX350	Midsize SUV 3.5-liter V6	0.065	7.6	8.6	10.2	8.1	16%
Mercury Mountaineer	Large SUV 4.6-liter V8	0.044	9.9	11.1	13.2	10.4	16%

In addition, Berry (2010) showed When the average speed of an actual driving pattern is changed without the change in the ratio of the acceleration, The fuel consumption, it will follow the same U-shaped curve was observed for driving speed. This is shown in Figure1.

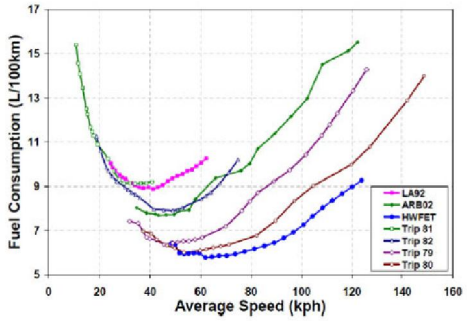

Figure1: The relationship between fuel consumption and average speed

Each point represents the average speed and fuel consumption for a specific route. For the seven paths-speeds, the minimum fuel consumption has been seen at intermediate speeds between 30km/h and 60km / h. Jones 1980, showed that fuel consumption, by constant acceleration, almost will increase linearly with the acceleration. The software results of simulation by Barry have been emphasizing the impact for various paths - speed.

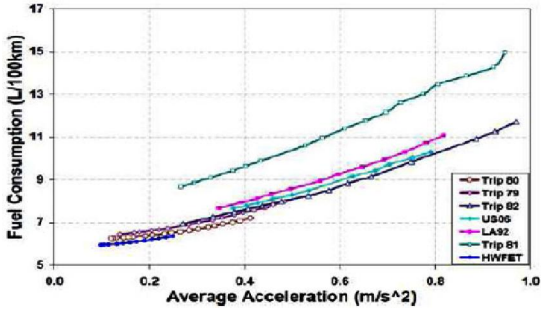

Figure 2: The relationship between fuel consumption and average speed for The seven pahts-speed

It has been seen in Figure 3, which has been a constant acceleration cycle at different speeds from the 8km / h to speed 132km / h. In each of these speeds is made of 8 or 9 cycles, with acceleration amplitude 0.7 $\frac{m}{s^2}$. the method of the construction of cycles is that to achieve the specified speed, the variety of acceleration is caused. To achieve more accurate results, this cycle can be repeated up to 10 times. 2 seconds per cycle is considered to stop

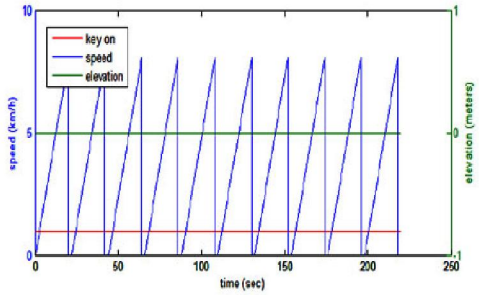

Figure 3: The example of a cycle with constant acceleration (The Acceleration: 0.8 $\frac{m}{s^2}$ and the maximum speed: 8 km / h)

2. The Simulation results:

The values of the fuel consumption can be changed by changing the parameters. The Figure 4 shows, the graph of fuel consumption changes associated with accelerated for 13 various speeds. As you can see the whole range of speed, a momentum increment leads to increased fuel consumption. It should be noted that perfect fuel consumption can be achieved when driving at a constant speed much as possible.

The Figure 5 shows that in a range of speeds (below km/h32), velocity variations strongly effect on the fuel consumption. So that with increased speed is being reduced fuel consumption. Another range of speeds (32 km/h to 72km / h), the changes of speeds will have a significant impact on fuel consumption. In the high speed 72km/h, increased speed will cause an increase in fuel.

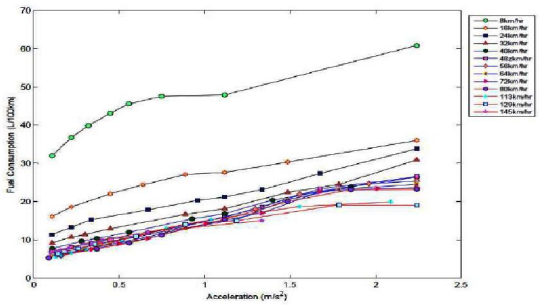

Figure 4: The fuel consumption for the different values of acceleration

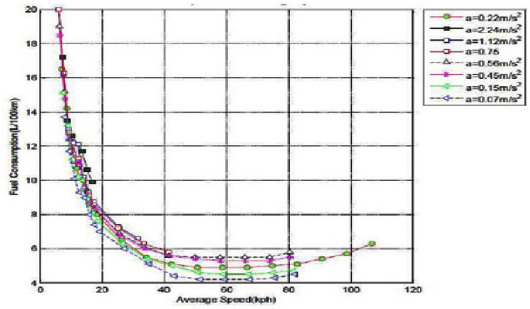

Figure 5: Relationship between fuel consumption and different speeds

3. Low speed driving (local):

Speed is less than 32km/h. As shown in Figure 6, fuel consumption for the four ranges of speed from 8km / h to 32km /h is calculated for a range of acceleration 11.5 $\frac{m}{s^2}$ to 24.2 $\frac{m}{s^2}$.

The relationship between fuel consumption and acceleration is incremental. In this range of speeds, acceleration increases fuel consumption. In low-speed driving, the average speed plays the main role on fuel consumption.

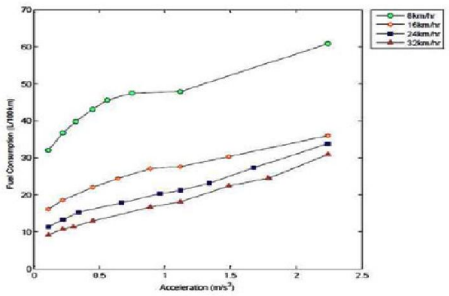

Figure 6: Fuel consumption for different values of acceleration in the low speeds(32 km/h)

4. Average speed driving

The speed in the this group is Between 32km / h to 72km / h. In the low-speed driving, Speed has any significant effect on fuel consumption but acceleration, it plays the main role on fuel consumption.

5. High speed driving (highway):

Figure 7 shows the more speed 72 km / h.

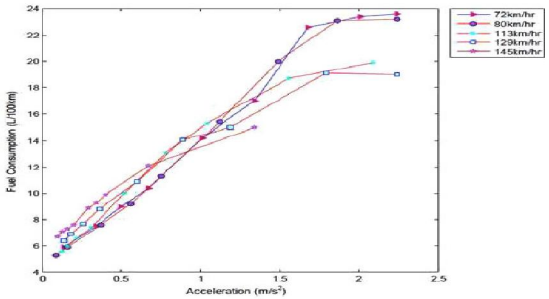

Figure 7: The more speed 72 km / h

5. Discussions

The variable-speed drive, the impact accelerations and speed are different. In driving at low speed (the average speed: 32km / h), the acceleration increases fuel consumption. In this type of driving, the average speed plays a major role. In high speed driving (average speed more than 72km/h), both parameters (i.e., speed and acceleration) are effective on fuel consumption.

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11/28/2013

Histological Characterization and Embryonic Development In the fertilizing eggs of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Oliver)

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Abstract: This research paper is a laboratory description for the characteristics, the tissues structures and the successive phases for the embryos development of the Red Palm Weevil, *R. Ferrugineus* (Oliver) in the deposited fertilized eggs, by the light and transmission electron microscopy. The study included a description of the initiation of cleavage, blastula, gastrula and the formation of extra-embryonic membrane before hatching. The 6h old egg is typical of undifferentiated cell, containing regular reticulum of much cytoplasm and a thick periplasm. The zygote daughter nucleus (energids) and the yolk granules are in cytoplasmic continuity. The energids and yolk granules spread regularly all round the egg periphery and arrange in a layer in the 12 h old eggs. However, the rest of irregular cytoplasm remains at the center. The cleavage energids move apart as they divide and form the cellular blastoderm, while the yolk granules appear around them in the 24 h old eggs. Large masses and spherules of vitellophages and vacuoles are also observed through the clear cytoplasm. In 30 h old eggs, many folds of the plasma membrane are developed, extended between and beyond the preblastoderm nuclei. The preblastoderm nuclei of the 48h old eggs are bounded by the cytoplasmic islands and form the cellular blastoderm which differentiate into germ band and the extra -embryonic membranes in the 60h old eggs. Gastrulation and, differentiation of the ectodermal and mesodermal tissues e.g. the trachea, fore and mid gut become overgrown in the 72h old eggs.While, the ectodermal layer, the connective tissue, the muscles , the fat body and the malppighian cells are differentiated in the 84h old eggs.

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Keywords: *Rhynchophorus ferrugineus,* Embryo, Cleavage, Energids, Blastoderm, Germ band, Gastrulation, Differentiation, Embryonic membranes.

1. Introduction

The Red palm weevil, *Rhynchophorus ferrugineus* (Olivier) is one of the most destructive pests of date palms, *Phoenix sylvestris* Rox., and *Phoenix dactyleferous* in the Arab Gulf States (Bokhari & Abuzuhari, 1992), Egypt (Salama & Hamdy, 2001), and of coconut, *Cocos nucifera* L., in South and Southeast Asia (Sivapragasam et al., 1990; Sadakathulla, 1991). During the last decade, multiple introductions of *R. ferrugineus* to the Middle East from India and Pakistan have occurred. Often, palm weevil infestations are not detected before extensive damage caused by larval mining in the trunk and it is not possible for the tree to recover (Sivapragasam et al., 1990). In date palms, the only visible sign of attack may be oozing out of palm sap from the trunk, and infestations are often not discovered until trees are blown over. Delay between detection and destruction permits emergence and migration of adult weevils prior to destruction. Transporting infested trees and offshoots for burning introduces the weevil to new areas.

Insect eggs are typically large relative to the size of the females that produce them because they contain a great deal of yolk. It is generally believed that the eggs of Endopteryogota contain less yolk and are smaller than those of Exopteryogota (Anderson, 1972b). Immediately following fertilization, as the egg is laid, the zygote nucleus divides and the daughter nuclei migrate to the periphery of the egg to form a layer of cells, the blastoderm, surrounding the yolk. Part of this cell layer becomes thickened to form the germ band from which the embryo develops (Anderson, 1972a, b; Haget, 1977; Nagy, 1995; Sander et al, 1985).

The purpose of the present study is to investigate the embryogenesis for fertilizing 6 to 84 hour old eggs. The details of cleavage and formation blastoderm cells, the germ band and extraembryonic, gastrulating, structural bodies in the eggs of Red Palm Weevil, *R. ferrugineus* (Oliver) were described.

This study may be considered a standard reference in the egg embryology descriptions of the insect, however, no ultrastructures embryogenesis or

histological characterized of egg were described before, for this species.

2. Methodology

2-1. Source and Insects rearing

Adults of the Red Palm Weevil, *R. ferrugineus* (Oliver) were obtained from infested wild date palm in Al-Kharj, and maintained on sugarcane in the laboratory. The adults were fed with fresh sugarcane and later allowed to mate. Isolated females were supplied with pieces of split sugarcane to oviposit on it. The sugarcanes were replaced every day.

2-2. Collection of eggs

After mating the adult females laid their eggs between sugar cane fibers and stuck them so strongly. The process of collecting eggs by minutes, brush placed inside the Petri dish on a layer of cloth moistened with water, Eggs were taken on the ages of 6,12, 18, 24.30, 36, 42.48, 54, 60.66, 72, 78.84 hours and then placed each group in the installer in preparation for the histological study.

2-3. Preparation the samples using binocular microscope of BM

Taken different ages of eggs to be photographed with a binocular microscope (BM) and monitoring changes of the external egg, was used with a microscope specifications.

2-4. Preparation the samples using Transmissing Electron Microscope

Primary Fixative by buffered Glutaraldehyde 2.5% over night in refrigerator ,wash by phosphate buffer pH=7.2, secondary fixative by buffered Osmium Tetraoxide 1% over night in refrigerator ,then dehydration using series conc. of ethanol, embedding by resin mixture from SPI (SPI-Pon™ - Araldite® Epoxy Embedding Kit), the block well cutting by (leica UC6 ultramicrotome) the section thickness is between 70-80 nm and it lode in cupper grid then stain by aqua's uranyl acetate and lead citrate, examined under Transmission Electron microscope (TEM) (Jeol JSM-1011 electron microscope).

3. Results and Discussion

3-1. Description of Egg Stage

The Red Palm Weevil eggs is 2 mm long and 1.6 mm wide characterized by elongated, creamy in color. It has two poles: the anterior pole has tapered a respiratory openings, the posterior pole has the slot to enter the sperm (Fig. 1). The insect lays its eggs individually in the Palm's tissues where embedded it strongly and eggs hatch over a period of 3-5 days.

Few hours later, they begin to harden and become white, calm and transparent at the bilateral two external edges (Al- Dossary et al, 2010). Corley and Tinker (2003) described the oblong eggs of *Rhynchophorus* weevils, which were 2–3 mm long. They added that, the eggs of the Red-Stripe Weevil *Rhynchophorus* schach Oliv., were whitish-yellow, ovocylindrical in shape and measured 2.4 mm long and 0.9 mm wide.

3-2. Incubation Period of Eggs

The incubation period ranged between 3-5 days where the fetus swallows the amniotic fluid and part of the air that spreads inside the egg, during that period, major divisions at the level of the cell and the cytoplasm result in the formation layers of the body wall, and leads to the rupture and torn of the outer shell of the egg, as well as rupture of fetal membranes (Fig. 2A- F). According to Al- Dossary et al., (2010) the hatching region of *R. ferrugenius* was observed on the anterior part at the opposite side. The hatching region was observed on the opposite side of the micropylar apparatus and appeared as a set of imprints separated by furrows and oriented along the longitudinal axis of the egg (Fig 2- F). Chorion morphology and the polygonal ornament of the outer chorionic surface show broad apparent phylogenetic trends in various insect eggs [29,16,17] reveal the imprints of the follicle cells that have participated in the egg shell formation [11]. This hatching region was not clearly visualized on the eggs of other species[18].

3-3. Embryogenesis

Includes all the developments that occur between the zygote formation and totally grown individual out of the egg. Red Palm Weevil eggs contain a large amount of yolk (Fig. 3), and thus the split occurs at the level of the nucleus and cytoplasm, and this meroblastic is called partially meroblastic.

3-4. Cleavage and formation blastoderm cells

After 12-13 hours of incubation, the first cleavage occurred. Fig. (4) shows the stages of the indirect division in the nucleus and cytoplasm of the embryo 12 h- old. The chromosomes appears in the primary development (Fig. 4 a). Fig 4 (b) shows the chromosomes in the final development, where they arrays on both sides of the spindle. By repeating this division, there are numerous of new cells that migrate towards the outer edge of the egg (Fig. 5), and arrange themselves in a single layer below the yolk membrane. The cytoplasm adjacent to the outer membrane combines with the divided nucleuses to form the blastoderm cells that surround the yolk while the yolk is concentrated in the center of the embryo 12h- old (Fig. 6 a, b).

Chapman (1998) stated that few eggs of insect does not happen by cleavage surface spread in eggs of most insects, and the surface is characterized by eggs containing copious amounts of yolk, but in races of

less yolk another type of cleavage occurs. Takesue (1890) observed that the morphology of the just-completed blastoderm cell was very different from the above and under its nucleus of *Bombyx mori*. Above the nucleus the cytoplasm was dense, full of a great number of mitochondria and poor in vacuoles, and microprojections on the surface were few and small. On the other hand, the nucleus have small densely-stained dots, a lot of vacuoles of different sizes and, sometimes, some yolk granule in the peripheral region.

3-5. The germ band and Extra embryonic

The blastoderm wrap around the egg is completed to form blastula and the internal vacuum is filled with yolk to form what is known the Blastocoele after 30 hours (Fig. 7). Within 12–18 h-old the germ band and formation of extra-embryonic membranes were detected, After remaining for a short period in its extended state, the germ band reverses its movement and shortens (Fig. 5). The beginning of shortening of the germ band, the contraction of the yolk this time and, leaving a spacious haemocoele between itself and the body wall. As shortening of the germ band is nearing completion in the embryo of 36–42 h-old, the lateral body walls begin to grow up and eventually fuse in the mid-dorsal line. The germ band consists of some cells blastula and takes the shape of columnar, while the other cells involved in the formation of blastula extra embryonic membranes (Fig. 8) where the amnion is formed towards the inside to surround the embryo and the serosa outward. After the germ-band had invaginated and sunk completely into the yolk, the serosa formed an uninterrupted outer layer to the yolk. The serosa was a very thin cellular layer, and its nuclei are extremely flattened. In some Lepidoptera, the blastoderm is differentiated into germ band and extra- embryonic tissue from the time of its first appearance, In some other insects, such as Mallophaga and *Apis*, the whole blastoderm is thick initially but subsequently becomes thinner except for the germ band (Chapman, 1998).

3-6. Gastrulation

Gastrula formation in eggs of red palm weevil in the early stage of embryonic development, begins after 30 hours of laying eggs where the formation of the middle layer starts, as well as the formation of the inner layer by failing indentation layer of the outer layer and this is illustrated in both Fig. (9 and 10). As illustrated in the Fig. (9), the ectoderm layer and the beginning of formation of the trachea, while in (Fig. 10) it shows the formation of the mesoderm layer and the beginning of the emergence of fat bodies after one day and six hours from laying eggs. Johannsen and Butt (1941) stated that there was a difference in the way Gastrula formation in insects than in animals where the insect sharp indentation does not occur. It consists of only an internal layer of cells below the germ band. In most insects, these cells become columnar and then migrate inward so that a mid-ventral groove is formed. They are isolated progressively from the outside by more lateral cells spreading beneath them. In most beetles, the invagination is so marked that it is at first almost tubular, while in *Apis* a broad middle plate sinks in the embryo (Jura, 1972).

3-7. Structural bodies

3-7-.1. Cuticle

Fig (11) shows the beginning of cuticle formation and the internal surface after 39 hours of the egg-laying in a rate of one day and fifteen hours, which means that the cuticle layer is the skin secretion, which in turn formed from ectoderm layer. In fact, that the secretion of cuticle coincides with the formation of organs of its constituent, which means that the formation of the blastoderm layer after - one day and six hours, then after nine hours, the cuticle formation begins, which appears as winding sheaves arranged on top of each other. Also, the cuticle surface is observed which appears dark (Fig.12). (Fig.13) shows the completion of cuticle formation that appears dense sheaves and other light sheaves after 84 hours of egg-laying and the formation of the pore channels, as evidenced by formation foraminalis in the same figure for the cross section. According of Chapman (1998) and Hoffmann and Laguex (1985), the first cuticle to be produced during the embryonic development of many insects is the serosal cuticle which is secreted by the blastoderm and forms a continuous layer on the inside of the eggs hell until the first stage larva hatches. Another very thin cuticle is produced by embryos of Acrididae after about 35% of the developmental period has elapsed. A similar cuticle is present in Phasmatodea and at least some Heteroptera and Lepidoptera (Chapman, 1998; Hoffmann and Lagueux, 1985).

3-7-.2. Respiratory system

Fig. (9) shows the beginning of the composition of the trachea and chtinidia after 30 hours of the egg-laying and continue to grow until they are completely configured Sell after 66 hours (Fig. 14, and created the trachea, which are the most important components of the device layer of the bronchial ectoderm, They were formed and emerged after 24 hours of embryonic development (Fig. 15). Chapman (1998) stated that the respiratory system in insects arises as a double bending on the segments and takes the form of a letter T and T ribs adjacent to the brain to be established and longitudinal logs bindings, to create finer branches of the bronchial system (Chapman, 1998)

3-7-.3. Fat bodies

Also, the formation of the Fat bodies begin after 30 hours of embryonic development (Figure 10),

and continue to do so until the composition is completed after 66 hours of its growth (Figure 14 B, C). These bodies are created from mesoderm layer which is the layer, which in turn originated from the inner layer of the wall of the body. Chapman (1998) said that the fat body in insects is a loose or compact clusters of cells associated with endothelial membrane suspended in the cavity haemolymph in terms of food stores within the body as in some insects directorial store materials.

3-7-4 muscles

Fig. (15) shows the completion -of the muscles configuration in the embryo of 78 h- old, the muscle composition of the tapes and the presence of nuclei. the muscles configuration is formed from the inner layer (layer mesoderm). The mesoderm is derived from the inner layer of the germ band which forms two lateral strands running the length of the body and joined across the midline by a thin sheet of cells. In some insects orders a pair of coelomic cavities is present in each segment of the protocorm, while, in the protocephalon, pairs of cavities develop in association with the premandibular and antennal segments. Sometimes, one or two more pairs are present in front of the antennae. Subsequently, in Coleoptera, the thoracic and abdominal coelomic cavities become confluent forming a tube on either side (Chapman, 1998). once the muscles formed , the walls of the coelomic sacs break down so the outer walls of the coelomic sacs form the somatic muscles and the inner walls of the coelomic sacs form the visceral muscles.

3-7-.5. Alimentary canal

Fig. (16) shows the divisions alternation of nuclei in cytoplasm of embryo of 66 h- old primary formation of foregut, mid and hind gut (Figure 17-18), and notice of epithelial layer which coated alimentary canal provider numerous microvilli (Figure 19- 20). The foregut and hind gut arise early in development as ectodermal invaginations, the stomodeum and proctodeum. These invaginations carry the anterior and posterior rudiments of the mid gut into the embryo. Then, these rudiments extend towards each other forming two longitudinal strands of tissue beneath the yolk and above the visceral mesoderm. From these strands, midgut tissue spreads out over the surface of the yolk, eventually completely enclosing it (Skaer, 1993).

Malbigian tubules originated of receptor for hind gut. Normally originated to 2- 3 pairs in embryo but may be formation of malbigian tubules may appear in larval stage (Savage, 1956).

4. Conclusion

Table (1) shows that our results in this study agree with what we have mentioned in a previous study (Al- Dossary et al, 2010), germ band formation, gastrulation and the extension of the germ band and formation of extra-embryonic membranes were detected at the embryo of 12–18 h-old (Figure 4, 5, 6). After remaining for a short period in its extended state, the germ band reverses its movement and shortens (Fig. 7, 8). The beginning of shortening of the germ band, the contraction of the yolk plasmodium at this time, leaving a spacious haemocoele between itself and the body wall, characterize the embryo of 18– 24 h–old. As shortening of the germ band is nearing completion in the embryo of 36–42 h-old, the lateral body walls begin to grow up and eventually fuse in the mid-dorsal line (Fig. 8). Organs formation and segmental divisions begin to appear in the embryos epidermis of the embryo of 48–72 h-old.

The embryological studies of insect orders are the most important for understanding the ground plan of insecta as well as for clarifying insect evolution (Weissling and Davis, 1995; Stanley and Grundmann, 1970; Handel et al., 2000; Kobayashi et al., 2002; Tojo, 2003).

Table 1: *R. ferruginues*: time-table of early development

No. of Hour after being Laid.	Stage reached in Development
6	large amount of yolk and thus the split occurs at the level of the nucleus and cytoplasm, and this is meroblastic.
12–18	germ band formation, gastrulation and the extension of the germ band and formation of extra-embryonic membranes.
18– 24	The beginning of shortening of the germ band, the contraction of the yolk at this time, leaving a spacious haemocoele between itself and the body wall.
36- 42	As shortening of the germ band is nearing completion in the lateral body walls begin to grow up and eventually fuse in the mid-dorsal line .
48- 84	Organs formation and segmental divisions begin to appear in the embryos epidermis.

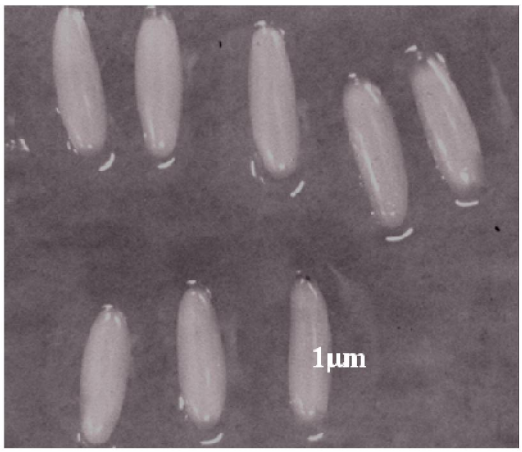

Figure 1: General formation egg of *R.ferrugeuus*, show elongated, cream color, with a length of 1.9 mm and width 0.43 mm.

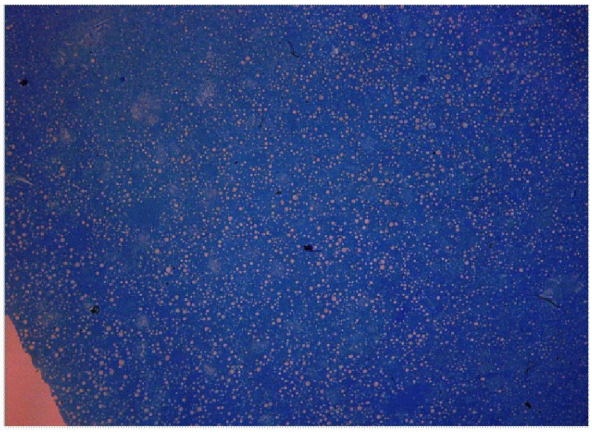

Figure 3: Semi thin section for embryo 6 h- old appear density of yolk and arranged reticulum from cytoplasm which contain of nucleus (N).

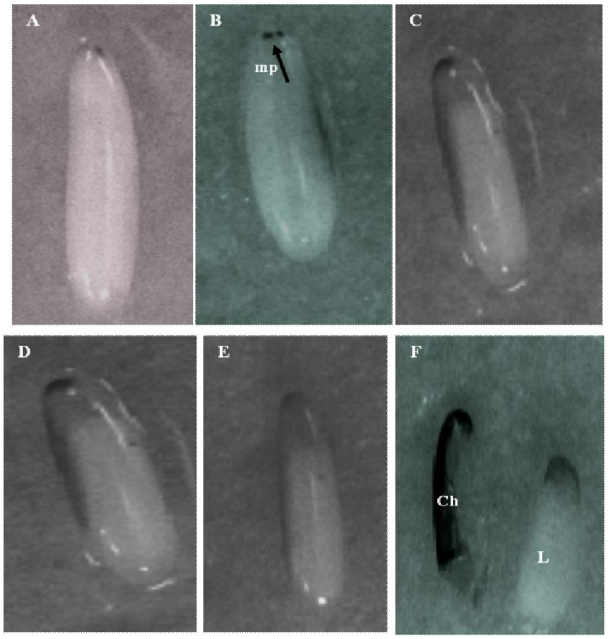

Figure 2: A- F: Photo of binocular microscope of egg red palm weevil show phase development embryo until hatching. A: primary formation of mouth parts. B: appearance of mouth parts (mp). C – E: 18–24 h-old embryo showing the beginning of shortening of the germ band. The contraction of the yolk plasmodium at this time, leaving a spacious haemocoele between itself and the body wall. F: hatching egg and larva (L) outer from hatching region see chorion (Ch)after incubation period.

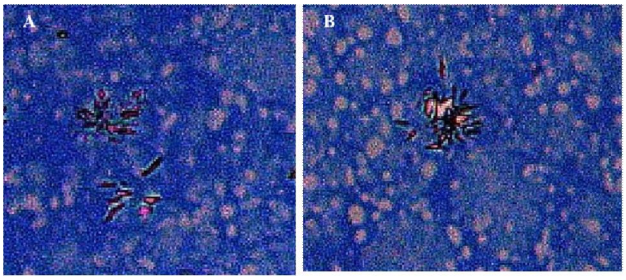

Figure 4: Semi thin section for embryo 12 h- old, A: appear decreased mitosis of cytoplasm and nucleus

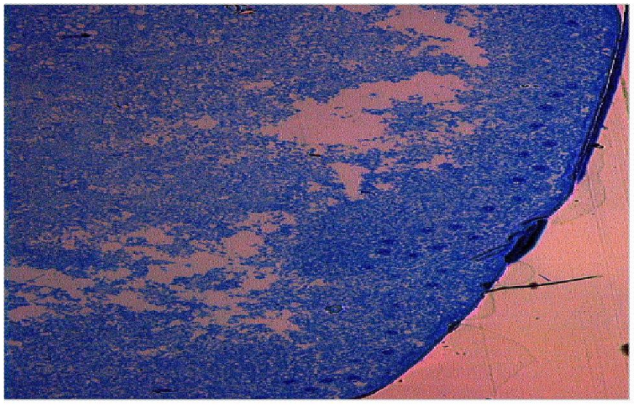

Figure 5: Showing the process of blastoderm formation and germ band in embryo 12 h- old after oviposition. Yolk granules; (Y)

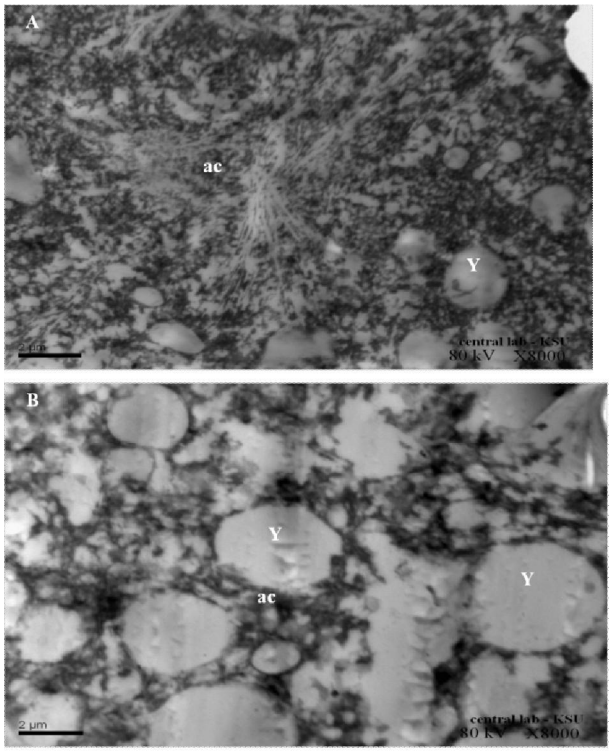

Figure 6: A: TEM showing the process of blastoderm formation in the embryo 12 h- old after oviposition. See density of yolk, y; associated cytoplasm, ac. B: Part magnification from A.

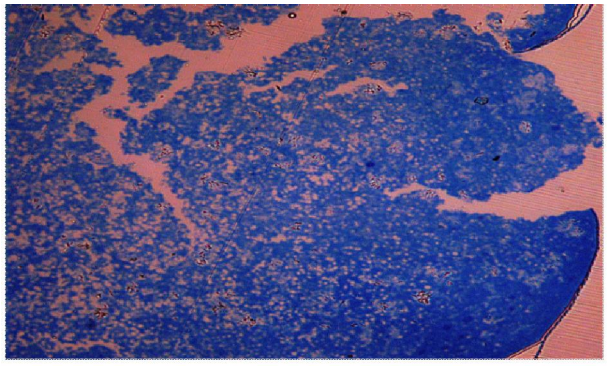

Figure 7: semi- thin section of the embryo 30 h- old reflecting the continued divisions in the cytoplasm.

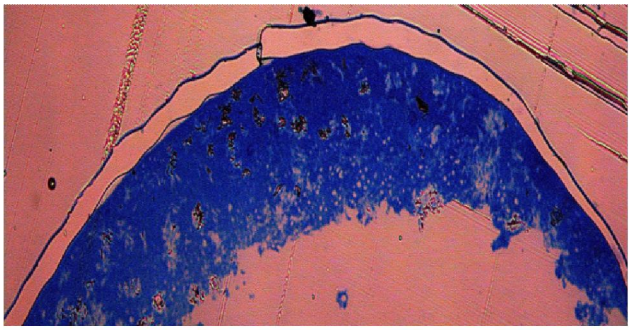

Figure 8: semi- thin section of the embryo 30 h- old gathering of clear cells bulastoderm (cells resulting from division of the cytoplasm) on the outer edge of the egg. As evidenced by the presence of the extra embryonic membranes.

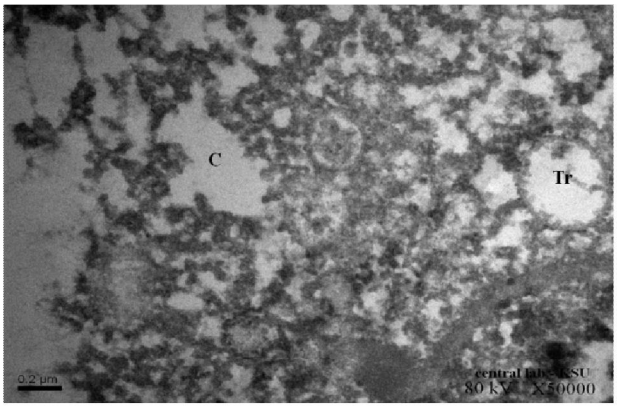

Figure 9: TEM of embryo 30 h- old illustrates the beginning of the trachea (Tr) and the composition of which originated from ectoderm layer. Cavities (C).

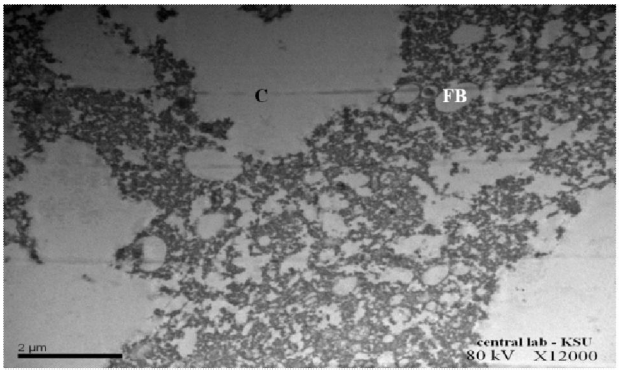

Figure 10: TEM of Gastrulation in embryo 30 h- old where the notes are the internal layer of cells that are scattered ectoderm layer, endoderm and mesoderm which ones start to be members of the body. As can see the cavities (C) of the body that represents the coelom, as show the fat bodies (FB).

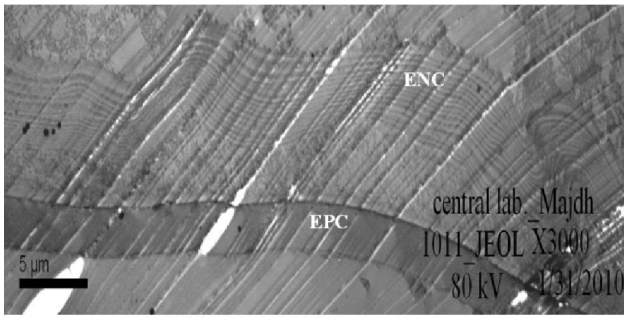

Figure 11: TEM embryo 39 h- old showing Epicuticle (EPC) and Endocuticle (ENC).

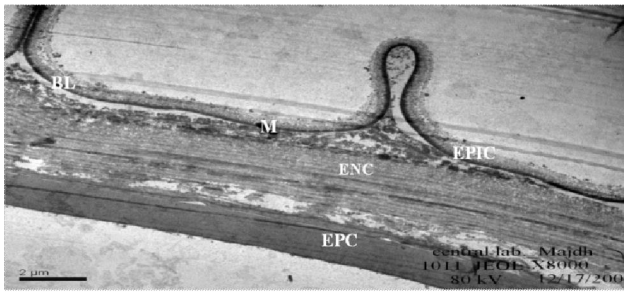

Figure 12: Section embryo 78 h- old, dense tow layers are they Endocuticle (ENC) and Epicuticle (EPC) with part of the underlying epidermal cell. The thicker laminated cuticle (CU) is comprised of fine chitin- protein micro fibrils which are arranged in different directions in each lamella. A thin epidermal cell (EPID) with a distinct basal lamina (BL) underlies the cuticle. The epidermal cell contains some mitochondria (M) but other organelles are not clearly illustrated.

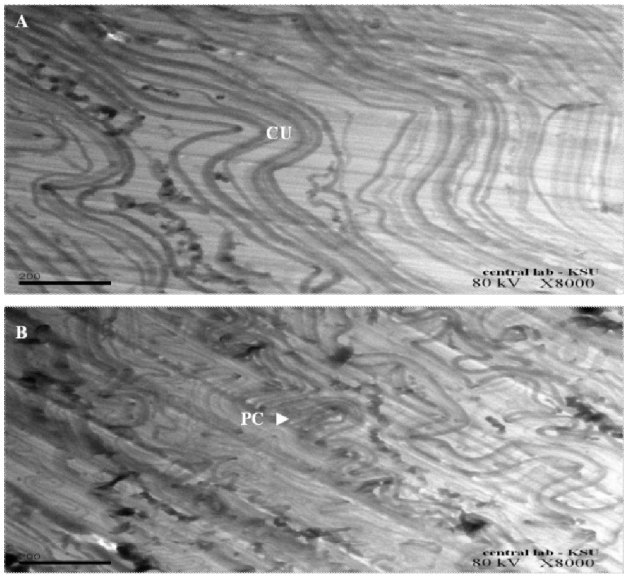

Figure 13: TEM transverse for embryo 84 h- old. The cuticle (CU) is comprised of dense and light laminae which are uniformly spaced. Pore canals (PC) appear as small light areas in the cuticle picture (B) arrow.

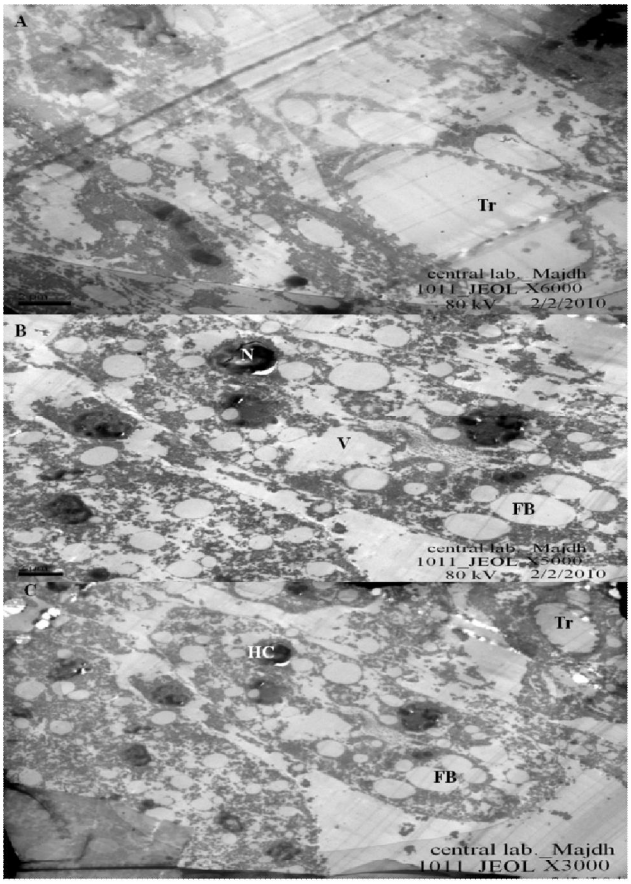

Figure 14: TEM of embryo 66 h- old. A: appear complete of Trachea (Tr) and contains of ictinidia. These Trachea contain of Ectoderm layer. B and C: show Fat body (FB) is flooded in haemolymph, haemocytes (HC), Nucleus (N). Fat body formation from Mesoderm. Vacuole, V

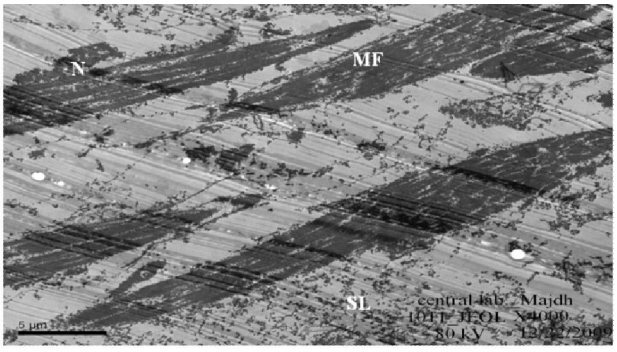

Figure 15: Electron micrograph for embryo78 h- old. appear of tubular striped (TS). From the located nucleus (N) the myofibrils (MF) radiate as flattened bundles to the sarcoplasm (SL)

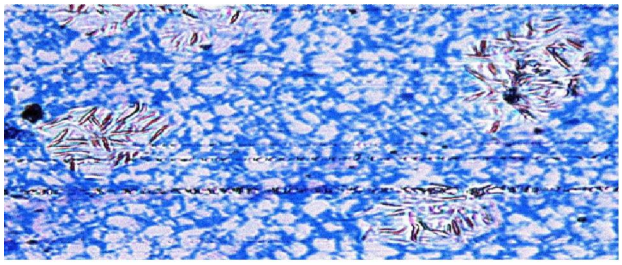

Figure 16: semi- thin section embryo 66 h- old clear division mitosis cell nuclei.

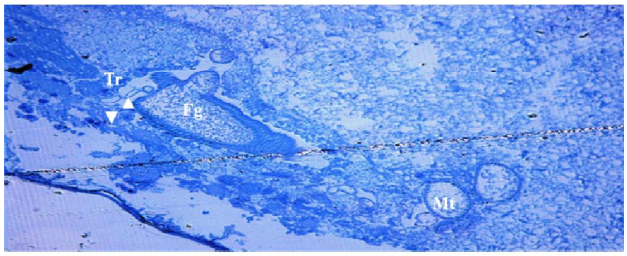

Figure 17: Semi- thin section with light microscopy of embryo 78 h- old showing primary formation of Fore gut, Fg, Malpigian tube; Mt; trachea, Tr (arrow).

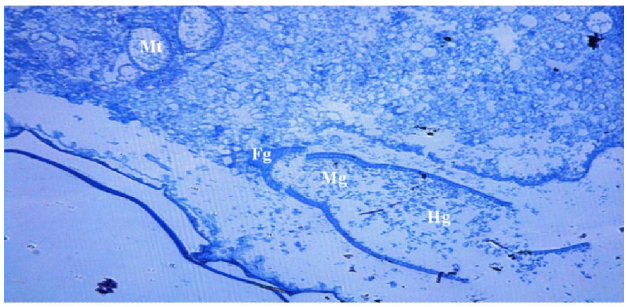

Figure 18: Semi- thin section with light microscopy of embryo 78 h- old showing completely formation of Fore gut, Fg, Med gut, Mg, Hind gut, Hg. Appear of Malpigian tube, (Mt).

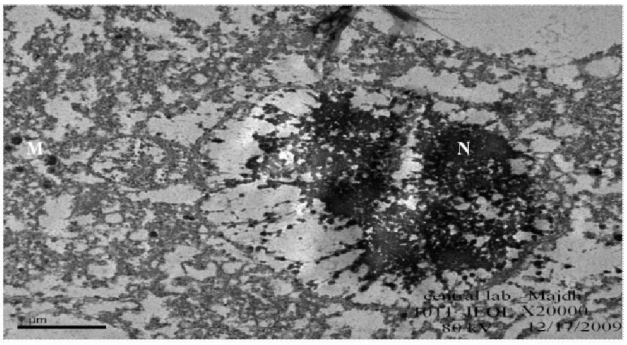

Figure 19: Micrograph Of gut epithelial cell with a dense cytoplasmic. Mitocondria (M) are numerous and a centrally located nucleus (N) is present.

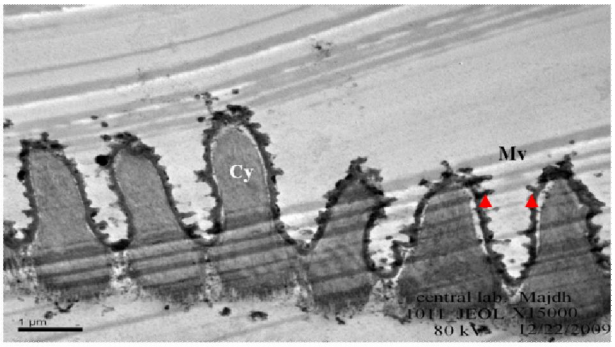

Figure 20 : Photograph of TEM for embryo 78 h- old of part from epithelial layer of gut. The luminal border has numerous microvelli, Mv (arrow), projecting into lumen filled with a cytoplasm, Cy. Nucleus, N.

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10/11/2013

The Relationship between Corporate governance and Conservatism in the Listed Companies in Tehran Stock Exchange

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Abstract: The aim of this study was to investigate the relationship between the components of corporate governance and accounting conservatism. Conservatism in this study is measured by Basu standard model. Corporate governance characteristics compared with non-duty members of the Board, CEO and Chairman of job separation and the ownership of institutional shareholders has been used as independent variables. Research data, using the population consists of 106 companies listed in Tehran Stock Exchange analyzes for the period 2001 to 2006 using a combination of data and ordinary least squares regression analyzes. The results of the regression estimates suggest that the timing of the financial reporting of listed companies in Tehran Stock Exchange will affect the index of profitability. The results reject the hypotheses of the study and showed that significant relationship does not exists between the proportion of non-duty members of the Board, CEO and Chairman of the Board and the task of separating ownership of institutional shareholders as a component of corporate governance and accounting conservatism .

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Keywords: corporate governance, conservative, non-duty members of the board of directors, institutional shareholders

Introduction

Useful information to users of financial statements includes quality properties. The main qualitative characteristics associated with the content, relevance and reliability and prudence or conservatism is one of the main features of reliability. In theoretical concepts of financial reporting conservatism used for estimating the degree of care in the exercise of judgment is required in conditions of uncertainty. So that income or assets presented no greater than the fact and cost or liabilities no less than the fact. Cautious conservatism is a cautious response to ambiguity. If there is no ambiguity, Conservatism does not require and there is much more uncertainty and risk there is a greater need to Conservatism [2].

Basu (1997) argues that accounting conservatism at least a hundred years ago rose with the aim of preventing and reducing the risk of presenting an unrealistic situation in business bankruptcy raised by the various groups, especially the suppliers of capital. In addition, The Basu conservatism requiring high degrees of support for the recognition of good news, such as earnings, the recognition of such losses is bad news [3].

One of the most important functions of corporate governance is to ensure the quality of the financial reporting process. If there is good structure and good performance monitoring role of the board members and the quality of financial reporting of these violations can be reduced somewhat. The International Organization for Economic Co-operation and Development, corporate governance defined as "The complex relationship between the management (executive) board of directors, shareholders and other stakeholders in a company or the concerned parties." Corporate governance, a wide range of checks and balances on the performance and incentives affect firms' managers [7].

Research Theoretical principle

The information with qualitative characteristics that is useful to users of financial statements. The main qualitative characteristics associated with the content, relevance and reliability and prudence or conservatism is one of the main features of reliability.

Accounting conservatism is one of the basic concepts that consider the Financial Accounting Standards Board and in conceptual statement Number2. Financial Accounting Standards Board defines conservatism: Cautious to ensure that the economic and financial situation of the company is to provide sufficient [4].

Feltham and Ohlson (1995) in terms of balance sheet defuned conservatism: In case of doubt, there is a choice between two or more reporting methods, the

method must choice to be the least favorable effect on stockholders [5].

Another definition of conservatism that based on the profit and loss perspective, Basu (1997) have stated that such conservatism requiring high degrees of verification for recognizing good news such as profits, in the face of recognizing bad news such losses.

Third definition of conservatism by Givoly and Hayn (2000) based on the combined balance sheet and profit and loss perspective. In this view, conservatism is an accounting concept leading to a reduction of retained earnings reported by later identify and recognize revenue faster than expenses, evaluation low assessment and over liabilities [6] .

The International Organization for Economic Cooperation Development (OECD), corporate governance defined as "The complex relationship between the management (executive) board of directors, shareholders and other stakeholders or the concerned parties in a company." Corporate governance affect a wide range of checks and balances on the performance and incentives affect firms' managers. If poor corporate governance, where the owner not the manager, the manager may not attempt to maximize shareholder profits and reduce costs not and will not perform their duties to the fullest. In such circumstances, even theft and fraud may also arise in the interests of managers. The difference between investment purposes (the owners) and their representatives (managers) is called "agency problem". Align managers' incentives, so that instead of looking to their own interests to act in the interests of owners, corporate governance is a major challenge. Corporate governance mechanisms can divide into two groups: 1 - internal governance (eg, board structure and independence) and 2 - external authority (such as institutional ownership concentration) [9].

Other institutional investors in corporate governance mechanisms that can monitor the management of the company, they can also have a significant influence on the management to provide the interests of shareholders. Although the concentration of ownership of corporate governance can notice as an important mechanism to control agency problems and improves the protection of investors, however, such a focus can also be negative. For example, access to confidential information of major shareholders and shareholder information asymmetry between them is smaller conflict of interest between managers and shareholders altered to the majority shareholder and minority interests' conflict [7].

Corporate governance as an engine of economic enterprises play a major role in determining corporate policy in terms of both operational and reporting. However, the corporate governance is stronger lead to better quality of financial reporting. Level of conservatism applied in the financial statements of one of the parties will determine the quality of accounting earnings (Dechow, Ge and Schrand, 2010). Conditional conservatism (or conservatism depends News) refers to the idea that profits reflect bad news faster than good news. According to the above discussion, it expected that corporate governance might have meaningful relationship with the degree of conservatism practices on businesses [11].

Research hypotheses

According to the theoretical assumptions of the study and the previous study and answer to the research questions, research hypotheses formulated as follows:

Main hypothesis:

There is a significant relationship between corporate governance and accounting conservatism.

Sub-hypotheses:

First hypothesis: there is a meaningful relationship between the number of board members and the applied non-mandated conservatism.

Second hypothesis: there is a significant relationship between the separation of CEO and corporate board of directors of applied conservatism.

Third hypothesis: there is a significant relationship between the proportion of institutional investors and the level of applied conservatism.

Literature reviewe

Some researches has done within and outside the country in the field of corporate governance and conservatism to be expressed in countinue.

In these researches, there was a positive relationship between Corporate Governance and Conservatism. From them cited to Lara et al (2005) and Lim (2006) that conducted using Australian examples [12].

The research by Lafond and Richvdary (2007) carried out in total and the results were consistent with previous studies itself. Moreover, they expect to see a negative relationship between the survivals of the company's managers is conservative and the percentage of ownership.

One of the wide fields that in recent year researchers have studied about conservatism, the problem of transfer agency and wealth management incentives and the company pays for their own benefit; hence, in this case something has done whether conservatism can serve as an effective mechanism for strengthening corporate governance

and use information asymmetry as well as the balance. Lara et al (2006) study a research and concludes that conservatism in financial reporting as collateral reactions to represent problems that the information asymmetry between informed and uninformed investors [11].

The result of Klein and Marquardt (2006) research in the United States on the subject of the relationship between accounting conservatism and the loss of 846 firms during the period 1997 to 2005 shows that there was a direct relationship between accounting losses and accounting conservatism. In other words, the results showed that the more conservative, the reported losses of tested company is more [10].

Lara, Osma, and Penalva (2006) in their study examined the relationship between conservatism and quality financial information on 420 Japanese companies over a period from 1989 to 2002. The results indicate that the conservatism in addition to increasing the quality of financial information cause to reduces the of risk liquidity and securities transactions costs. They ultimately concluded that conservatism reduces the cost of capital to companies.

Balachandran and Mohanram (2008) in their study examined the relationship between unconditional conservatism and income information content of the 438 Canadian firms over the period 1996 to 2005. They presented evidence that showed a significant positive association between accounting non-conditional conservatism and net benefit content information [1].

Variables and model research

In this study, measures of corporate governance, including the non-duty members of the Board, CEO and Chairman of job separation and ownership of institutional shareholders as independent variables and the dependent variable were used as accounting conservatism.Variables mentioned above based on the breakdown of the assumptions are shown in Table 1.

Table 1: independent and dependent variables of the study

Dapendent Variables	Indapendent Variables	Hyphotises
Accounting Conservatism	Other members of the board shall	Hypothesis 1
Accounting Conservatism	The task of separating the CEO and Chairman of the Board	Hypothesis 2
Accounting Conservatism	Ownership of institutional shareholders	Hypothesis 3

To test three sub- hypotheses and and main hypotheses of research, the three following model is estimated respectively using combined data:

Sub-model 1

$$X_{it} = \alpha + \beta_1 Dum + \beta_2 outdir_{it} + \beta_3 Dum \times outdir_{it} + \beta_4 R_{it} + \beta_5 Dum \times R_{it} + \beta_6 outdir_{it} \times R_{it} + \beta_7 Dum \times outdir_{it} \times R_{it} + + \varepsilon_{it}$$

Sub-model 2

$$X_{it} = \alpha + \beta_1 Dum + \beta_2 Duality_{it} + \beta_3 Dum \times Dualityit + \beta_4 R_{it} + \beta_5 Dum \times R_{it} + \beta_6 Duality_{it} \times R_{it} + \beta_7 Dum \times Dualityit \times R_{it} + \varepsilon_{it}$$

Sub-model 3

$$X_{it} = \alpha + \beta_1 Dum + \beta_2 Ins_{it} + \beta_3 Dum \times Ins_{it} + \beta_4 R_{it} + \beta_5 Dum \times R_{it} + \beta_6 Ins_{it} \times R_{it} + \beta_7 Dum \times Ins_{it} \times R_{it} + + \varepsilon_{it}$$

Main model

$$X_{it} = \alpha + \beta_1 Dum + \beta_2 Totgov_{it} + \beta_3 Dum \times Totgov_{it} + \beta_4 R_{it} + \beta_5 Dum \times R_{it} + \beta_6 Totgov_{it} \times R_{it} + \beta_7 Dum \times Totgov_{it} \times R_{it} + + \varepsilon_{it}$$

Where in :

X: Net profit after tax is equal to the stock price at the beginning of the period.

Rit: is on common return equity that obtained of the difference between the beginning and the end of the financial year and their prices and cash dividends to shareholders.

Dum: is a dummy variable equal to 1 when the outcome is negative or zero, and is zero otherwise.

Outdirit: is the percentage of the Board of Directors shall that the percentage of non-executive board members is obtained by divid the total number of board members in the end.

Dualityit: Duality Managing Director and Chairman: The Chairman or Vice-Chairman and Managing Director of the company, not a single person (the two side posts, one is not provided). If the Chairman or Vice-Chairman and Managing Director of the company's board of directors is one, number one, and zero otherwise be attributed to this variable.

Insit: percentage of institutional ownership. This value is the percentage of shares that preserved by institutional investors - in particular investment.
Totgovit: overall corporate governance index that is derived from a combination of the above. If the coefficient is significant in these models, the hypothesis was not rejected.

Conducting personal or institutional investors pay such a huge amount of public and private banks, pension funds, insurance companies and social security funds and companies, and foundations investing and institutions.

Methodology

The study is application based on aim and the nature and methods is descriptive - correlational. This study is based on a quasi-experimental research design and performed using to casual approach (from the past).

Statistical population and samplesize

Statictics population in this study included all companies that admit between the years 2001-2006 in Tehran Stock Exchange and preserved his membership in this period. The reason for selecting and surveying of stock companies, allowing easier access to the financial information of the company and having a more homogeneous due to the provisions of the Stock Exchange of Tehran. The sampling method in this study is a systematic deleting method. Thus, among all the listed companies that it meets any of the following conditions have been removed and finally the remaining companies were selected for testing:

• Companies must have complete information for all financial statements including balance sheet, income statement and cash flow statements. • The fiscal year end is 19th March.

• Research companies must be active on the Stock Exchange during the period when they are active. • During the research period not change the financial year. • The types of companies are not investment companies or financial intermediation and insurance.

Due to the constraints mentioned in the study, 106 selected companies have studied as statistical sample.

Research findings

Descriptive statistics

In order to better understand the community and learn more about the research variables, before analyzing the data, it is necessary to describe the data. Describe the data, a step in the direction of the pattern recognition and basis for explaining the relationship between the variables used in the study. Thus, before testing the research hypotheses sometimes in recognizing descriptive statistics of the variables used for calculating in the study presented in Table 2. Descriptive statistics presented overview of the research data.

Table 2: Descriptive statistics results of research variables

Totgov	Ins	X	R	
1.261	0.470	0.192	0.374	Average
1.243	0.511	0.166	0.160	Middle
2.800	1.000	1.001	5.666	Maximum
0.200	0.000	-0.228	-0.984	Minimum
0.520	0.324	0.134	0.788	Standard deviation

Source: Research finding

The results of testing the first hypothesis

In this study, the type of data and methods of statistical analysis, data econometric methods combined (total study period) to the estimated model is used to investigate the hypothesis testing. In this research, a quantitative value of independent and dependent variables on the one hand, about 106 different companies and on the other hand, covers the period 2001-2007. Thus, the total number of observations in regression analyzes 636 company-years. The results of the first study in are presented in Table 3.

According to the model to test the research hypotheses, the model parameters or the model coefficients represent the independent variables related to the severity and type of relationship between the independent variable and the dependent variable. Thus, if the coefficient of the independent variables βn is positive, the independent variables accounting conservatism (the dependent variable) and the direct relationship between the coefficients is negative, it will have an inverse relationship.

Table 3: Results of testing the research first sub-hypothesis

D-W	F-Prob	F-static	Adjusted R^2	R^2	t- static p-value	Cofeince	Description
1.58	0.00	52.87	0.38	0.39	8.047 0.000	0.213	Intercept
					-1.252 0.211	-0.048	**Dum**
					-1.648 0.163	-0.065	**Outdir**
					0.741 0.459	0.044	**Dum× Outdir**
					3.161 0.002	0.068	**R**
					0.513 0.609	0.065	**Dum× R**
					0.777 0.437	0.025	**Outdir× R**
					-0.004 0.997	-0.001	**Dum× Outdir× R**

Source: Research findings

Adjusted coefficient of determination of the model test research indicates that some of the dependent variable is explained by the independent variables. Much more dependent variable will be correlated with the independent variables. The adjusted R2 of the model test is equal to 0.38. In other words, 38% of the change in accounting conservatism (the dependent variable) is due to changes in the independent variables (indicators of corporate governance, respectively). According to the estimation results of the research model to the data model, the F statistic is significant at 1% error. Thus, research model was overally significant. To examine the significant relationship between each independent variable with the dependent variable use the resulted values for the t-statistic and the p-value.

As Table 3 it can be seen, the t-statistic to test the model variables Dum × Outdir × R based on the relationship between the number of directors shall not be rejected and conservatism. Therefore, the results were not confirmed the first sub-hypothesis. Result findings related to this hypothesis is similarto the results of Garcia et al (2007) Qi et al (2007) researches.

The results of testing the second hypothesis

The results of the second model are presented in Table 4.

Table 4: Results of testing the research second sub-hypothesis

D-W	F-Prob	F-static	Adjusted R^2	R^2	t- static p-value	Coeifence	Description
1.84	0.00	53.96	0.38	0.39	19.637 0.000	0.166	Intercept
					-1.027 0.310	-0.013	**Dum**
					1.687 0.092	0.034	**Duality**
					-1.399 0.163	0.048	**Dum× Duality**
					13.276 0.000	0.094	**R**
					1.249 0.212	0.045	**Dum× R**
					-4.423 0.713	-0.073	**Duality × R**
					0.718 0.476	0.071	**Dum×Duality×R**

Source: Research findings

Adjusted coefficient of determination test the research model is equal to 0.38. In other words, 38% of the change in accounting conservatism (the dependent variable) was due to changes in the explanatory variables. The estimation results of the research model to the data model, the F statistic is significant at 1% error. Because of its significance level is less than the error level. As in Table 4 show, the t-statistic to test denied the model variables Dum × Duality × R based on the relationship between the roles of CEO and chairman of the board and conservatism. Therefore, the results represent not cofirm the second sub-hypothesis. Research findings related to this hypothesis, the results of Garcia et al (2007) is similar. Research findings related to this hypothesis is similar to the results of Garcia et al (2007).

The results of testing the third hypothesis

The results the third hypothesis is presented in Table 5.

Adjusted coefficient of determination test the research model is equal to 0.38. In other words, 38% of the change in accounting conservatism (the dependent variable) was due to changes in the explanatory variables. The estimation results of the research model to the data model, the F statistic is significant at 1% error. Because of its significance level is less than the error level. Thus, the research model was significant overally. The t-statistic to test denied the model variables Dum × Duality × R based on the relationship between the roles of CEO and chairman of the board and conservatism. Therefore, the results represent not cofirm the second sub-hypothesis. Research findings related to this hypothesis, the results of Garcia et al (2007) is similar. Research findings related to this hypothesis is not similar to the results of Garcia et al (2007) but the results are consistent Bix and colleagues (2004).

Table 5: Results of testing the third sub-hypothesis research

D-W	F-Prob	F-static	Adjusted R^2	R^2	t- static p-value	Cofeince	Description
1.86	0.00	52.25	0.38	0.38	13.199 0.000	0.177	Intercept
					-1.644 0.101	-0.033	**Dum**
					-0.369 0.712	-0.009	**Ins**
					0.731 0.436	0.027	**Dum× Ins**
					8.738 0.000	0.088	**R**
					0.723 0.470	0.034	**Dum× R**
					-0.486 0.628	-0.019	**Ins × R**
					0.368 0.720	0.040	**Dum× Ins × R**

Sources: Researcher,s Findings

Results of testing basic hypothesis of the research

The estimation results of the research model are presented in Table 6.

Adjusted determination coefficient results of testing the research model is equivalent to 0.39. In other words, 39% of the change in accounting conservatism (the dependent variable) was due to changes in the explanatory variables. According to The estimation results of the research model to the data model, the F statistic is significant at 1% error level. To examine the relationship between each independent variable with the dependent variable, the values for the t-statistic and the p-value use associated with it. As in Table 6 show, deny the t-statistic to test the model variables Dum × Totgov × R based on the relationship between corporate governance and conservatism. Thus, the results indicate the main research hypothesis not confirmed. Findings related to this hypothesis are inconsistent with the results of Garcia et al (2007) and Qi et al (2007) respectively.

Table 6: Results of testing basic hypothesis of the research

D-W	F-Prob	F-static	Adjusted R^2	R^2	t- static p-value	Coefficient	Description
1.86	0.00	54.59	0.39	0.39	8.439 0.000	0.160	**Intercept**
					-0.259 0.796	-0.007	**Dum**
					0.741 0.459	0.010	**Totgov**
					-0.552 0.581	-0.023	**Dum× Totgov**
					8.029 0.000	0.141	**R**
					-0.248 0.746	-0.025	**Dum× R**
					-3.682 0.000	-0.0483	**Totgov × R**
					0.866 0.387	0.064	**Dum× Totgov × R**

Source: Research findings

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South American Leaf Blight: Identification and Management of disease outbreak in Nigeria

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Abstract: South American Leaf Blight is identified as one of the world's five most threatening plant diseases that affect rubber. Until today it is the single factor responsible for the failure of economically rubber cultivation in its motherland, South America. The hectares of rubber plantations in Nigeria are over 247,000 hectares with more than 70% of the holdings by small-scale farmers. Nigeria contributes more than 60,000 tons of rubber to the international market. The rubber industry provides employment opportunities in the non-oil sector in Nigeria. An outbreak will have a far-reaching economic effect on the resource poor farmers and poverty alleviation program in the country. Presently Nigeria is currently free from South American Leaf Blight. However, based on the level of interaction between Nigeria, and South and Central America in such aspect as economic and cultural relationship, the spread of South American Leaf Blight to Nigeria is possible. The major means to prevent the spread of South American Leaf Blight if eventually introduced is to continually educate those involve in rubber production especially our small scale farmer who form the bulk of rubber producers in the industry in the country of the symptoms and biology of the dreaded disease South American Leaf Blight.

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Keywords: Disease, Identification, Management

1. INTRODUCTION

South American Leaf Blight is identified as one of the world's five most threatening plant diseases that affect rubber. Until today it is the single factor responsible for the failure of economically rubber cultivation in its motherland, South America. The causal agent responsible for SALB is the obligate parasite *Microcyclus ulei* (P. Henn.) v. Arx. It affects all the above ground parts: leaves, Petioles, Stem, Young Bark, Inflorescence and Fruit. It causes repeated defoliation during favourable condition leading to the death of the rubber tree.

Ford motor company in Brazil established a plantation of 3,200 ha at Fordlandia in 1927 and the plantation was abandoned in 1933. Another plantation of 6,478 ha was established by Ford motor Co. at Belterra in 1936 and was abandoned in 1943. Under PROBOR programme (1967-1987) 150, 000 ha were planted of which 100,000 ha suffered severe SALB by 1967. In Surinam, a plantation (40,000 trees) was established in 1911 and was abandoned in 1918.

The hectares of rubber plantations in Nigeria are over 247,000 hectares with more than 70% of the holdings by small-scale farmers. Nigeria contributes more than 60,000 tons of rubber to the international market. The rubber industry provides employment opportunities in the non-oil sector in Nigeria. This is more so as the bulk of the field labour is derived from the rural areas.

Any threat to the rubber industry will have a significant adverse effect on the resource poor farmers in the rubber belt, the agricultural sub sector of the economy and serious socio-economic consequences in Nigeria.

The dangers posed by the threat of outbreak of *Microcyclus ulei* in Nigeria, could be imminent if adequate phytosanitary control measures are not taking into account. An outbreak will have a far-reaching economic effect on the resource poor farmers and poverty alleviation program in the country. SALB would directly affect rubber plant mortality, reduction in latex yield, reduction of rubber wood production, investment eradication, increases in production costs, Loss of income and employment within affected regions, environmental impact, loss in aesthetic value, loss in foreign exchange; and indirectly affect loss of market opportunity (International trade), intensified research and development, social dislocation-urbanization and migration of rubber labour force, and eventual decline in the standard of living of people involved in rubber industries, especially small holders.

PRESENT SITUATION OF SALB

Nigeria and indeed the whole of Africa and Asia are currently free from South American Leaf Blight (SALB); aside the malady of SALB one can observe the incidence of *Colletotrichum gloeosporioides, Corynespora cassiicola* and white root rot disease. The last field survey carried out in

late 2008 in the rubber growing region in the country revealed that the country is free from SALB (Ogbebor *et al.,* 2009). However, despite phytosanitary measures, the spread of SALB to Nigeria is possible. This is based on the level of interaction between Nigeria, and South and Central America in such aspect as economic and cultural relationship.

FUTURE ACTIVITIES TO PREVENT ENTRY OF SALB INTO NIGERIA

Currently only two possible checks to SALB are in place, the first are the quarantine services of each country including Nigeria. This exercise if normally targeted at introduction of live materials through regular checks for foreign bodies before they are allowed entry or if found, infected the materials are destroyed.

However, apart from live materials, sources of inoculums could be inanimate or human carriers who may bear such inoculums unconsciously on their persons or personal effects. The second check to the possible spread is the consensus under the Association of Natural Rubber Countries (ANRPC) that scientist of rubber producing countries that visit Brazil for studies should spend some time in Europe before coming to their home country. It is expected that during such stay in Europe, spores of the causal organism of SALB carried on the person or personal effect will be dislodged. Since the environmental condition in Europe is not conducive for the growth of the spores, such natural quarantine will not predispose the transit country to the treat of SALB. The Rubber Research Institute of Nigeria has complied with this regulation. Consequently a stake holder's forum was held on the 3rd of March, 2009 at RRIN main station, Iyanomo, Benin City to raise the awareness of SALB and quarantine protocol for introduction of SALB tolerant rubber clones to the country.

It is necessary for the African countries that produce rubber to create a network concerned with SALB to strengthen the measures that up until now have prevented the introduction of SALB. The training that I received on the SALB and management in Michelin Plantations at Bahia, Brazil is very important in terms of continuing the future activities towards management practices of SALB and in effectively operating quarantine measures.

The major means to prevent the spread if it is eventually introduced is to continually educate those involve in rubber production especially our small scale farmer who form the bulk of rubber producers in the industry in the country of the symptoms and biology of the dreaded disease SALB.

BIOLOGY OF SALB

Hosts

M. ulei affects the genus *Hevea*, namely *H. brasiliensis* Muell, *H. benthamiana* Muell Arg., *H. guianensis* Aubl and *H. spruceana* (Benth.) Muell. among the several thousand genera in the Plant kingdom. Inoculation of other plants of the same family (Euphorbiaceae) as *Hevea* rubber was unsuccessful. *M. ulei* failed to successfully infect and sporulate on cassava plant (Jens, *et al*., 2003)). On *Hevea* rubber, *M. ulei* infects the young aerial part of the plant. Infection is most common on young leaves; however leaf petioles, young stems, inflorescences, flowers and young fruit are also infected.

Spore

Microcyclus ulei is in the group Ascomycete and produces three types of spores in sequence on the same leave; the conidia (Plate 1a), the pycnospores (plate 1b) and the ascospores (Plate1c). The conidia are produced abundantly during the asexual state of the disease while the pycnospores and the ascospores are produced during the sexual state of the fungus. The conidia are mainly two celled with a broad proximal cell and a tapered distal cell. The unique character of the conidia is that they are twisted. The length of the conidia is 23-62 µm and the width is 5-10 µm (Holliday, 1970). The size of the conidia varied with location and season. The conidia, sometimes, have only one cell and the one-celled conidia are more common during dry weather conditions. One celled conidia are also more common from laboratory cultures.

The pycnospores are dumbbell shape and small (6-10 µm long and 2-5 µm in width). The ascospore is oblong shaped and is made up of two cells of unequal size. The ascospores are 12-20 x 2-5 µm in size. The ascospores are protected from desiccation by thick wall of ascocarp and are responsible for the survival of the pathogen from one season to another.

Plate 1. Spores of *Microcyclus ulei*: (a). The two-celled conidia of *Microcyclus ulei* with a characteristic "twist" (b). Pycnospores (c). Ascospores

Symptoms

Shortly after infection of young rubber leaflets, the first visible symptom is the distortion in shape of the leaflets (Plate 2a). Two to 12 days old leaves showed symptoms of SALB about 2-3 days after inoculation (Blazquez and Owen, 1963). A few days later, irregular-shaped disease lesions developed on the undersurface of the young brown colored leaflets. Heavily infected susceptible leaflets shriveled turn black and dropped off. The petioles remain on the stem for several more days before they also drop-off (Plate 2b). The characteristic lesions with abundant conidia are visible on young green leaves remaining on the plant. Then, the lesions produce abundant conidia and appear dark to olive green in colour (Plate 2c). The size of lesions and the amount of conidia produced is influenced by the age of leaflets, the susceptibility of the clones and the prevailing weather conditions. The infected leaves are deformed in shape and smaller in size than healthy leaves.

Plate 2. Symptoms: (a). The early symptoms of SALB on young rubber leaflets (b). Petioles remaining on the stems after the infected leaflets fall-off (c). Disease lesions covered with conidia on the undersurface of young leaflets.

About two to three weeks after infection started, the lesions stop producing conidia (Plate 3a). Then, the leaf tissues on the upper surface of leaf immediately above the disease lesions on the lower leaf surface turn yellowish (Plate 3b) and later small round black raised structures called the pycnidia are formed (Plate 3c). The pycnidia are 120-160 µm in diameter and these fruiting bodies produce the pycnospores. Several weeks later, the round dark raised structures enlarge and form another dark colored raised bodies called the perithecia especially around the edges of the disease lesions (Plate 3d). The perithecium produces the fruiting structure called the ascus that bears the ascospores. The number of perithecia varies with infection and susceptibility of leaves. In certain cases, the whole upper surface of the lamina is covered with numerous perithecia. As the leaf aged, the leaf tissues at the centre of the lesions die and turn papery white and later tear off leaving shot-holes in the leaf (Plate 4a).

Plate 3. (a). Older lesions on the lower leaf surface of leaflets that had ceased to produce conidia (b). Yellowish discoloration form on the upper leaf surface immediately above the lesions (c). Pycnidia formed on the upper surface of leaflets immediately above the lesions on the lower leaf surface (d). Perithecia forming on the upper surface of leaves immediately above the lesions on the lower leaf surface.

Plate 4. The leaf tissues at the centre of the colonies of perithecia died and form a “shot hole” (b). stem (c). Infection of SALB on the fruits of rubber (d). Dead trees caused by severe infection of SALB

M. ulei also infects other parts of the plant, the leaf main vein, leaf petiole, bud, stem (Plate 4b), inflorescence and fruit (4c). The process of infection and defoliation occur repeatedly under favourable weather condition in susceptible clone. If control measures are not adopted the result will be the shot die back and death of rubber tress (Plate 4d).

Disease Development

The conidia and the ascospores are responsible for disease infection. The pycnospores do not caused infection even under artificial inoculation, but germinated *in vitro* (Holliday, 1970). The reaction of the host to infection is influenced by the degree of resistance of the leaves. In susceptible leaves, the fungus spreads intercellularly in the leaf without apparent hindrance. However, in certain highly resistant or immune clones, the fungus penetrates into the leaf but disease spread was inhibited.

In infected rubber plantations ascospores are present throughout the year with peak concentrations occurring during the wet seasons. The wet season also marks the period of maximum production and dispersal of conidia (Chee 1976a,b).

2. CONCLUSION

Application of fungicides is the most popular strategy to control SALB. Farmers are advised to regularly look out for these common symptoms of SALB in their plantations/ farms and quickly report any detectable symptom or resemblance in symptoms of SALB in Nigeria to Rubber Research Institute of Nigeria who in turn after confirmation of the detected symptoms of SALB shall report to the plant quarantine service. As early detection is the surest remedy in the eradication of the disease in case of its eventual introduction into the country.

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Clinical Features Of Patients Diagnosed With Vesico Vaginal Fistula (Vvf) In South East Nigeria

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Abstract: This study examined the clinical features of patients diagnosed with Vesicovaginal Fistula (VVF). 125 medical records of patients with VVF were reviewed. The patients were between the ages of 17 to 65 years (M = 34.05; SD = 5.84). The participants were sampled using convenience sampling technique from National Obstetric Fistula Centre, Abakaliki and Gynaecology department Nnamdi Azikiwe University Teaching Hospital, Nnewi. Ex-post facto design was adopted while descriptive (%) and quantitative (Chi-Square (X^2) statistics was applied in testing the hypotheses. The result revealed that clinical features (rejection = 64.29%; X^2 = 21, and depression = 73.81%; X^2 = 31.85) all at p<.001 level of significance had significant difference on patients diagnosed with VVF. The findings were discussed and recommendations made. Based on the outcomes, it was concluded that clinical features had a remarkable difference on the prevalence and incidence of VVF among diagnosed patients.
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Keywords: vesico vaginal fistula, depression, rejection, early marriage, south east nigeria

1. Introduction

Confinement is a life-changing event which is a pleasant and joyful experience for many mothers. On the other hand, it is a difficult and regretful period for others particularly, when it is accompanied by serious illness, debilitating injuries and death of the baby, mother or both. About half a million women die yearly from causes related to pregnancy and delivery and for each maternal death approximately 10–15 other women sustain serious morbidity including vesicovaginal fistula (VVF) (Ashford, 2002; Lewis & de Bernis, 2006; Aboyeji, Ijaiya & Fawole, 2007; Orji, Adeloju & Orji, 2007; Rizvi, 1999). Thus, vesicovaginal fistula (VVF) can be described as aftermath of a 'near miss maternal death'. However, VVF appears to have been in existence since antiquity evident by references made to genital fistula in Ebers papyrus and in an Egyptian mummy in 2000BC and 2050BC respectively (Rizvi, 1999). Fistulas destroy the lives of many young women in the developing world. While obstetric vesicovaginal fistulas have vanished from the industrialized world, despite the efforts of many charitable organizations, they continue to occur in epidemic numbers in developing countries. The national and local governments of these countries do not have either the resources or the political will to address this problem and help these outcast women. The number of vesicovaginal fistulas in a region reflects the quality and the level of prenatal care delivered by the local health systems. In regions where health care (particularly maternal health care) is poor or absent, the number of obstetric fistulas is likely to be high. Vesicovaginal fistula is a preventable disease but is prevalent among the less privileged and marginalized members of the population; the poor, young, illiterate girls and women in the remote rural areas of the world, where access to emergency obstetric care, family planning services and skilled birth attendance are unavailable and where available poorly utilized (Lewis & de Bernis, 2006). From the foregoing, it is worthy to understand that, a fistula is an abnormal communication between the vagina and the bladder (or rectum) of a woman that results in a constant leakage of urine and/or faeces. The term "vesico" according to the medical profession is called urinary bladder. Vesicovaginal fistula is thus the abnormal connection between the urinary tract and the vagina such that there is an uncontrollable leakage of urine into the vaginal tract. According to Valerie and Riley (2004), "VVF is an abnormal communication between the urinary bladder and the vagina that results in the continuous involuntary discharge of urine into the vaginal vault". The incidence of fistula varies from country to country and continent to continent as do the main causative factors. There are large differences in the incidence of VVF among populations. Globally, over two million women are estimated to be living with vesicovaginal fistula and majority is in Sub-Saharan Africa and South Asia (Kelly & Kwast, 1993). The reported incidence rates of vesicovaginal fistula in West Africa range between 1– 4 per 1,000 deliveries (Ijaiya, 2004; Margolis & Marcer, 1994; Ijaiya, 2002). An annual obstetric fistula incidence is estimated at 2.11 per 1000 births

(Tsui, Creanga, & Ahmed, 2007); from 100,000–1,000,000 Nigerians live with obstetric fistula (Wall, 1998). Over 70,000 Bangladeshi women live with obstetric fistula (UNFPA, 2004; Technical Report, 2001) and about 9,000 new cases occur each year in Ethiopia (Technical Report, 2001). It is however not impossible that some of these incidences/ prevalence are under reported. World Health Organization (WHO, 2006) estimates that in developing countries each year five million women suffer severe maternal morbidity, obstetric fistula being on the top of the list. It is also estimated that currently more than 2 million women are waiting for surgery worldwide and about 50 to 100,000 new cases are added each year mostly in Africa and Asia (Nawaz, Khan, Tareen, & Khan, 2010). In developed countries on the contrary, fistulae are related to Gynaecologic surgery or radiation therapy (Wall et al. 2004). The clinical and psychosocial consequences of this morbidity are also very detrimental to women. The women are often rejected by their husbands, family and community, while suffering the loss of their stillborn baby. Although husbands and family members may initially be supportive and compassionate to these women, when it becomes clear that the constant loss of urine or faeces is a chronic condition (viewed as incurable in the context of the traditional local culture) these women are usually divorced or abandoned by their husbands and are often cast out by their families These attitudes might lead to depression, low self esteem, and other psychopathological issues as the case maybe. There is a great need on highlighting the importance of investigating and attending to psychological health issues among women who have sustained an obstetric fistula/VVF. VVF is considered a major public health problem in Nigeria, with the prevalence rate on the increase because of rising poverty and declining quality of maternal care. However, since the national safe motherhood conference in Nigeria in 1990, the problem of maternal mortality has been placed on the national agenda, but very little has been done to address the problem of VVF. Victims of obstetrics VVF are usually the lucky survivors of traumatic prolonged childbirth, but oftentimes without the joy of a baby as the baby invariably dies during childbirth. They become social outcasts. Divorced and rejected by families, they travel long distances in search of treatment, which often eludes them. They often have to take to begging or prostitution for survival. Although the treatment of women with obstetric fistulas is a worthy endeavour, the ultimate goal should be to eliminate fistulas entirely by providing adequate maternal health services and prenatal care. As some Africans say: 'Treating obstetric fistulas is like taking a serpent by the tail—you can only control the snake by taking it by the head." The ultimate goal must be fistula prevention. Therefore, this study was designed to investigate the psychosocial features of patients diagnosed with VVF.

Purpose of the Study

The aim of this study is to determine the Clinical features' of patients diagnosed with VVF.

- Will there be significant difference in clinical features of patients diagnosed with VVF?

Clinical Features and VVF

Recent publications (Wall,& colleagues, 2004; Browning, Fentahun, & Goh, 2007; Goh, Sloane, Krause, Browning, & Akhter, 2005; Murphy, 1981) and a review by Ahmed and Holtz (2007) have documented the physical, social, economic, emotional and psychological consequences of fistulas in affected women. A meta-analysis of the literature published between 1985 and 2005 showed that 36% (95% CI: 27%-46%) of women afflicted with fistulas were divorced or separated and foetal loss occurred in 85% of cases in which a fistula developed. Low self-esteem, feelings of rejection, depression, stress, anxiety, loss of libido and loss of sexual pleasure were commonly reported by these women.

It also appears that the rates of separation or divorce increases the longer a woman lives with a fistula, particularly if she remains childless (Browning, & Menber, 2008). Not surprisingly, successful fistula repair reduces the prevalence of these psychosocial pathologies (Ahmed & Holtz, 2007).Three recent articles further document the presence of these problems in women with fistulas. In their 2007 article on the health and social problems of women with fistulas in Ethiopia, Muleta, Fantahun, Tafesse, Hamlin and Kennedy (2007) reported 69.2% of fistula victims were divorced, only 19.2% were members of a local community association, and 44.2% ate separately from other family members. Forty-eight of 52 women felt listless and 28 had suicidal thoughts. Goh & colleagues (2005) conducted a prospective observational study to screen women in Bangladesh and Ethiopia with fistulas for mental health dysfunction. Of the 68 women with fistulas screened, 66 were at risk for mental dysfunction as measured by the General Health Questionnaire (GHQ-28) compared with only 9 of 28 controls. In a prospective interventional study, 51 women with fistulas in the north of Ethiopia were screened for mental health issues before and 2 weeks after surgery using the GHQ-28. Prior to surgery, all women had signs of mental dysfunction, but two weeks after fistula surgery, only 36% still had signs of mental distress. Among the 45 women who were cured of their incontinence, only 27% had signs of mental dysfunction two weeks after surgery, whereas

all of the six patients who remained incontinent continued to screen positive for mental distress on the GHQ-28 (Browning & colleagues, 2007).

In a study by Alio, Merrell, Roxburgh, Clayton, Marty, Bomboka, Traore and Salihu (2007) in Niger, women reported many psychological consequences of VVF including depression, feelings of shame, and loneliness. Others reported feeling devalued as a woman and wanting to end their lives. Social consequences of fistula reported by these women included rejection from society, isolation, rejection from husband and/or divorce. Almost half of the women reported of having lost their social network and support as a result of the fistula. Women with VVF were deemed unworthy, and their illness was often attributed to some fault of their own.

The theoretical explanation hinges on the classification system, causes/development of VVF (physical and sociocultural factors) were highlighted. This study tapped from the wealth of literature on this reviewed literature in establishing basis for the current study. Again, from the empirical studies reviewed, they showed that many studies have been carried out in the sub Saharan African countries and Nigeria in particular. However, the paucity of research in the area of the clinical aspect of patients diagnosed with VVF triggered of the passion for this study and aimed at closing the gap created by the dearth of research.

Hypotheses

The following hypotheses were tested:

- There will be no significant difference in clinical features of patients diagnosed with VVF.

2. Material and Methods

Participants:

A total of 125 medical records of patients with VVF were reviewed. The patients are between the ages of 17 to 65 years with a mean age of 34.05 and standard deviation of 5.84. The participants were sampled using convenience sampling technique from National Obstetric Fistula Centre, Abakiliki and Gynaecology department Nnamdi Azikiwe University Teaching Hospital, Nnewi, South East Nigeria.

Instrument

This study adopted qualitative method in data collection. The qualitative data were gathered from the secondary sources (medical records) of VVF patients.

Ethical Standards

The researchers obtained oral permission/approval as waivers to written permission from the two institutions to access the medical file of their patients in the study. The management consented orally to permit the study since it is purely for academic purpose and teaching and there were no form of external funding from any agency to the study. The oral permissions for this study were given as a waiver to written permission by the medical directors of these centres who directed the records/data departments of these centres to allow us have access to the medical records of their patients. It is important to note here that VVF is a condition that happens to the uneducated poor people who dwell in the rural areas. As a special research centre for the treatment of VVF, the management informed us that all the patients do sign at their admission to permit the hospital management to keep their data and use it for research purpose only.

Procedure:

A total of 125 medical records of VVF patients were reviewed and 100 copies of the questionnaire were administered within 7 weeks. This recorded review and administration of questionnaire was carried out in National Obstetric Fistula Centre, Abakaliki and Gynaecology Department Nnamdi Azikiwe University Teaching Hospital, Nnewi. The medical records reviewed covered from 2008 to 2012.

Design/Statistics:

The design adopted for this study was Ex-post facto design. This entails gathering of detailed information about one individual or group and might include a detailed account of experiences relevant to the issue, which makes the person of a particular research interest (Coolican, 2009). However, descriptive statistics and inferential statistic, percentage (%) and Chi – Square (X^2) were applied respectively in testing the hypotheses. This position was upheld by Patton (2002) who states: qualitative analysis transforms data into findings.

3. Results

Summary tables of percentage and Chi – Square on clinical features' of patients diagnosed with VVF.

Table I: VVF patients' perception of societal reaction towards patients diagnosed with VVF.

Hypothesis I:

Societal reaction	Frequency	%	X^2	P
Sympathetic	12	28.57		
Rejected	27	64.29	21	<.001
Indifferent	3	7.14		
Total	42	100		

From table I above, it was observed that rejection received the highest frequency accounting for 64.29% of the patients' responses on perceived

societal reaction toward patients diagnosed with VVF. Furthermore, X^2 – calculated value of 21 at p<.001 level of significance was found to be greater than X^2 – critical value of 10.83, indicating that rejection as a clinical feature's had a significant difference on patients' diagnosed with VVF. This means that VVF patients perceived themselves as been rejected by the society.

Table II: patients diagnosed with VVF reaction to their conditions.
Hypothesis II:

VVF patients' reaction	**Frequency**	**%**	**X^2**	**P**
Fate	8	19.05		
Depressed	31	73.81	31.85	<.001
Indifferent	3	7.14		
Total	42	100		

From table II above, depression received the highest frequency accounting for 73.81% of the VVF patients' reaction towards their condition. Also, X^2 – calculated value of 31.85 at p<.001 was found to be greater than X^2 – critical value of 10.83, indicating that depression as a clinical feature had a significance on patients diagnosed with VVF. This means that patients diagnosed with VVF have depression as a serious clinical problem.

4. Discussion

The hypothesis which stated that "There will be a significant difference in clinical features of patients diagnosed with VVF" was confirmed, on the basis that the clinical features had significant difference on patients diagnosed with VVF. Patients in this study reported high rate of rejection and depression as a result of their medical condition. These outcomes are consistent with previous studies by Kabir & colleagues (2003) in Kano, who reported that one third of VVF patients had psychological depression and over half suffered from societal negative reaction. In a study by Alio & colleagues (2007) in Niger, women reported many psychological consequences of VVF including depression, feelings of shame, and loneliness. Others reported feeling devalued as a woman and wanting to end their lives. Social consequences of fistula reported by these women included rejection from society, isolation, rejection from husband and/or divorce. Almost half of the women reported of having lost their social network and support as a result of the fistula. Women with VVF were deemed unworthy, and their illness was often attributed to some fault of their own. However, the outcomes of this study were not surprising as the plight of these unfortunate victims can be so devastating and dehumanizing that even when cured after surgery some of them never regain their self esteem and as such shun social life. The high level of perceived rejection observed in this study could be as a result of separation, divorce or low self-esteem common among these patients. Economically they cannot work because they cannot stay in the public and will not be employed. The patient thus becomes an economic burden to others. These social problems were also observed by Murphy (1981) among VVF patients in Zaria.

Implications of the Study

Government at all level should launch a campaign against teenage marriage, create special counseling and enlightenment programme for the people on the benefits of prenatal health care and VVF, developing and building functional health care centers in the rural areas to reduce traditional form of midwifery. The study has also shown that VVF patients suffer physically, emotionally and socially. They suffer from unnecessary and avoidable clinical complications such as loss of self esteem,divorce/separation and depression. Most patients cannot work or attend social gathering because of smell of urine. A campaign similar to that of anti HIV/AIDS campaign should be instituted by government and non governmental agencies to help in curbing the menace of stigmatization on VVF victims. Families should rarely round VVF patients to give them moral support and encouragement to live with their medical condition happily. Fistula centers should be built and manage by the federal government as this will give hope to the patients and their relations and help eradicate the hopelessness being faced by patients and care givers. Also, adequate advertisement should be carried out on the successfully repaired cases of fistula as this will increase the awareness and hope of concerned persons. Fistula centers should be adequately funded and equipped with both human and material resources, roads leading to all health care facilities should be properly done especially those in the rural areas to allow ease access to these facilities. As this will go a long way in reducing the incidence and increase the repair of existing VVF among patients.

Recommendations:

Based on the outcomes of this study, the researcher recommended that;

Government should pass a bill against teenage marriage. The campaign for the girl child education should be doubled and monitored to achieve effective compliance. Any divorce that has to do with VVF should be handled by the court of law and a bill should be passed to protect VVF victims from the agony of divorce coupled with their condition. While surgical repair helps victims to get on with a normal

life, it is not enough to deal with the effect the scourge has had on their psychological well-being. Therefore, patients diagnosed with VVF should be assigned a psychologist/social worker who will work on their psychological aspect of the problem. Victims of VVF need people to give them the confidence to relate their experience. With this opportunity, bottled up emotions are let out and victims would be able to gradually gain a good confidence level. Also, they should build network of people with the same problem as victims often tend to relate with one another, this form of association helps them to discuss their problems freely, thus they are able to alleviate the stigma associated with their condition. This type of network can be strengthened and empowered by the local authority to mount campaign against the incidence of VVF. Awareness campaign should be carried out to curb stigmatization among patients diagnosed with VVF. Local communities should be enlightened on the problems faced by VVF victims. Afflicted women are only victims of their socio-cultural circumstances; therefore the society should be enlightened to accept them. Hospital should be built specifically for fistula cases in all the 36 states of the federation and located in the prone areas for this disease as this will increase the hope of victims and their relations thereby reducing rejection and depression.

Limitations of the study

Research of this nature comes with a lot of limitations and challenges;

First, the data that produced the outcome of this study are generated from the medical files of the patients which limited the direct interaction with the victims. Therefore, generalization of the result should be done with caution as the result were presented qualitatively and sampled from available sample. Second, locating the VVF victims for direct interaction was an uphill task and the nature of their condition made them to decline participating in the study. Finally, time constraint, distance location of the fistula centers, bureaucratic bottle neck in assessing these patients medical records.

Suggestion for further Studies

Based on the outcome of this study, a community based study should be carried out to determine the true incidence and prevalence of the condition in the community and need for a prospective study with multivariate analysis to ascertain the level of involvement of the possible contributory factors. This will guide in making informed recommendations towards prevention and eradication of the condition. In other to cross validate the outcome of this study, future researchers should sample from other fistula centers so as to increase the sample size. They should also consider a retrospective study of VVF patients in this part of the country. Finally, a complete psychological investigation should be carried out this will help in developing counseling and psychological intervention for the identified psychosocial features.

Conclusion

From the outcomes of this study clinical features showed a remarkable percentage difference in patients diagnosed with VVF. This significance difference showed that the incidences and prevalence of VVF as a disease are anchored on these variables. The findings here largely agree with findings of earlier studies. The lack of skilled supervision and adequate obstetric emergency facilities are to blame. The medical and social consequences of the disease amount to agony and unqualified tragedy of its unfortunate victims while the disease is largely preventable (Kabir & Colleagues, 2003). The clinical conditions reported showed to be greatly significant in destroying their emotional well-being. It should be noted that the pains and suffering endured by VVF victims is the result of the social isolation and abandonment and subsequent loss of self-esteem coupled with economic deprivation that results from this social isolation (Wall & colleagues, 2004). The outcome of this study should be seen as a complement to other studies by other researchers with reference to the circumstances surrounding the incidence and prevalence of VVF and its impact on the psychological well-being of Nigerian women. It is a complement in the sense that it serves as direct report that gives the victims voices of their own, since the researchers adopted a qualitative analysis unlike the conventional quantitative reports. To this end, the researchers concluded that clinical features had influence on the incidence and prevalence of VVF in Nigeria with regard to diagnosed patients of VVF. Therefore, it is imperative that constituted authority should hearken to this study's recommendations and chart a course towards eradicating the scourge as obtained in the developed countries.

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Čerenkov Radiation: The Space-Time Paradox

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Abstract: Čerenkov radiation has become the interesting phenomenon which can be occurred in many aspects, where in this article, a new model of space-time paradox concept is proposed to describe the <conscious | subconscious> situation, where the connection between whispering gallery mode (WGM) probe and brain signal can be formed and the mind and mater interfacing information described, which is useful for possible mind and dream investigations. The uncertainty of the paradox pair is also involved and discussed.
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Introduction:

In general, the natural phenomenon is basically localized by a couple (pair), which is defined by the orthogonal state of the possible outcomes, which is an early and influential critique leveled against quantum mechanics, where only one in each pair can be seen, i.e. measured in the certain situation. In which Albert Einstein and his colleagues (Podolsky and Rosen) designed a thought experiment intended to reveal what they believed to be inadequacies of quantum mechanics, which is known as EPR paradox. To that end, they hypothesized a consequence of quantum mechanics that its supporters had not noticed but looked unreasonable at the time (Einstein et al., 1935). The routine explanation of this effect was, at that time, provided by Heisenberg's uncertainty principle. Physical quantities come in pairs which are called conjugate quantities. Examples of such conjugate pairs are position and momentum of a particle and components of spin measured around different axes. When one quantity was measured, and became determined, the conjugated quantity became indeterminate. Heisenberg explained this as a disturbance caused by measurement. The other pairs of EPRs can be categorized by the followings such as Particle | Anti-particle: **<P | A>**; Dark | Bright soliton pair: **<D | B>** (Phatharaworamet et al., 2010); Entangled photon: **<0 | 1>**; polarization: <H | V>; Conscious | Sub-conscious: **<C | SC>**; Čerenkov radiation: **<S | T>**, where few of them have been proposed.

Čerenkov radiation:

Two states of radiation can be occurred only one state in each appearance, so we use the terms of space-time paradox for the two states as **<S | T>**. Čerenkov radiation is the electromagnetic radiation, which is emitted when a charged particle (such as an electron) passes through a dielectric medium at a speed greater than the phase velocity of light in that medium. The charged particles polarize the molecules of that medium, which then turn back rapidly to their ground state, emitting radiation in the process (Georgescu, 2012). Čerenkov luminescence imaging is an emerging optical preclinical modality based on the detection of Čerenkov radiation induced by beta particles when traveling though biological tissues, for instance, human brain or body tissues with a velocity greater than the speed of light. We present the first human Čerenkography obtained by detecting Čerenkov radiation escaping the thyroid gland of a patient treated for hyperthyroidism. The Čerenkov light was detected using an electron multiplied charge coupled device and a conventional C-mount lens.

Rabi oscillation:

A two-state atom (an atom in which an electron can either be in the excited or ground state) in an electromagnetic field with frequency tuned to the excitation energy, the probability of finding the atom in the excited state is found (Fischer et al., 1998), which means that the ripple of beta particles in brain may cause the Rabi oscillation and finally the Čerenkov radiation is formed, where in this case the Rabi oscillation is forced the particle (beta particle) move(oscillate) with speed exceed the speed of light in the brain liquid.

Meditation:

This is the practical method of human noise reduction, where in this case the propagation of beta

particle in the human tissues can travel faster than light within the medium, where finally it is possible to obtain a planar image of Čerenkov beta particle escaping from a human tissue. Čerenkography is a potential novel medical tool to image superficial organs of patients treated with beta minus radiopharmaceuticals and can be extended to the imaging of beta plus emitters (Spinelli et al., 2013). In Buddhism, there were number of monks can obtain the Čerenkography after meditation, for instance, Buddha, where the Čerenkov radiation is appear as shown in Figure 1, which is known as an "Enlightenment". In the physical interpretation concept, if the model of beta particle propagate (circulate) in human brain (or body) is configured by a shiny path as shown in Figure 2, so the Čerenkov radiation can be taken place as shown in Figure 3. When the Buddha was in the Enlightenment status, the bright light around Buddha can be occurred as shown in Figure 1. In Figure 4, it presents the leaky or whispering gallery modes (WGMs) of brain signals can be generated and concentrated (Tamee et al., 2013; Rosch, 2009; Yupapin, 2013; Tamee et al., 2013). By using the proposed conceptual model of WGMs, the extrasensory perceptions such as telepathy, clairvoyance, precognition, and psycho-kinesis can be described, which can be used to make many researches and investigations.

Whispering gallery mode (WGM):

WGM of light can be easily generated by using a PANDA ring resonator (Spinelli et al., 2013; Tamee et al., 2013), where the left (R_1) and right (R_2) rings are placed as the nonlinear coupling modulation part. Figure 4 shows the simulation result of light when the leaky modes and whispering gallery mode within a PANDA ring waveguide are generated**,** the material is I***nGaAsP/InP***, where the used parameters are the radius $R_1 = R_2 = 1.0$ m and $R_3 = 2.0$ μm, $A_{eff} = 0.30$ μm^2, $n_{eff} = 3.14$, $n_2 = 1.3 \times 10^{-17}$ cm^2/W, all coupling coefficients ($_i$) are 0.5, $\gamma = 0.01$, $\lambda_0 = 1.50$ μm. The obtained result of the Gaussian pulse with center wavelength of 1.50 μm and power of 10 mW is input into the input port of the PANDA ring circuit as shown in Figure 4, where (a) leaky and whispering gallery modes, (b) WGM probe for particle trapping and transportation.

Dreaming is the situation occurred while we are sleeping, which may be in the gap between Conscious │Subconscious, which is under the Čerenkov radiation. It can be described by the two states of conscious and subconscious, <C│SC>, in which there is no time in the gap between the states <CS│C>, which is may be caused by the **Č**erenkov radiation. Apparently, there are 3 dimensions in space localized and 1 time, where time is a free dimension that can go through. During the dream, a 4D (dimensions) person would be capable of passing through walls, disappearing and reappearing at will, seeing through buildings and finding hidden objects, and performing a surgery without even cutting the skin (Hallman, a, b, c, 2012). One can only imagine what it would be like if doctors could observe cancerous growths within the body and then surgically remove them without leaving any bodily scars simply by learning to access the fourth dimension. To date, many dreamers have reported manipulating objects with some degree of control during lucid dreaming.

Figure 1: Buddha enlightenment situation (Enlightenment, Google search)

Furthermore, the aurora (Frank et al., 1986) borealis is also caused by the Čerenkov radiation, when the atmosphere is bombarded by a lot of high energy charged particles, which occurs predominantly at the earth's poles, where may also be caused by the earth's magnetic fields. Aurora borealis which is also seen in the living creatures (Kollareddya et al., 2008), for instance, when the Buddha was in the enlightenment situation, however, there is no memory during this situation, the enlightenment person cannot have self-sensory of such a status. In healthcare, the aurora status is also seen because the beta particle can have the speed exceed the speed of electromagnetic wave within the human organ medium.

Conscious │Subconscious:

It can be defined by the EPR form as **<C │SC>**, which means that it can be occurred only one event in each appearance. In human, the subconscious can work while they are sleeping, where sometime they

can obtain good outcomes better than they are in the waking situations, for instance, good works or ideas can be generated and obtained good after waking up but they have lost the connection of the paradox states, in which all details or procedures of the result methodology were lost, which is no time in the subconscious state, where the imagination can move freely in time within the localized space under the Čerenkov radiation, i.e. a space-time paradox, **<S | T>**. The proposed detection scheme can be plugged to the required paradox state by using the WGM probe, where the condition is that the measurement instrument must be in the same paradox generated by the Čerenkov radiation, otherwise, it will be disappear, i.e. the information is lost.

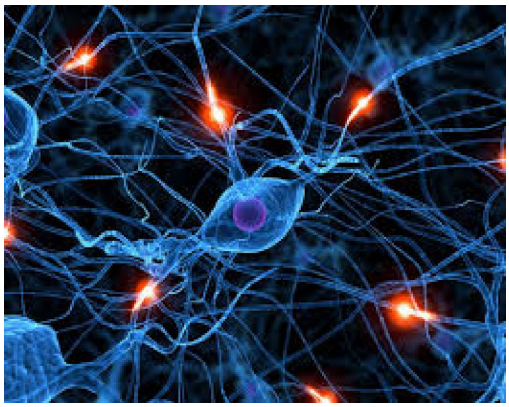


Figure 2: Brain electrical signals (beta signals) (Brain signal, Google search)

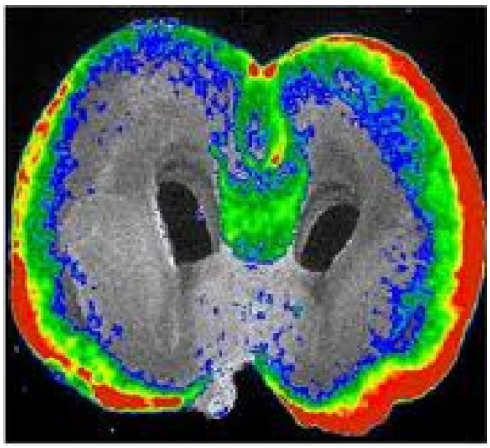
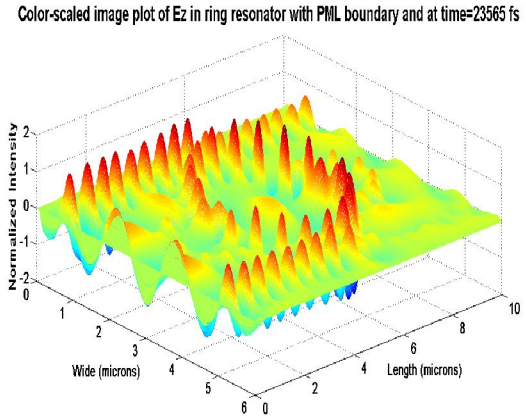
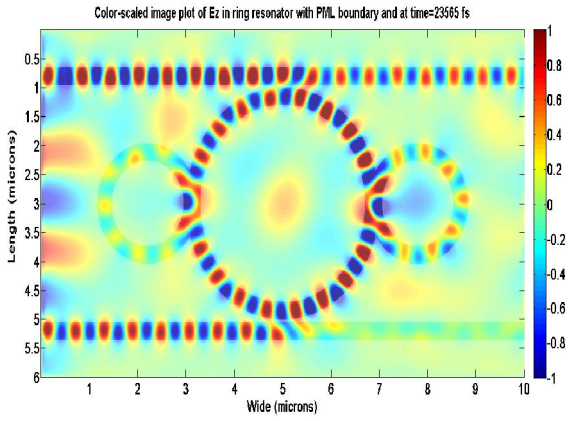


Figure 3: Brain signal propagation structure form (Brain signal, Google Search)

We have proposed that the extrasensory perception (imagination) can be occurred when we are in the sub-conscious state, where we can travel freely in time with noiseless condition, which means that there is no time involved in this situation, so there is no recorded data (memory), so we cannot perfectly remember after waking. Imagination is recognized as the most important human creative thinking generation, which was also said by Albert Einstein as "Imagination is more important than knowledge". However, the extrasensory imagination is not generally found in the common people, in fact, it is an extraordinary case, where sometime it cannot occur in the ordinary life, for instance, it can appear during the sleeping period. Imagination can be described by the paradox pair of Čerenkov radiation (**<S | T>**), which means that these two states are in the orthogonal (entangles) states, which can be randomly occurred in the realistic situation.



(a)



(b)

Figure 4: Brain signal propagation model: leaky and whispering gallery modes, where WGM probe, (b) particle travelling track

In conclusion, an element in each pair of paradox states can be investigated by using WGMs within a thin optical film device, in which the interaction between the state of interest and WGM

probe can be established and investigated, however, the introduced measurement will cause the uncertainty of the remain state due to the Heisenberg's uncertainty principle, i.e. the remaining states will be lost.

Acknowledgment:

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A Comparison of Smart and Traditional Schools in Enhancing Learning Physics: a Study of Third Grader Girls of Experimental Science in Shahryar High Schools

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Abstract: The present study attempts to compare smart schools and traditional schools in terms of improving physics learning skills among female students of third grade in experimental science in Shahryar high schools. In terms of its objective, this study is an applied one, while it is a descriptive study with causal-comparative design in terms of data collection. The statistical population consists of 142 high school third grade girls who study experimental science in smart schools (Hazrat Zeinab and Farzanegan) and traditional schools (Hazrat Masumeh and Parvin Etesami) in Shahryar in the academic year 2012-2013. The statistical sample includes 100 girls in third grade (50 studying in smart schools and 50 going to traditional schools), selected using convenience sampling and sample size formula. The data were gathered using student scores in teacher-designed physics tests for the second semester of 2011-2012 and the first semester of 2012-2013. The data were analyzed through inferential and descriptive statistics, and one-way analysis of variance and Tukey's post-hoc tests were used to compare mean physics scores for students in smart and traditional schools. Our findings suggest a significant difference between physics scores of third graders in smart high schools and those of girls going to traditional schools. The results indicate effectiveness of smart education systems in promoting learning among third grader girls in high schools of Shahryar.
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Keywords: Smart Schools, Traditional Schools, Smart Education, Learning, High School

1. Introduction

Knowledge and technology have changed the form of life on the planet in a way that it is now almost impossible to think of life without technology in the twenty-first century, an era which requires computer skills and knowledge for most jobs. This calls for a new form of education which cannot be implemented through the traditional means of education, particularly those currently found in Iranian schools (Mortazavi & Movaqar, 2011).

Furthermore, current limitations on electronic systems used in basic educations dictate application of suitable technologies based on the existing infrastructure and current conditions. Significant advancements in communication and information technologies and their vast applications have led many organizations and governments to large investments in such technologies. As such, effective management will depend on effective investment in communications and information technologies.

Smart schools adopt a new approach to education which combines IT and curricula in order to bring substantial changes into teaching and learning processes. In this approach, teacher acts as a guide rather than someone who merely communicates knowledge while students act as creative actors who actively engage in learning instead of passively consuming this knowledge, and appraisal systems are process-oriented instead of result-oriented ones (Garison & Anderson, 2000).

The ultimate objective of establishing smart schools is to equip students with computer skills and literacy required to meet the requirements of the twenty-first century. If the goals of e-learning are defined as facilitating learning process, focusing on individual potentials, preparing the stage for learner growth, creating time- and place-independent means of learning, developing life-lasting education, improving education quality, and finally using all effective means of learning, then education agency will be the best target for realizing these goals particularly in Iran with its large student population, suitable infrastructures for growth and development, and young population (Alirezayi, 2008). Smart schools are among the most important achievements of IT developments in educational programs with advantages and effects that will go beyond the education environment to provide students with new experiences and access to unlimited information (Taleqani, 2010).

Our young society needs smart schools since the growth of computer technologies has provided everyone with easy and quick access to vast amounts of information which were often unavailable in past schools where teachers passed information, knowledge,

skills, and values of their choice to students. Today, economic and socio-cultural frameworks together with mass communication tools play an important role in shaping student perceptions (Moayednia, 2005).

This enhances standard level of student knowledge and with training courses left behind, IT and informatics used in smart schools will be quite helpful in updating teachers' knowledge and enhancing their teaching skills by making use of the existing resources, gaining a more accurate understanding of students knowledge, and developing courses and their contents based on this level of knowledge (ibid).

On the other hand, the information society of the future will require people who can creatively use IT to promote growth and development. In this era, lack of up-to-date knowledge, understanding, and skills will result in unemployment and social inequality, thereby leading to tension and dissatisfaction. Smart schools are usually designed based on these requirements as students at these schools can learn how to extract the information they need from information networks, how to interpret them, and how to apply their findings to solve their problems and to help the development of their society (Alirezayi, 2008).

Many problems faced in IT development in Iran, including lack of cultural packages, insufficient skilled human resources, unfamiliarity with foreign languages, low levels of research motivations and morale, lack of practicality, and in one word, lack of life skills, stem from inefficient traditional education system which cannot meet the requirements of an ever changing society (ibid).

Iranian education system is still largely based on books, and students are required to memorize the contents of books in order to pass their courses. The teaching approach is still a teacher-oriented one, leaving students with only a minor role to play in teaching and learning processes. Therefore, greater emphasis must be made on education and on reinforcing stable and lasting infrastructures for e-learning. Smart schools can create significant changes in the education system. Research Hypothesis: There is a significant difference between smart and traditional schools in terms of improvement in learning physics.

3. Methods

In terms of its objectives, the present study is an applied one that seeks development of knowledge applicable to education. The study is a descriptive one with causal-comparative design in terms of the data collection method as the researchers used the dependent variable (improved learning capabilities for physics) to examine the dependent variable (creating smart schools).

The statistical population consists of third grader girls studying experimental science in two smart schools, namely Hazrat Zeinab (49 students in two classes) and Farzanegan (23 students in one class) and two traditional schools, namely Hazrat Masumeh (36 students in one class) and Parvin Etesami (34 students in one class) in Shahryar in the academic year 2012-2013. In total, 142 students -72 students in smart schools and 70 students in traditional schools - were studied (Shahryar Education Department, 2012).

Convenience sampling was used to create a sample of 100 students in third grade of experimental science in Shahryar (50 students from smart schools and 50 students from traditional schools). The following formula was employed to determine the sample size:

This formula is used for one-way analysis of variance or in studies where the measurement scale is an interval scale (Human, 2005).

To calculate sample size using the formula above, one needs the variance σ^2. For this purpose, we performed an initial evaluation of physics scores of 20 students from smart and traditional schools in order to obtain an initial estimation of the variance and select the final sample. The table below shows the results of this stage.

Standard deviation	5.4
Standard error of the mean	1.148936
Alpha level	1.96
D-value	2.251915
Sample size	49.7448

By substituting the values indicated in the table above into the sample size formula, we get a sample size of 50. We used simple random sampling to select individuals by drawing lots.

4. Results

The idea of using computers and the internet at schools and academic communities dates back to early 1960s and twentieth century. England was among the pioneers in establishing smart schools and using IT as a part of its nationwide education plan. The country rapidly implemented research and practices for reforming training methods and educational approaches, course subjects, and curricula, and adopting more effective learning approaches.

England quickly understood the requirements for ITC and its application to innovations in education and promoting effective methods through establishment of smart schools. The country claims that these schools are helpful in achieving education goals in an effective and stable manner.

Perhaps, other factors such as quick and easy access to implementation tools and systematized schools play a role too, but it should be noted that movement toward smart schools is inevitable and

therefore effective mechanisms must be developed to supervise and assess establishment of smart schools in order to ensure a balance between powers and responsibilities of smart school staff and to move toward a more dynamic and novel approach to educational development (Saeid Zand Vakili, 2011).

Smart schools represent a development in school architecture (*i.e.* structure, culture, role, *etc.*) and moving toward organizational learning and promotion (creating a learning organization) which contribute to changes in learning-teaching approaches by promoting creativity, research, and critical thinking in a knowledge-based nationwide education system, and to transition from memory-based approaches to research-based ones and from teacher-based methods to student-based one for creating a dynamic and attractive environment which helps actualization of potentials and collective and individual creativity by facilitating development of knowledge and technology across the society and the education system.

Furthermore, new technologies (*e.g.* ITC) provide opportunities for improving education quality, access to learning and teaching opportunities, and enhancing scientific capabilities and skills among teachers, parents, and members of society (Mashayekh, 2000).

Using their flexible curricula, novel teaching methods, and a broad range of programs and learning methods, smart schools focus on the role of students and their individual differences, needs, interests, and capabilities to effectively bridge the gap in the education system.

The information society of future requires people who can creatively use IT for growth and development. In this era, lack of up-to-date knowledge, understanding, and skills will result in unemployment and social inequality, thereby leading to tension and dissatisfaction, and therefore, human societies of today need human development more than ever before (ibid).

Samadi and Nemati (2010) argue that smart schools are often designed to meet planning requirements since in these schools, students learn how to obtain the information they need from information networks, how to reflect on this information, and how to apply them to solving problems and moving toward development and progress.

Arefi (2010) notes that a smart school is a fundamental step toward correct and effective training for making pre-planned use of knowledge and technology by learning meaningful techniques, thinking, and setting goals for life in order to assist students to draw on their real social capital to successfully prepare for a global competition by the help of electronic systems, smart supervision, and intelligent appraisal system which improve learning efficiency in students

5. Discussion

Theoretical definitions variables

Smart school is a school where all processes including administration, management, control, teaching, learning, development of educational materials, appraisal, office documents and supplies, and communications and their developments are based on IT in attempt to promote research-based education system. In other words, smart school is a school which, in addition to physical facilities and programs used by other schools, applies computer equipment and related technologies to control and manage the school; the content of most courses are in electronic form and smart systems are used to assess and supervise the system. In such schools, the system is installed on a central computer which is connected to many phone lines to provide 24/7 service. The system enables students, parents, teachers, and school employees to interact on a continuous and dynamic basis (IT and Statistics Center, 2011).

Learning is a process of relatively stable and experience-based changes in potential behavior of individuals (Hilgard & Marquis, 1961).

Learning enhancement consists of general or special knowledge or skills acquired for learning course subjects, often measured through tests or indicators, or both, set by students (Shoarinejad, 1985).

High School Education System in Iran

The existing education system in Iran is a relatively new social and educational phenomenon which is over one decade old. The Iranian Ministry of Education's proposal for new high school system was approved by High Council of Cultural Revolution in 1990 and the plan was launched on experimental basis in different regions of the country in September 1991. The timing and stages for implementation of this new system were defined in the General Education Framework:

(1) In 1992, the new system was tested for the first grade in all majors.
(2) In 1993, the new system was launched for first and second grades in all majors.
(3) In 1994, the new system was tested for first, second, and third grades in all majors.
(4) In 1995 to 1997, the system was gradually developed into a uniform system for all high schools in the country.
(5) In 1998, a program was launched to implement pre-university, associate degree program, and public education in a broader scope, and finally in the academic year 1999-2000, most high school students were covered by the new program (Vista e-Zine, 2011).

In this section, we discuss the reasons put forth by the High Council of Cultural Revolution for implementing the new education system for high schools:

(a) To provide sufficient resources to improve quality of high school education (theoretical, technical, and vocational) and to develop the system based on economic, social, and cultural needs of the country, geographical features, course materials, and gender and age-based needs of students;
(b) To create flexibility in high school orientation for useful employment or continuing education, and to determine majors based on the country's requirements and individuals' interest and competence while taking into account the existing conditions and scientific and technical advancements;
(c) To improve quantity and quality of technical and vocational training; and
(d) To set the stage for optimal use of available resources in high school education and organizing out-of-school training and promote education by making use of resources available to authorities. High school education system in Iran is divided into three branches: theoretical, technical and vocational, and theoretical-practical training (ibid).

The major goals of each branch, which indicate employment or education orientation, can be summarized as follows:

Theoretical branch: the courses in this branch are intended to promote culture, general knowledge, moral virtues, political and social vision, and better understanding of students' potentials and interests in continuing education.

Technical and vocational training: this branch seeks the same goals as the theoretical branch in addition to directing students toward useful employment and preparation for continuing education in scientific or applied (technological) majors.

Practical – theoretical training: in addition to the goals of theoretical and vocational training, this branch seeks to educate human resources at semi-skilled, skilled, and professional levels for industries, agriculture, service sector, and preparing students for continuing education in applied and scientific majors (Vista e-Zine, 2012).

One major goal and function of education system is to prepare students to grasp and understand scientific changes of the future world. However, the most important goal of this system is to fully actualize student personalities and values. Social and emotional development of students is closely linked to cognitive aspects and even to educational progress. According to Bloom, any student completes learning a subject with a set of its affective features. Therefore, educational planning and practical programs require close attention to special quality and results of each method (Kadivar, 2003).

What is a smart school?

Broad applications of information and communication technology (ICT) into education together with recent development in global educational approaches have led to formation of smart schools which are among the key requirements of today's knowledge-based societies and seek to develop entrepreneurship and knowledge-based skills among students (Ministry of Education, Tehran Department of Education, 2011).

At these schools, teaching-learning process is promoted and an interactive environment is developed to enhance students' key skills by focusing on group activities in a knowledge-based era. The current teacher-based conditions in the education system of the country call for updating school systems, using state-of-the-art technologies, and applying innovative approaches to education while focusing on students capabilities. The term "smart school" has been recently introduced into education literature in Iran and several valuable, and yet scattered, works have been carried out in this area. Inspired by the religious teachings and current requirements, the Ministry of Education has drawn on international standards and local requirements to define a meaningful structure for developing smart schools in order to realize the goals defined in 2025 Vision Plan as well as the goals set by the education system in terms of promoting educational equality. Iranian Fifth Development Plan requires the Government to realize educational equality and facilitate the existing processes by making use of ITC in training courses and in educational processes (ibid).

According to the existing documents, a stand-alone program was developed for IT sector of development plans in 1972 with IRR 470 million fund as well as in the Second Development Plan which was not implemented to due Islamic Revolution. In post-Revolution period, the First Development Plan did not emphasize the importance of IT. Although a High Council of Informatics was established and found legal status after the Revolution, however, the Second Development Plan addressed the goals and policies for IT development and its applicable programs only in a limited manner (Ebadi, 2003).

In the Third Socioeconomic Development Plan greater importance is attached to IT which then found a more proper position in the proposed plan that focused on the requirements for new attitudes, application of IT to all areas, reviewing previous performance, identifying the current conditions, problems and issues, structural strengths and weaknesses, and analysis of the existing IT performance (ibid).

An important aspect of the Educational Development Document is greater attention to ITC as a facilitating factor. EDD regards ITC as an important factor which catalyzes optimal education and can serve as a starting point for movement toward a modern education system that underlines research and innovation (Planning Council, Ministry of Education, 2007).

EDD refers to recent civilization changes and new professional requirements which call for use of ITC and fundamental modifications in the educational materials and methods of managing schools. In addition, stakeholders will be given a new role and students will have a more prominent role in their own learning as they must be allowed to choose their growth path based on their interests and to develop their creativity, capabilities, and abilities to create and manage information (ibid).

Smart Schools: a World History

Although used in different ways and to refer to different concepts, the term "smart school" found its first broad application in 1997 in Malaysia as a tool for assisting students to prepare for the new information age and to make fundamental changes in Malaysian schools (Attaran, 2011).

The first smart school was established in England in 1996, followed by a similar motion by Malaysia that defined a basic project for establishment of smart schools within its development plan. In 1998, Malaysia became the first country to launch a smart school within its education system which set a successful model for other countries like France that successfully followed Malaysia.

The advent of data processing systems and their development over three decades paved the way for computers to enter many social and individual areas, and in 1990s many countries equipped their schools, even the elementary ones, with computer tools. The advent and development of computer led to establishment of computer networks and the emergence of the internet (Tehran Department of Education, Ministry of Education, 2011).

The idea of using the internet and computers in schools and academic environments dates back to the twentieth century and early 1960s. The internet emerged in 1969 and grew rapidly beyond anyone's imagination to serve 180 million users in 2000. The fast growth continued to cover about 500 million users in 2003. This rapid development in IT together with other factors such as transition from industrial society to information society, demographic changes, globalization of professional activities, growth of labor market in the education area, or in other words commercialization of education all played a significant role in education.

Smart schools in Iran

Over the past few decades, the scope of education and learning has grown just like academic, cultural, economic, and social activities inspired by rapid developments in technology and emergence of satellites, computers, and the internet.

Application of technology to Iranian education system dates back to the use of audiovisual education aids including slides and films at schools. Later, TV received attentions from education society and Iranian National TV formally launched programs for public nationwide education.

Following the introduction of computer industry to Iran and penetration of PCs among different social and cultural communities, computer-based education started to grow by introduction of training CDs in about ten years ago. In general, it was not until 2001 that computer-based training was taken seriously and operational activities began in internet-based education and using communication bandwidths to offer training courses around the country. In 2004, a smart school project was launched for the first time for development of ITC in education system (Tehran Department of Education, Ministry of Education, 2011).

The draft of Smart School Strategic Plan defines smart school as an educational organization with a physical, as opposed to virtual, environment where students can learn using novel education tools. In a smart school, control and management is based on computer and network technology, contents and materials are often in electronic format, and a smart system is used for appraisal (Tehran Department of Education, 2005).

In the academic year 2004-2005, Tehran Department of Education was assigned implementation of a pilot smart school plan in 4 high schools. According to official statistics, in September 2011, over 120,000 schools were running in the country and about half this number were operating as educational complexes (Tehran Department of Education, Ministry of Education, 2011).

Characteristics of Smart Schools

In smart schools, blackboards are replaced with computers and notebooks are replaced with CDs. Students can obtain large amount of information on any subject from the internet. In such a system, student and teacher both contribute to the electronic materials and present the courses in CD formats. In smart schools, teaching is not limited to teachers; rather, teaching and learning occur in an interactive way and students play a fundamental role in learning practical subjects (Sadeqimoqaddam, 2008).

Teachers in a smart school use electronic materials to improve understandings and save time and

students are given opportunities to exhibit their capabilities and create contents. Instead of trying to find answers to students' questions, teachers in smart schools ask students for finding the answers using computers and then communicating these answers to other students.

Smart schools are "student-oriented" schools where teachers merely direct and leave the choice of materials to students. Parents can communicate online with school teachers and principal and get information on the progress of their children. Libraries of smart schools are electronic libraries that can be accesses online by students. Online discussions and threads are also accessible online (ibid).

One can summarize the characteristics of smart schools as follows:

(1) Creativity
(2) Novel learning methods
(3) Focus on understanding
(4) Exchange of information
(5) Centralization of learnt materials (Mehri, 2012)

In a smart school, learning stems from thinking and a good idea can be learnt by all students. Schools are places which help students grow and smart schools provide this opportunity for administrators and teachers as well.

Structure of Smart Schools

Establishment of smart schools requires a long-term plan. For example, a smart school established today may need more than 10 years to complete changes in terms of communication infrastructures, suitable materials, teacher training, training methods, and parent culture. These schools differ from the traditional ones both in physical form (*e.g.* arrangement of seats and classrooms) and architecture. Classroom spaces should be designed in a way that provides students with a laboratory next to their desks. On the other hand, teachers of smart schools act as guides who should engage students in learning relevant materials. This training method helps students understand materials both theoretically and practically and teach the students ways to access information when they encounter a problem in future (Sadeqimoqaddam, 2011).

Structurally, smart schools consist of four general sections:

Education: systems for managing and developing education materials

Administration: administrative and financial automated systems as well as systems for registration, teaching fees, payments to staff, *etc*.

Control: physical security and attendance check for human resources

Community: participation of stakeholders, parents-school relations, and web-based procedures

Advantages of Smart Schools

(1) Giving back teachers their position as academic references and education guides
(2) Using computers as teaching aid and for better communication of materials
(3) Institutionalizing administrative automation in all activities
(4) Decentralizing local decision making processes
(5) Resolving problems related to lack of administrative staff
(6) Basing decisions on fundamental thinking
(7) Assisting families in using computers
(8) Increasing productivity in application of technology into educational and other forms of activities (ibid)

Disadvantages of Smart Schools

(1) Digital information gap between teachers and students
(2) Lack of skills among colleagues
(3) Lack of professional users in schools
(4) Absence of proper technology models in schools
(5) Poor decision making due to lack of knowledge on IT
(6) Insufficient cultural infrastructures to develop technologies for teachers and other persons
(7) Adverse effects of websites on moral and religious identities
(8) Lack of necessary skills for technology management and using information systems
(9) Inconsistencies between smart schools and traditional schools

A smart school is not simply a tool or hardware; rather, it is a progressive idea for facilitating learning and teaching processes (ibid)

Smart text messages

Smart Schools vs. Traditional Schools

In traditional schools there are several limitations, particularly in terms of access to information since students only use textbooks and have access to limited knowledge of teachers. There are also limitations on physical environment and educational resources which lead to other forms of limitations, while many of such problems are resolved in smart schools through various networks that provide access to information (Tehran Department of Education, 2007).

It should be noted that smart schools differ from traditional ones in many ways. In smart schools, course materials are often developed based on students' capabilities and therefore, there may be, for example, 40 different training approaches for a class with 40 students in order to provide more capable students with more opportunities to use the materials and to help the less capable ones catch up with others (Jalali, 2009).

Automated education system and voting and assessment of each student's condition are among other major differences between the traditional and smart schools. Such systems may be used to promote parent roles in guiding students. Thus, when we speak of a smart school we mean, in fact, a school where there is a different system for each student to teach students how to gain access to global information without needing help from others (Zabeti, 2009).

Here we summarize the differences between smart schools and traditional ones. In traditional schools

(1) Students learn and teachers educate;
(2) Curricula are limited (to textbooks and prescribed notes);
(3) Teaching method is teacher-based (knowledge and information);
(4) Data are exchanged slowly;
(5) Chalks, blackboards, and scores are used as training aids (overworked materials);
(6) Research organization develops course contents;
(7) Classrooms are not active (students are passive);
(8) Creativity and theorizing are limited;
(9) Supervision and assessment of education and trainings are relative;
(10) Parents have limited engagement;
(11) The focus is largely on information and knowledge;
(12) The whole class constitutes one single group;
(13) Teaching is based on notebooks, assignments, textbooks, and so on; and
(14) Verbal approaches dominate the teaching process (Smart Schools Secretariat, 2011)

Descriptive and Inferential Findings

An explanation of hypothesis is presented in this section, followed by the results of the statistical tests presented in SPSS tables as well as discussion on the findings.

H_1: Smart school and traditional schools differ in terms of promoting learning physics.

H_0: Smart school and traditional schools do not differ in terms of promoting learning physics.

Table 1: Mean and standard deviation for Physics II and III scores in smart and traditional schools

Type of school	Course	Mean score	Standard deviation	N
Smart school	Physics II	12.9000	3.77593	50
	Physics III	11.9850	4.14175	50
	Average	12.4425	3.96972	100
Traditional school	Physics II	17.0600	1.79821	50
	Physics III	17.8000	1.75109	50
	Average	17.4300	1.80455	100

As seen in the table above, average scores for Physics II and III in traditional schools are 12.90 and 11.985, respectively, while the respective values for smart schools are 17.06 and 17.80, with an average of 12.4425 and 17.43 for two subsequent semesters for Physics II and III in traditional and smart schools, respectively. This obviously indicates a 5-point difference in favor of smart schools. One-way analysis of variance was used to determine whether these differences are statistically significant or not. The results are presented in Table II.

Table 2: Sources of variance, mean of squares, degree of freedom, and statistical significant level

Source of variance	Sum of squares	Degree of freedom	Mean of squares	F	Significance level (α)
Intergroup	1,278.378	3	426.126		
Intra-group	1,847.871	196	9.428	45.198	0.001
Total variance	3,126.250	199			

Given the value of F (45.198) in the table above, the difference is significant at 0.001 with the degrees of freedom 3 and 196. More specifically, the difference between smart and traditional schools is significant at 0.001, indicating the prominent role of smart schools in enhancing learning physics. The following table presents the differences between the two types of schools in terms of physics scores. This table complements the previous table. In other words, the table above only shows the differences in average physics scores while the following table which is based on Tukey's test indicates differences in Physics II/Physics III as well as the differences in individual semester scores. Table IV contains more details.

Table 3: Tukey's post-hoc test

Type of school	Course i	Course j	Mean difference (i-j)	Significance level (α)
Traditional	Physics II	Physics III	0.9150	0.445
Smart		Physics II	4.1600	0.001
Smart		Physics III	4.9000	0.001

As seen in this table, there is no significant difference between mean scores of Physics II and Physics III in traditional schools (α=0.445) while average Physics II score of traditional schools is significantly different from average Physics II and III scores in smart schools (α=0.001) by more than 4 points.

Table 4: Tukey's post-hoc test

Type of school	Course i	Course j	Mean difference (i-j)	Significance level (α)
Traditional	Physics III	Physics II	0.9150	0.445
Smart		Physics II	5.0750	0.001
Smart		Physics III	5.8150	0.001

In addition, average Physics III score of traditional schools is significantly different from average Physics II and III scores in smart schools (α=0.001) by more than 5 points.

Table 5: Tukey's post-hoc test

Type of school	Course i	Course j	Mean difference (i-j)	Significance level (α)
Smart	Physics II	Physics III	0.7400	0.624

On the other hand, there is no significant difference between Physics II and Physics III scores in smart schools (α=0.624).

Recommendations

Based on the findings of this experimental study and the theoretical bases, education authorities are recommended to implement smart schools in order to benefit from its positive effects, and to prepare comprehensive and professional plans based on an appraisal of needs and requirements, preparation of infrastructures, and using experiences and models employed in other countries, particularly Islamic countries, to facilitate the movement toward a smart education system.

Directions for Future Research

(1) Currently, there are some smart schools in the country but these schools employ primitive tools which are incomplete compared to successful implementations in other countries like England and Malaysia that are far ahead of our country in terms of smart school implementation. Iran effectively implemented smart schools only on paper and therefore, comparative studies are needed to incorporate experiences of other countries and available research into making reasonable decisions.

(2) Future research may investigate necessary trainings in terms of skill, attitude, and cognitive requirements in order to prepare staff for working at smart schools.

(3) Research should also examine the types of educational environments and infrastructures required for smart education.

(4) The scope of the present study is limited to Shahryar and its particular economic and social conditions. The study can be replicated to other cities and provinces.

(5) We studied high school third graders who study experimental science. Other studies may cover other grades and majors.

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Morphological and Histological Changes in the Camel Testes In Relation To Semen Characteristics During Breeding and Non-Breeding Seasons

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Abstract: A total number of 65 clinically healthy male dromedary camels were used in the present study. The age of these camels ranged from 5 to 10 years and their weights were approximately 500 kg an average. The experimental work aimed to investigate the effect of breeding and non-breeding seasons either hot-humid or hot-dry months on testicular measurements, epididymal semen characteristics and histological changes in the right and left testes of the dromedary camels. The obtained results showed that, the testis weight, testicular volume, scrotal circumference, testis tone firmer score and the percentage of sperm motility, sperm-cell concentration of the dromedary camels were significantly (P<0.05) higher during breeding than non-breeding season either hot-dry or hot-humid months, while the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa were significantly (P <0.05) higher during hot-humid months as compared to both hot-dry months and breeding season. Semen colour was creamish white, milky white and watery white during breeding season, hot-dry and hot-humid months, respectively. Semen consistency was viscous during breeding season and hot-dry months and semi-viscous during hot-humid months. Seminal pH value was significantly (P <0.05) higher during hot-humid months as compared to both breeding season and hot-dry months. The testis of the male dromedary camels during breeding season showed consisted of numerous semineferous tubules (ST) with different shapes and size (oval, ovaid and circular) were highly active. The ST lined by spermatogenic cells at different maturation stages (spermatogonium, spermatocytes, spermatid and spermatozoa) are present as compared to camels during non-breeding season either hot-dry or hot-humid months.

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1. Introduction:

The camel is a domesticated animal whose full agricultural reproductive potential has not yet been achieved. It is fully adapted to the rigours of the extreme diurnal variations of temperature of the arid zones of Africa and Asia and therefore requires little expenditure in terms of housing or shelter. The *dromedarius* camels are regarded as seasonal breeders (Wilson, 1984). The impression gained is that decreasing day length is the stimulus to seasonally, but it is obvious that, in dromedary camels near the equator factors such as rainfall, nutrition and management (Wilson, 1984), may override the effect of photoperiod (Merkt *et al.,* 1990) and allow breeding to occur throughout the year (Arthur *et al.,*1982).

The breeding season of camels varies geographically, since the environmental factors affect temporally the pattern of reproduction in this species (Gombe and Okelo, 1977). Daylight ratio and temperature are the two main climatic factors influencing the seasonal physiological and biochemical changes which, in turn affect the sexual behavior. However, numerous investigations have shown that the most efficient climatic factors are the variation in the daylight ratio (Hafez, 2000), although the length of daylight seems to be the primary stimulus for seasonally in reproduction.

Testicular sperm achieve fertilizing ability through the epididymis (Bedford, 1975). Although the process of sperm maturation is not clearly understood, it is believed that the epididymis provides a specific milieu (Ariyaratna *et al.,* 1996), this milieu results from interepithelial ion exchange and secretory and absorptive activities of the epithelium and is regulated by androgenic hormones (Brooks and Higgins, 1980).

The present study aimed to investigate the effect of breeding and non-breeding seasons either hot-humid or hot-dry months on testicular measurements, epididymal semen characteristics and histological changes in the right and left testes of the male dromedary camels.

2. Materials and methods

The present study was conducted in the Department of Animal Production, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The experimental work was carried out in Private Camel

Farm and Abattoris in Belbies City, Sharkiya Governorate, located in the north eastern part of the Nile Delta (30°N).

The present study aimed to investigate the effect of breeding season (December to May) and non-breeding season either hot-humid (June to August) or hot-dry (September to November) months on testicular measurements (testis weight, testicular volume, scrotal circumferences and testis tone firmer score), epididymal semen characteristics (semen colour , semen consistency, hydrogen-ion concentration (pH), percentages of sperm motility, dead spermatozoa, sperm abnormalities, acrosomal damage of spermatozoa and sperm cell-concentration) and histological changes in the right and left testes of the dromedary camels. A total number of 65 clinically healthy male dromedary camels were used in this study. The age of these camels ranged from 5 to 10 years and their weights were approximately 500 kg an average.

Testicular measurements:

Testes were weighed to the nearest gram by an ordinary balance after slaughtering. Testicular volume was determined as the method described by Weibel (1989) using the following formulae:

$$\text{Testicular volume} = \frac{\pi \times L \times B \times T}{6}$$

Where: $\pi = 3.14$

L = Length of the longitudinal axis of the testis,

B = Breadth of the testis,

T = Thickness of the testis.

Scrotal circumference was measured with a flexible cloth measuring tape around the largest diameter of the testis and scrotum placed after pushing the testes firmly into the scrotum (Mickelson *et al.,* 1982).

Testes tone firmer (score) was determined via manual palpation (scored from 1: very soft and 9: very firm) as described by Wildeus and Hammond (1993).

Camel semen collection:

Epididymal semen was collected from the dromedary camels after slaughtering. A total number of 65 clinically normal testes were collected during breeding (n=23) and non-breeding seasons (n=42) either hot-humid (n=22) or hot-dry (n=20) months.

Transportation of the samples:

The genitalia (epididymes attached to the testes) were removed from the carcass and transferred to the laboratory for semen analysis in a thermos flask containing sterile physiological saline (0.9% NaCl) supplemented with 100 µg/ml streptomycin at 25°C according to Goto *et al.* (1989) within 2-3 hours post-slaughtering.

Sperm recovery:

The epididymis (n=65) was thoroughly cleaned and the superficial blood vessels of the cauda were punctured, so that most of the blood could be wiped off. Epididymal semen was collected directly after slaughtering from the body of epididymis (corpus of epididymis). Each corpus epididymis region was cut to allow the escape of its contents in buffer citrate solution (0.5 ml NaCl) for the determination of semen characteristics immediately after collection (Kaabi *et al.*, 2003).

Semen colour of the dromedary camels was determined directly from the collecting tube. Semen consistency of the dromedary camels was qualified as viscous when semen did not drop from a Pasteur pipette, semi-viscous when semen dropped from the Pasteur pipette to glass slide and liquid when semen was fluid and dropped readily from the Pasteur pipette according to Bravo *et al.* (1997).

Hydrogen-ion concentration (pH):

The pH value of the semen of the male dromedary camels was measured by using universal indicator paper and standard commercial stains according to Karras (1952).

Percentage of sperm motility (%):

Generally, camel sperm motility (%) detected as an oscillatory motion the flagellum, but not progressive due to the viscous materials according to Campbell *et al.* (1956). Sperm motility was estimated by adding one drop of the diluted fresh semen with physiological saline (0.9% sodium chloride) on the dry, clean and pre-warmed (37°C) glass slide.

Sperm motility was estimated by observing the approximate percentage of spermatozoa moving forward motion across the field of vision with a normal vigorous swimming motion according to Plasson (1975).

Percentages of dead spermatozoa and sperm abnormalities were recorded according to Salisbury *et al.* (1978). The percentage of acrosomal damage was done according to Watson (1975).

Sperm-cell concentration ($\times 10^6$/ml):

The spermatozoa were counted using haemocytometer according to Khan (1971).

Histological changes of the camel testes:

For histopathological studies, the testes (right and left) were taken and put in neutral formal saline (10%) to be preserved, then it passes in ordinary histological set (puttig small pieces of the fresh tissues in the proper fixative as 10% formaline saline). Then after, fixed tissues were washed in running tap water to remove fixative from them and then the water was removed by treatment with ethyl alcohol (70, 80, 90 and 100%). These ascending grades of alcohol prevent shrinkage of tissues and they remove the water completely from the fixed tissues. Then the tissues were treated with clearing agents as xylol to remove alcohol and to allow the fixed tissues to be miscible with paraffin wax which will be used in the next step.

Then the tissues are put in melting soft paraffin wax at 60°C. The paraffin will penetrate in between the cells of the tissue. This process of paraffin wax infiltration is a necessary step to harden the tissues before their embedding. The tissues were then embedded in the center of melted paraffin. The paraffin wax was then allowed to be cooled-down in order to form a block of hard paraffin with tissues in its center. The block of hard paraffin with tissues in its center was cut into thin sections by means of rotatory microtome. The thin paraffin sections (4-6 micrometer) were placed on clean glass slides smeared with albumin and glycerin mixture (1:1) to flow beneath the sections and then slides were warmed on hot plate. Thereafter, the sections were preserved for several hours in the incubator to dry. The sections were stained by Haematoxylin and Eosin (H&E) stains according to Culling (1975). After staining, the slides were examined by binuclear microscope and photographed by magnification x 10 & 40.

Data were statistically analyzed by one way ANOVA, using General Linear Model (GLM) procedure of SAS (Goodnight *et al.,* 1986) and Duncan's New Multiple Range test (Duncan, 1955) was used to detect significant differences among means.

3. Results and Discussion

Testicular measurements

Data presented in Table (1) showed that, the testis weight, testicular volume, scrotal circumference and testes tone firmer score of the dromedary camels were significantly (P <0.05) higher during breeding than non-breeding season either hot-dry or hot-humid months. The testis weight, testicular volume and scrotal circumference during non-breeding season in hot-humid months were significantly (P<0.05) lower than hot-dry months. The highest (P<0.05) values of the testis weight, testicular volume and scrotal circumference were recorded during breeding season, while the lowest (P<0.05) values were recorded during hot-humid months. Similar trend was reported by Volcani (1954) who found that the testis size was bigger during rutting season in winter than hot summer months (non-breeding season). Similarly, Ahmadi (2001) and Zeidan *et al.* (2001) found that the testis weight was significantly higher during winter and spring than summer and autumn seasons. The variation of the testis weight in between the active and inactive stages amounts to about 30% (average of 96.0 and 66.0 gm, respectively). El-Sherief (1997) and Zeidan and Abbas (2004) also found that the testis increased in winter (breeding season) and decreased in summer (non-breeding season). These findings may be due to the increase in the amount of interstitial tissues and spermatogenesis and the growth of the soft palate that takes place during the rutting season (Charnot and Racadot, 1963 and Charnot, 1964). In addition, the reduction in testis weight during summer may be due to exposure to heat stress which due to degeneration in the germinal epithelium and to a partial atrophy in the seminiferous tubules (Chou *et al.,* 1974). However, Ismail (1979) found that testis weight of the camel in Egypt was highest in the summer (71.3gm) and lowest in winter months (56.0gm).

In respect to testicular volume, Charnot (1965) found that the size of the testis was greatly increased due to increased in the development of interstitial tissue during rut season. Zeidan and Abbas (2004) showed also that testicular volume was significantly higher during the rutting as compared with the non-breeding season in the dromedary camels. The increased of testicular volume during winter and spring may be attributed to the increase spermatogonia, spermatocytes, spermatids and spermatozoa. In addition, the interstitial tissue increased markedly with evidence of oedema (Hemeida *et al.,* 1985). The testes dimensions increased during breeding season reflecting higher spermatogenesis is activity affected by increasing testosterone concentration and development of interstitial tissues.

In scrotal circumference, Zeidan and Abbas (2004) showed that scrotal circumference was significantly higher during the rutting as compared with the non-breeding season in the dromedary camels. These results may be attributed to the high environmental temperature during late summer causing a more pendulous arrangement of the scrotum with reduced scrotal wrinkling (Zeidan *et al.*, 2001).

The testis tone firmer score during non-breeding season in hot-humid months was insignificantly lower than hot-dry months. The highest (P<0.05) value of the testis tone firmer score was recorded during breeding season, while the lowest (P<0.05) value was recorded during non-breeding season in hot-humid months. These results are in agreement with those reported by Ahmadi (2001) and Zeidan *et al.* (2001) who found that the testes tone firmer was significantly higher during winter and spring than summer and autumn seasons. Similar trend was reported by Zeidan and Abbas (2004) showed that testis tone firmer score was significantly higher during the rutting as compared with the non-breeding season in the dromedary camels.

Generally, photoperiod seems to play a major role in regulating the seasonal activity (rutting season) of the camel testes which are regarded as short day breeders. In which change from long to short day seems to stimulate synthesis and release of gonadotropins hormones from the anterior pituitary gland, which in turn stimulate testicular activity and sexual behaviour (Lincoln *et al.,* 1977).

Table 1. Effects of breeding and non-breeding seasons either hot-humid or hot-dry months on testicular measurements in the dromedary camels (Means ± SE).

Items	Season		
	Breeding	Non-breeding	
		Hot-humid months	Hot-dry months
Testis weight (gm)	128.61 ± 2.06[a]	102.27 ± 2.11[c]	114.15 ± 2.21[b]
Testicular volume (cm^3)	116.30 ±1.79[a]	82.18 ± 1.83[c]	101.75 ± 1.92[b]
Scrotal circumference (cm)	26.83 ± 0.95[a]	14.23 ± 0.96[c]	20.15 ± 1.02[b]
Testis tone firmer (Score)	7.80 ± 0.27[a]	6.45 ± 0.27[b]	6.82 ± 0.29[b]

[a-c]Values with different superscripts within a row are significantly different (P<0.05).

Epididymal semen characteristics Semen colour

Data presented in Table (2) showed that, semen colour of the dromedary camels was creamish white, milky white and watery white during breeding season, hot-dry and hot-humid months, respectively. These results are in agreement with those reported by Rai *et al.* (1997) and Zeidan and Abbas (2004) who showed that, semen was creamy in colour during the breeding season, while watery white during the non-breeding season in the dromedary camels. Similar trend was reported by Abd El-Azim (1996), Ahmadi (2001) and Zeidan *et al.* (2001) they found that semen colour was yellowish white, creamish white and milky white during winter and spring, grayish white, watery white and light milky white in summer and autumn in the dromedary camels at 3-5, 6-11 and 12-20 years old, respectively. The different colour of semen during different seasons of the year may be due to the different concentrations of spermatozoa and semen consistency (Zeidan *et al.,* 2000).

Semen consistency

Data presented in Table (2) showed that, semen consistency of the dromedary camels was viscous during breeding season and hot-dry months and semi-viscous during hot-humid months. These results are in agreement with those reported by Rai *et al.* (1997) who found that semen consistency was medium and thick jelly during breeding and non-breeding seasons, respectively. Zeidan *et al.* (2007) showed that semen consistency was viscous in the camels at 5 to 10 and 10 to 15 years and semi-viscous in the camels at 15 to 20 years old. Viscosity of the camel semen is usually attributed to the presence of mucopolysaccharides (Mann, 1964, Garnica *et al.,* 1993 and Hassan *et al.,* 1995) which only secreted from bulbourethral or the prostate glands. Immediately after semen collection, the ejaculate becomes aqueous in consistency. The physiological role of mucopolysaccharides is not clear.

The hydrogen-ion concentration (pH)

Data presented in Table (2) showed that, the hydrogen-ion concentration (pH) of the male dromedary camels semen was significantly (P<0.05) higher during hot-humid months than breeding seasons or hot-dry months. The hydrogen-ion concentration (pH) during breeding season was significantly (P<0.05) decreased as compared with the non-breeding season. The hydrogen-ion concentration (pH) during non-breeding season in hot-dry months was significantly (P<0.05) lower than hot-humid months. The highest (P <0.05) value of hydrogen-ion concentration (pH) was recorded in hot-humid months, while the lowest (P<0.05) value was recorded in hot-dry months. Similar trend was reported by Agarwal and Khanna (1993), Abd El-Azim (1996) and Abd El-Samee *et al.* (2006). The alkalinity reaction of the camel semen was increased during the sexual activity (rutting seasons) than during the sexually rest period (Musa *et al.,* 1992). Ahmadi (2001) found that the hydrogen-ion concentration (pH) was insignificantly higher during winter and spring than summer and autumn seasons.

Percentage of sperm motility and the sperm-cell concentration

Data presented in Table (2) showed that, the percentage of sperm motility and the sperm-cell concentration of the dromedary camels were significantly (P <0.05) higher during breeding than non-breeding seasons either hot-dry or hot-humid months. The percentage of sperm motility and the sperm-cell concentration during non-breeding season in hot-humid months were significantly (P <0.05) lower than hot-dry months. The highest (P <0.05) values of the percentage of sperm motility and the sperm-cell concentration were recorded during breeding season, while the lowest (P <0.05) values were recorded during the non-breeding season in hot-humid months. Similar trend was reported by Abd El-Raouf and Owaida (1974) and Abd El-Azim (1996) in camels. Ahmadi (2001) and Zeidan *et al.* (2001) found that the percentage of sperm motility of the dromedary camel was significantly higher during winter than spring, summer and autumn seasons. Similarly, Zeidan and Abbas (2004) showed that percentage of sperm motility was significantly higher during the rutting as compared with the non-breeding season in the male dromedary camels. These results may be attributed to increase of the mature Leydig cells and spermatogenesis process are increased significantly during the rut season than during the summer one

(non-breeding season). As the Leydig cells are mainly responsible for testosterone production. So, an improvement in the semen quality is expected to occur during the rut season (Charnot, 1965).

In addition, the low sperm-cell concentration of the camel semen during non-breeding season (summer) may be attributed to the long day length, as well as, heat stress which lead to reduction in the interstitial cells stimulating hormones and consequently, reduction in androgen production (Sinha and Prasad, 1993). Moreover, the increase of sperm-cell concentration during the rutting season (winter and spring) may be expected and parallel with the results obtained by Fat-Halla and Ismail (1980) who found that FSH concentrations in the male camels were the highest during the rutting season. A positive relationship between FSH level and spermatogenesis was reported by Franchimont (1972). The sperm concentration in the semen is affected by multitude of factors such as virility of the bull, frequency of services, season of the year and the intensity of sexual excitement. Some of these factors might have been responsible for the disparity in the findings of this investigation and those of other workers (Agarwal *et al.*, 2004).

Percentage of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa

Data presented in Table (2) showed that, the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa of the dromedary camels were significantly (P <0.05) higher during hot-humid months than hot-dry months or breeding season. The percentage of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa during non-breeding season in hot-humid months was significantly (P <0.05) higher than hot-dry months. The highest (P <0.05) values of the percentage of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa were recorded during hot-humid months, while the lowest (P <0.05) values were recorded during breeding season. These results are in agreement with those reported by Ahmadi (2001) and Zeidan *et al.* (2001) who found that the percentage of dead spermatozoa was significantly higher during summer than autumn, winter and spring seasons. Similar trends were reported by Abd El-Azim (1996), Rai *et al.* (1997), Zeidan and Abbas (2004) and Abd El-Samee *et al.* (2006) in camels. These results may be due to the decline of temperature during winter and short photoperiods which have effect on the pituitary gland and activity of spermatogenic process and the critical temperature that inhibits spermatogenesis (Rhynes and Ewing, 1973). In addition, heat stress during summer (non-breeding season) which may be cause disturbance in spermatogenesis process due to degenerative changes with diminished number of mature spermatozoa or destruction or even death of spermatozoa (Abd El-Raouf and Owaida, 1974; Musa *et al.*, 1992 and Zeidan *et al.,* 2001).

Zeidan and Abbas (2004) and Abd El-Samee *et al.* (2006) found that the percentage of acrosomal damage of spermatozoa was significantly higher during spring, summer and autumn than winter. These results may be attributed to the onset of rut which is marked by increase in activity in the Alpha and Beta secreting cells in the anterior pituitary and increase in Leydig cells active in the rut season with a resulting reduction in steriodogenic activity by the testes and high testosterone levels which due to improvement of spermatogenesis and decrease of acrosomal damage of spermatozoa.

Table 2. Effects of breeding and non-breeding seasons either hot-humid or hot-dry months on the epididymal semen characteristics in the dromedary camels (Means ± SE).

Items	Season		
	Breeding	Non-breeding	
		Hot-humid months	Hot-dry months
Semen colour	Creamish white	Watery white	Milky white
Semen consistency	Viscous	Semi-viscous	Viscous
Hydrogen-ion concentration (pH)	7.85 ± 0.102^{b}	8.27 ± 0.104^{a}	7.62 ± 0.11^{b}
Sperm motility (%)	80.43 ± 1.12^{a}	58.18 ± 1.15^{c}	72.25 ± 1.20^{b}
Dead spermatozoa (%)	12.83 ± 0.92^{b}	23.18 ± 0.94^{a}	15.20 ± 0.98^{b}
Sperm abnormalities (%)	8.91 ± 0.83^{b}	15.73 ± 0.85^{a}	10.35 ± 0.89^{b}
Acrosomal damage(%)	5.74 ± 0.77^{b}	11.05 ± 0.79^{a}	6.15 ± 0.83^{b}
Sperm-cell concentration ($\times 10^{6}$/ml)	426.30 ± 11.70^{a}	281.59 ± 11.96^{c}	345.25 ± 12.55^{b}

[a-c]Values with different superscripts within a row are significantly different (P<0.05).

Histological changes of the testes

The histological section in the right testis of the camel during breeding season revealed that, the right testis (Plate 1) was the highly active and consisted of numerous seminiferous tubules (ST) with the different shapes and size (oval, ovaid and circular). The ST lined by spermatogenic cells of different maturation stages (spermatogonium, spermatocytes,

spermatid and spermatozoa) in the left testis during breeding season (Plate 2). The ST surrounded by interstitial cells "Leydig cells" which characterized by numerous ovoid cells.

In the right testis of the camel during the non-breeding season in hot-humid months showed some degenerations and vacuolations in the ST (Plate 3). Meanwhile, the left testis during the non-breeding season in hot-humid months showed the number of the Leydig cells was numerous (Plate 4).

The right testis of the camel during the non-breeding season in hot-dry months showed that the ST were numerous of different shapes, sizes and physiological states (Plate 5). The left testis of the camel during the non-breeding season in hot-dry months showed that the cells of the ST characterized by depletion in the high season of non-breeding in the form of vacuolation and desquamation of some spermatogenic cells (Plate 6). Whereas, it appeared as inert with intact spermatogenic cells and numerous interstitial cells in the right testis in hot-dry months (Plate 5).

The left testis of the camel during the non-breeding season in hot-dry months showed that it also appeared in the same sample as vacuolated spots in the spermatogenic series of cells in the ST with increased interstitial connective tissue (Plate 6). It also appeared as depleted cells with desquamated spermatogenic cells and numerous connective tissues (CT) fibres, intact spermatogonium and atrophy in the Leydig cells (Plate 6). In addition, the left testes during breeding season showed that the ST were highly convoluted, more activity in the spermatogenesis, all stages of spermatogenesis, primary spermatocytes, secondary spermatocytes and mature spermatozoa (Plate 1). So, the left testes being to be more active than the right one (Plate 2).

In respect to the non-breeding season in the hot-humid months, the right testes revealed dormant stage and the ST less in activity than the left testes (Plate 3). The stage of spermatogenesis is also inactive and the interstitial tissues were less vascularized (Plate 4). With regard to hot-dry months, the left testes (Plate 5) showed highly active, all the spermatogenesis stages are present and spermatozoa inside the lumen of the ST were highly convoluted as compared to the right testes (Plate 6). Also in the left testes during hot-dry months showed slightly and nearly as the breeding season. Similar trends were recorded by Zayed (1994), Ahmadi (2001), Zeidan and Abbas (2004) and Abdel-Samee *et al.* (2006) in the dromedary camels. In addition, the histological status in the camel's testes during breeding season showed the ST with active spermatogenesis and the Sertoli cells increased in size and nuclei become large, enlarged with distinct nucleoli and light eosinophilic cytoplasm. The Leydig cells increased also in size more than in camel testes during the non-breeding season either hot-humid or hot-dry months.

In general, the histological status in the testes of the dromedary camels as affected by different seasons of the year and side of testes revealed highly active during the breeding season at the left testes, slightly active during the non-breeding season in hot-dry months and inactive during the non-breeding season in the hot-humid months and right testes. Similar trends were reported by Osman and Ploen (1986), Zeidan and Abbas (2004) and Abdel-Samee *et al.* (2006) in the dromedary camels.

In conclusion, the male dromedary camels *(Camelus dromedaries)* during breeding season (short day light) showed testicular activity and epididymal semen characteristics of the dromedary camels were better as indicated by higher the percentage of sperm motility, sperm-cell concentration and histological status and lower the hydrogen-ion concentration (pH), the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa than non-breeding season either hot-dry or hot-humid months (long day light). So, the epididymal spermatozoa of the dromedary camels has the potential of being used in the laboratory investigations related to *in vitro* fertilization (IVF) and artificial insemination (AI) as a useful tool in animal breeding programmes. Further detailed studies are required to establish the reproductive efficiency of the male dromedary camels through the non-breeding season especially in both hot-humid and hot-dry months under Egyptian environmental conditions.

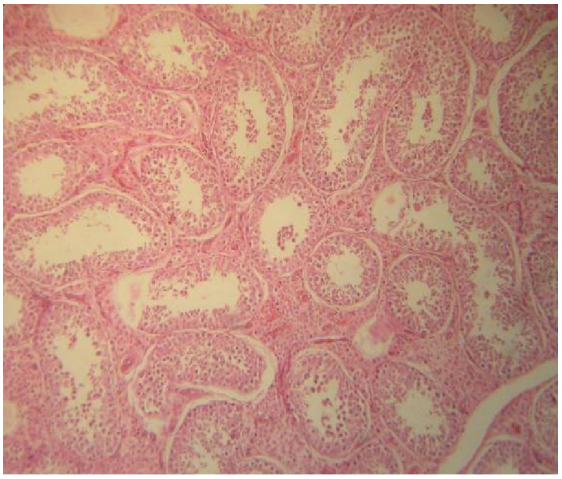

Plate 1. Photomicrograph of the right testis in the breeding season, showing; seminiferous tubules (ST) of different shape and size (H & E ×10).

Plate 2. Photomicrograph of the left testis in the breeding season, showing; the lining epithelium of the seminiferous tubules (ST), spermatogonium, spermatocytes, spermatid and spermatozoa (H & E ×40).

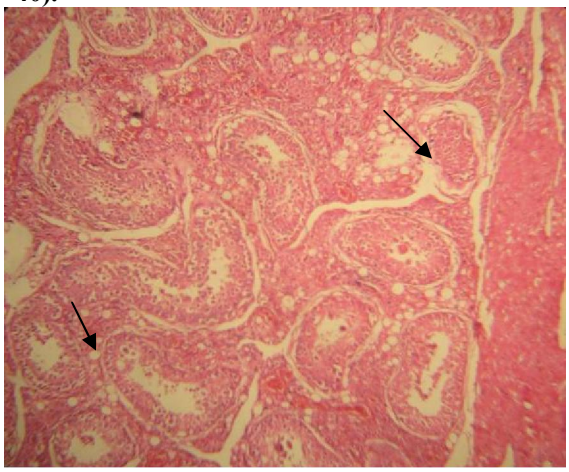

Plate 3. Photomicrograph of the right testis in the hot-humid months, showing; degeneration and vacuolation in the seminiferous tubules (ST) (H & E ×40).

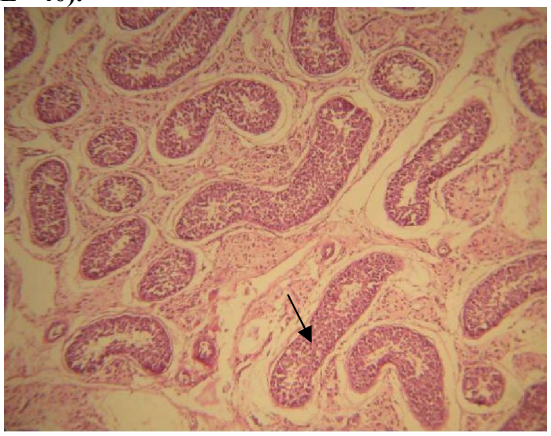

Plate 4. Photomicrograph of the left testis in the hot-humid months, showing; numerous, ovoid shape leydig cells (H & E ×10).

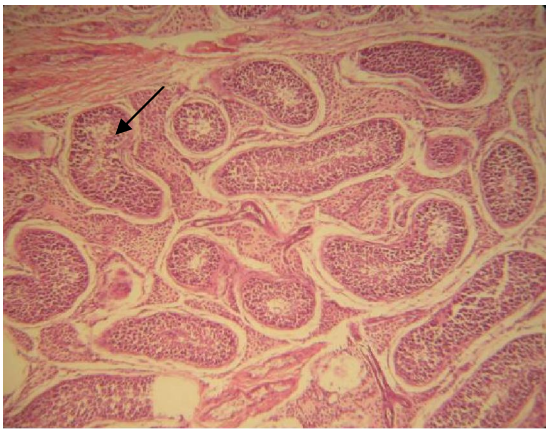

Plate 5. Photomicrograph of the right testis in the hot-dry months, showing; inert, non active spermatogenic series of cells (H & E ×20).

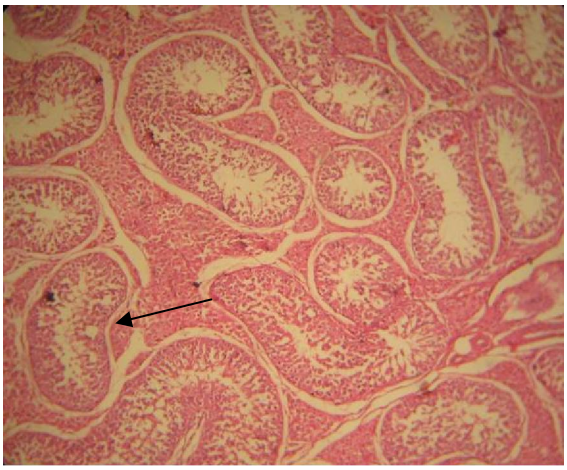

Plate 6. Photomicrograph of the left testis in the hot-dry months, showing; depletion and vacuolation in the spermatogenic cell series and desquamation of the cells (H & E ×20).

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Trends of Annual Mean Surface Air Temperature over Iraq

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Abstract: Iraq is one of the sensitive regions to climate variation particularly temperature change in the World. This study aims to show the change of the mean annual temperature in Iraq. Complete and homogenous time series of mean surface air temperature (T ^{o}C), for different eleven sites over Iraq have been used in this study. RH test software package used as a reference set of homogenous time series well correlated with a base series. Also, Sen's non-parametric estimator of slope has been frequently used to estimate the magnitude of trend, whose statistical significance was assessed by the Mann-Kendall test. The trends in temperatures at annual and seasonal time scales were examined and discussed. Trends of T showed a rising trend at all stations and it experienced an increase of 0.5 °C/decade.

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Key Words: Climate, Surface air temperature, Mann-Kendall Test, Trends, Iraq**.**

1.Introduction

The climate is always changing and has forever been a hot topic of discussion at all levels. In the late 20th century, the natural sciences have increasingly focused on the problems and risks of modern societies. Climate change is considered as the most serious environmental challenge that threatens the developed and less developed countries. It has reached a critical magnitude with a serious impact on society, human welfare and quality of human life. So, the impact on the environment, food security, and socioeconomic systems, at the present time, is seriously taken into consideration by international authorities and has been receiving considerable recent attention from governments.

The detection and attribution of global climate change resulting from anthropogenic activities are one of the main themes of current climatologically research. Recent investigations have shown an increase in global mean temperature of approximately 0.48–0.88 ^{o}C during the twentieth century (e.g., Panel on Reconciling Temperature Observations 2000); however, this trend has not been uniform either spatially or temporally. Many previous studies [including those incorporating general circulation models (GCMs)] focused on changes in long-term average (i.e., annual, seasonal) temperature (Kattenberg *et al.,* 1996). Another important aspect involves characteristics of daily temperature and in particular, changes to the extreme ends (or tails) of the daily temperature distribution. Numerous task groups, including the Intergovernmental Panel on Climate Change (IPCC), have identified the detection of trends and variability in extreme temperatures as critical factors toward an improved understanding of past and potential future global change. To describe accurately the spatial and temporal characteristics of daily and extreme temperatures, long-period time series of reliable and homogeneous daily values are required. Recently, there have been several observational analyses involving daily and/or extreme temperature trends and variability over various regions of the globe. The majority of the findings revealed significant decreases in days with extreme low daily temperature but no significant increases in the number of extreme warm days [e.g., over the contiguous United States (Karl *et al.,* 1996); Canada (Zhang *et al.,* 2000) Great Britain (Jones *et al.,* 1999); northern and central Europe (Brazdil and Coauthors, 1999); Australia and New Zealand (Plummer *et al.,* 1999); and China (Zhai *et al.,* 1999)].

The main objective of this paper is to identify whether or not the frequency or intensity of climate events have increased during a climate warming period over Iraq through. The detailed analysis and understanding of trends of climate events in the Iraq are important to reduce the climate-induced dryness and the impact of temperature extremes on society, agriculture, environment, and so on.

2. Study area and data

Iraq is located in southwest Asia between latitudes 29^{o} 5′ to 37^{o} 22′ N and longitudes 38^{o} 45′ to 48^{o} 45′ E. Iraq, with a total area of 438 317 km^{2}, is bordered by Turkey to the north, Iran to the east, the Persian Gulf to the southeast, Saudi Arabia and Kuwait to the south, and Jordan and the Syrian to the west. (Figure1). Topographically, Iraq is shaped like a basin, consisting of the Great Mesopotamian alluvial plain of the Tigris and the Euphrates rivers (Mesopotamia means, literally, the land between two rivers). This plain is surrounded by mountains in the north and the east, which can reach altitudes of 3611

m above sea level, and by desert areas in the south and west, which account for over 40 percent of the land area. Iraq is characterized by four distinct topographic features:

1. Mesopotamian plain: Alluvial plain occupies a quarter of the area of Iraq.
2. Desert plateau: Located in the west of Iraq and occupies about less than half the size of Iraq.
3. Mountainous region: Mountainous region is located the northern and the north-eastern part of Iraq.
4. Undulating region: A transition zone between the low-lying Mesopotamian plain in the south and the high mountains in the far north and the north-eastern Iraq.

Diverse topography of Iraq plays an essential role in its climate. Iraq being situated in the north part of semi tropic region which distinguishes it by winter of relatively low temperature, dry and hot summer, and with two short seasons which they are spring and autumn. It seems that the differences in temperature have great impacts on Iraq's extreme climate. Iraq lies within the northern temperate zone, but the climatic is continental and subtropical. Winters are usually cool to cold, with an average daily temperature that might reach 16°C dropping at night to 2°C. Summers are dry and hot to extremely hot, with a shade temperature of over 43°C during July and August, yet dropping at night to 26°C (Al-Ansari et al., 2013).

The monthly mean daily values of surface air temperature, T (^{o}C) for different periods at eleven selected stations have been taken from four sources and used in this study. The data sources are:

a. Iraq Meteorological Authority (IMA),
b. Sulaymaniya meteorological station,
c. Arbil meteorological station,
d. Duhok meteorological station.

The stations have been chosen based on data availability and to cover the whole of Iraq. Worth mentioning that the site of data collection has remained the same, with almost negligible change since the beginning of the data measurements at each station. The selected stations and their geographical coordinates as well as the observation periods of temperature is given in Table 1 and Figure 1.

Table1. List of the eleven under study stations, their geographical coordinates and the observation periods of the surface air temperature at each station.

Stations	Longitude (E)	Latitude (N)	Elevation (m) above M.S.L	Observation period	Length of available recorded period (Years)
Zakho	42^{o} 41′	37^{o} 08′	434	1982-2010	29
Mosul	43^{o} 07′	36^{o} 19′	223	1941-2010	70
Arbil	44^{o} 00′	36^{o} 11′	420	1982-2010	29
Sulaymaniya	45^{o} 26′	35^{o} 32′	885	1973-2010	38
Kirkuk	44^{o} 23′	35^{o} 28′	331	1941-2010	70
Baghdad	44^{o} 25′	33^{o} 19′	32	1941-2010	70
Rutba	40^{o} 17′	33^{o} 02′	631	1973-2010	38
Al-Hai	46^{o} 02′	32^{o} 01′	17	1941-2010	70
Diwaniya	44^{o} 59′	31^{o} 59′	20	1973-2010	38
Nasiriya	46^{o} 14′	31^{o} 03′	5	1941-2010	70
Basra	47^{o} 50′	30^{o} 30′	3	1941-2010	70

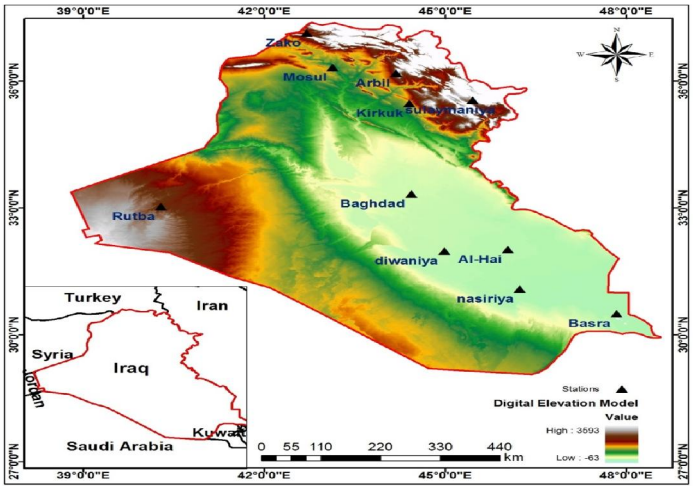

Figure1. Map of Iraq including the eleven selected stations (▲).

3. Methodology

3.1. Homogeneity testing

Data homogeneity is assessed using an R-based program, RHtest, developed at the Climate Research Branch of Meteorological Service of Canada, and available from the ETCCDMI Web site. This program is capable of identifying multiple step changes at documented or undocumented change points. It is based on a two-phase regression model with a linear trend for the entire base series [Wang, 2003]. Detailed discussion about this model can be found in the work of Wang [2003].

We use the R software, which is one of the most flexible and powerful statistical package that currently exists to perform statistical tasks of all kinds, from the most elementary to the most advanced. It has been improved from previous versions and it is maintained by some of the most prestigious statesmen. It's free and open source software that can be download and installs easily from the web page http://www.r-project.org/

3.2. Significance of trend

a. Mann-Kendall test (M-K)

By M-K test, we want to test the null hypothesis H_0 of no trend, i.e. the observations x_i are randomly ordered in time, against the alternative hypothesis, H_1, where there is an increasing or decreasing monotonic trend. The data values are evaluated as an ordered time series. Each data value is compared with all subsequent data values. If a data value from a later time period is higher than a data value from an earlier time period, the statistic S is incremented by 1. On the other hand, if the data value from a later time period is lower than a data value sampled earlier, S is decremented by 1. The net result of all such increments and decrements yields the final value of S (Shahid 2011). The M-K test statistic S is calculated using the formula:

$$S = \sum_{k=1}^{n-1}\sum_{j=k+1}^{n} \operatorname{sgn}(x_j - x_k) \quad (1)$$

$$\operatorname{sgn}(x_j - x_k) = \begin{cases} +1 & if \quad (x_j - x_k) > 0 \\ 0 & if \quad (x_j - x_k) = 0 \\ -1 & if \quad (x_j - x_k) < 0 \end{cases} \quad (2)$$

Where x_j and x_k are the annual values in years j and k, j > k, respectively.

If n < 10, the value of |S| is compared directly to the theoretical distribution of S derived by Mann and Kendall (Gilbert, 1987). The two tailed test is used. At certain probability level H_0 is rejected in favor of H_1 if the absolute value of S equals or exceeds a specified value $S_{\alpha/2}$, where $S_{\alpha/2}$ is the smallest S which has the probability less than α/2 to appear in case of no trend. A positive (negative) value of S indicates an upward (downward) trend (Salmi *et al.,* 2002, Luo *et al.,* 2008).

For n ≥ 10, the statistic S is approximately normally distributed with the mean and variance as follows:

$$E(S) = 0 \quad (3)$$

$$VAR(S) = \frac{1}{18}[n(n-1)(2n+5) - \sum_{p=1}^{q} t_p (t_p - 1)(2t_p + 5)] \quad (4)$$

Where q is the number of tied groups t_p is the number of data values in the p^{th} group.

The standard test statistic Z is computed as follows:

$$Z = \begin{cases} \frac{s-1}{\sqrt{VAR(S)}} & if \quad s > 0 \\ 0 & if \quad s = 0 \\ \frac{s+1}{\sqrt{VAR(S)}} & if \quad s < 0 \end{cases} \quad (5)$$

The presence of a statistically significant trend is evaluated using the Z value. A positive (negative) value of Z indicates an upward (downward) trend. To test for either an upward or downward monotone trend (a two-tailed test) at α level of significance, H_0 is rejected if the $|Z| > Z_{1-\alpha/2}$, where $Z_{1-\alpha/2}$ is obtained from the standard normal cumulative distribution tables. Trends of temperature (mean temp) time series over Iraq (11 stations) were computed from the available data, from table (1), as a long-term trend. Trend during this study has been presented as a rate of change per decade (10* Q/decade).

B. Sen´s slope estimator

If a linear trend is present in a time series, then the true slope (change per unit time) can be estimated by using a simple nonparametric procedure developed by Sen (1968). This means that linear model f (t) can be described as

$$f(t) = Qt + B \quad (6)$$

Where Q is the slope and B is a constant.

To derive an estimate of the slope Q, the slopes of all data pairs are calculated

$$Q_i = (\chi_j - \chi_k) / (j - k), \; i = 1,2,\ldots N, \; j > k \quad (7)$$

If there are n values χ_j in the time series we get as many as N = n(n-1)/2 slope estimates Q_i.

The Sen's estimator of slope is the median of these N values of Q_i. The N values of Q_i are ranked from the smallest to the largest and the Sen's estimator is

$$Q = Q_{[(N+1)/2]}, \quad \text{if N is odd}$$

Or (8)

$$Q = \frac{1}{2}(Q_{[N/2]} + Q_{[(N+2)/2]}), \quad \text{if N is even.}$$

A 100(1-α) % two-sided confidence interval about the slope estimate is obtained by the nonparametric technique based on the normal distribution. The method is valid for n as small as 10 unless there are many ties (Salmi *et al.,* 2002).
At first we compute

$$\sqrt{VAR(S)}$$

$$C_{\alpha} = Z_{1-\alpha/2} \quad , \qquad (9)$$

Where VAR (S) has been defined in equation (4), $Z_{1-\alpha/2}$ is obtained from the standard normal distribution.

Next $M_1 = (N - C_{\alpha})/2$ and $M_2 = (N + C_{\alpha})/2$ are computed. The lower and upper limits of the confidence interval, Qmin and Qmax, are the M_1^{th} largest and the $(M2 +1)^{th}$ largest of the N ordered slope estimates Q_i. If M_1 and/or M_2 are not a whole numbers, the respective limits are interpolated.

To obtain an estimate of B in equation (6) the n values of differences $\chi_i - Qt_i$ are calculated. The median of these values gives an estimate of B (Sirois 1998). The estimates for the constant B of lines of the 99 % and 95 % confidence intervals are calculated by a similar procedure. Data were processed using an Excel macro named MAKESENS created by (Salmi *et al.* 2002).

4. Results and discussion

4.1. Mean surface air temperature

The mean surface air temperature in Iraq increased gradually from lowest value during January month to highest value during July month over all parts of the country. It was noticed that the lowest value of mean surface air temperature (-2.6°C) occurred over Iraq in 1964 at Baghdad in the midst part of the country while the highest value (41.1°C) occurred in 2010 at Basra in the extreme southern part of Iraq (see Table 2). It could be noticed that the lowest value of mean surface air temperature occurred over Iraq before 1980 while the highest value occurred after 1990 year. This is attributed to the effect of war series which started in Iraq in 1980.

4.2 Tends of mean temperature (T °C)

Figure (2) illustrates the annual anomalies of mean surface air temperature (T °C). Statistical properties of the seasonal and annual T series were also tested and presented in Table (3). It was found that, according to Mann-Kendall test for trend; all stations have experienced positive significant trends (warming pattern) of the annual mean temperature.

In winter season, all stations have experienced significant positive trend. Also, it could be noticed that Arbil in the north has the highest positive trend (1.10°C/decade) while Mosul in the north has the lowest positive trend (0.13°C/decade) (Figure 2 and Table 3). On the other hand, there is no any station has significant negative trend (See Table 4).

During spring, all stations show strong upward tendency trends except Zakho station which reported non-significant trend (0.45°C/decade). Arbil which located in the northern part of the country experienced highest positive trend (0.97°C/decade), while Mosul in the northern part experienced lowest positive trend (0.24 °C/decade) (Figure 2 and Table 3). As in winter, there is no any station has significant negative trend during spring season.

Table 2. The lowest and highest values of mean surface air temperature (°C) that occurred at the selected eleven stations during their available study periods

Station	Period	Lowest mean surface air temperature (°C) (Year)	Highest mean surface air temperature (°C) (Year)
Zakho	1982-2010	2.75 (1992)	36.25 (2000)
Mosul	1941-2010	2.75 (1964)	36.90 (2000)
Arbil	1982-2010	4.35 (1983)	37.25 (2000)
Sulaymaniya	1973-2010	1.45 (1992)	35.70 (2000)
Kirkuk	1941-2010	4.35 (1964)	39.10 (2000)
Baghdad	1941-2010	-2.6 (1964)	37.40 (2000)
Rutba	1973-2010	4.45 (1992)	34.70 (2000)
Al - Hai	1941-2010	5.85 (1964)	39.75 (2001)
Diwaniya	1973-2010	7.95 (1977)	38.50 (2000)
Nasiriya	1941-2010	6.25 (1964)	39.85 (2000)
Basra	1941-2010	7.5 (1964)	41.10 (2010)

All eleven stations also showed strong evidence of a significant positive trend during summer season while there is no any station has negative trend during this season. It could be also noticed that the values of significant positive trend ranged between 1.25°C/decade at Arbil station and 0.18°C/decade at Baghdad station.

In autumn, the significant positive trend could be generally identified at all stations except Baghdad station which shows a very slightly non-significant negative trend (-0.03°C/decade) while the highest significant positive trend (1.05°C/decade) occurred at Arbil (Figure 2 and Table 3).

Annually, it is noticed that the mean temperature showed a significant positive trend in at 100% of the stations, while there is no any station has significant/non-significant negative trend in the whole Iraq (See Figure 2 and Tables 3 and 4). It was also found that the positive significant trends varied between the lowest value (0.16°C/decade) at Baghdad in the middle country and the highest value (1.18°C/decade) at Arbil in the north.

Generally, the analysis of the whole dataset in the Table (3) indicates that the annual mean surface air temperature over study period has spatially average of (0.50°C/decade) which is mainly controlled strongly by spring and summer temperatures and caused for such that high annual warming. This result indicates that the annual mean temperature increased, especially after 1995. In brief, study period located under enhanced of greenhouse warming conditions and the impact of years of wars on Iraq.

The findings of this result agree with the findings of Tomozeiu *et al.* (2002), who found a significant upward shift in the air temperature over Romania from 1985 onward. As well as Karaburun *et al.* (2012) who also found that the annual mean temperatures experienced an increase of 1.86°C over the 32-year period at Marmara region, which located in the north west of Turkey.

5. Conclusions

1. In general, trend of Iraq annual mean is 0.50°C/decade, which is mainly derived by summer mean (0.58°C/decade) temperature.
2. Positive high significant trends (warming pattern) of the mean annual temperature were observed at all studied stations (which controlled strongly by summer pattern).
3. There are clear increases in annual temperature for the 11 stations in Iraq.
4. Annual mean temperature series showed a rising trend at all of the stations and it experienced an increase of 0.50°C/decade. The annual mean temperature increased, especially after 1995.

Table3. Trends of mean surface air temperature (□C/ decade), by Mann- Kendall Sen's test

Station	Period	Winter	Spring	Summer	Autumn	Annual
Zakho	1982-2010	0.73*	0.45-	0.61***	0.71*	0.62***
Mosul	1941-2010	0.13*	0.24**	0.22***	0.19*	0.19***
Arbil	1982-2010	1.10***	0.97**	1.25***	1.05***	1.18***
Sulaymaniya	1973-2010	0.73**	0.62***	0.59***	0.43*	0.62***
Kirkuk	1941-2010	0.23**	0.34***	0.29***	0.15*	0.26***
Baghdad	1941-2010	0.13+	0.28***	0.18**	-0.03-	0.15**
Rutba	1973-2010	0.48*	0.64***	0.68***	0.70***	0.59***
Al - Hai	1941-2010	0.29***	0.57***	0.48***	0.25***	0.39***
Diwaniya	1973-2010	0.62**	0.65***	0.67***	0.54**	0.61***
Nasiriya	1941-2010	0.20**	0.46***	0.58***	0.23***	0.38***
Basra	1941-2010	0.21**	0.56***	0.78***	0.31***	0.47***
Average		0.44	0.53	0.58	0.41	0.50

The tested significance levels are 0.001, 0.01, 0.05 and 0.1 as follows: *** = 0.001 level of significance, ** = 0.01 level of significance,* = 0.05 level of significance, + = 0.1 level of significance, - =the significance level is > 0.1

Table4. Number of station with significant positive or negative trends (Mann-Kendall test).

	(Positive trend)					(Negative trend)				
α	0.001	0.01	0.05	0.1	>0.1	0.001	0.01	0.05	0.1	>0.1
Winter	2	5	3	1	0	0	0	0	0	0
Spring	8	2	0	0	1	0	0	0	0	0
Summer	10	1	0	0	0	0	0	0	0	0
Autumn	5	1	4	0	0	0	0	0	0	1
Annual	10	1	0	0	0	0	0	0	0	0

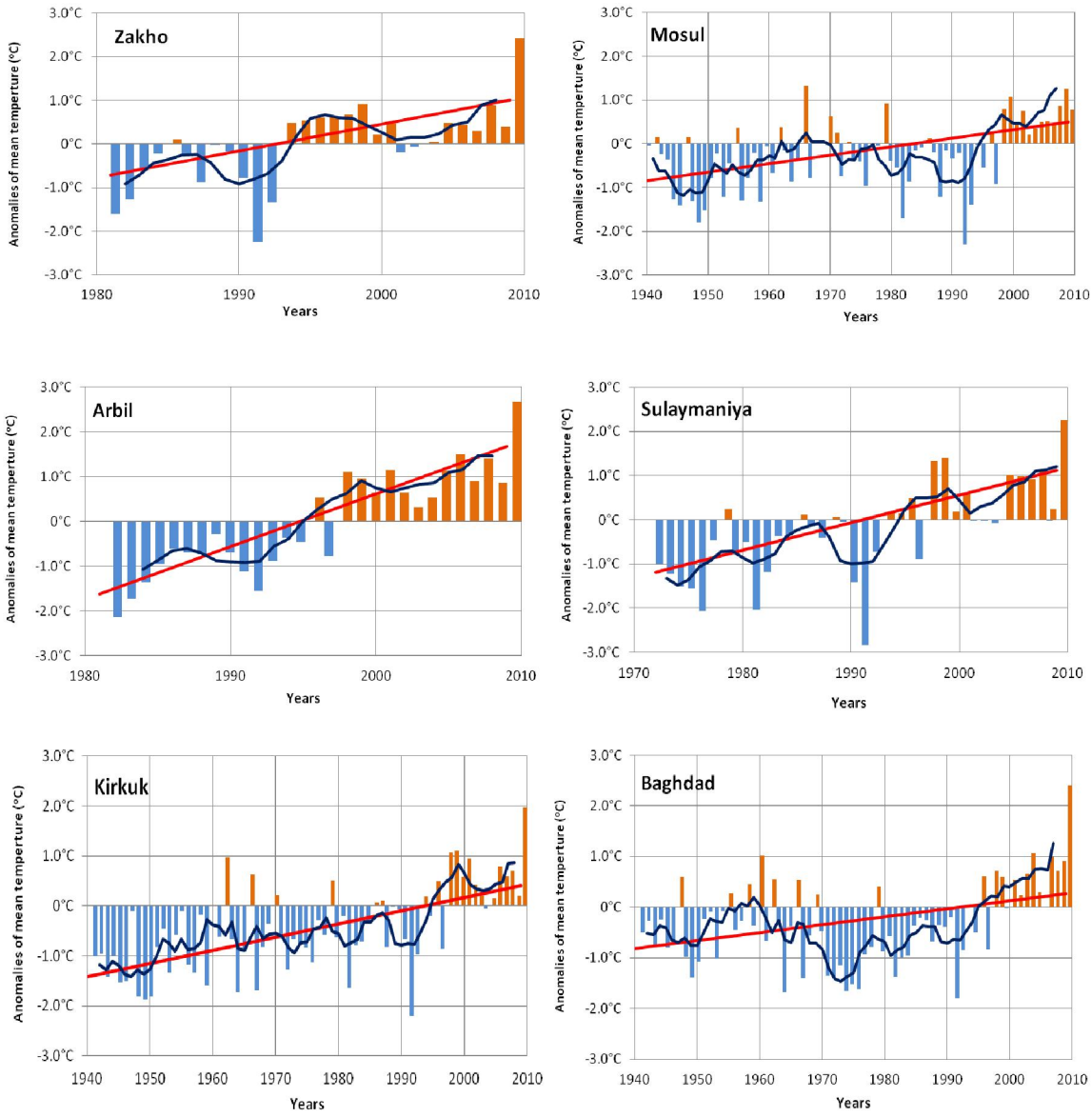

Figure (2a) Anomalies of mean annual temperatures (T °C) for Zakho, Mosul, Arbil, Sulaymaniya, Kirkuk and Baghdad.

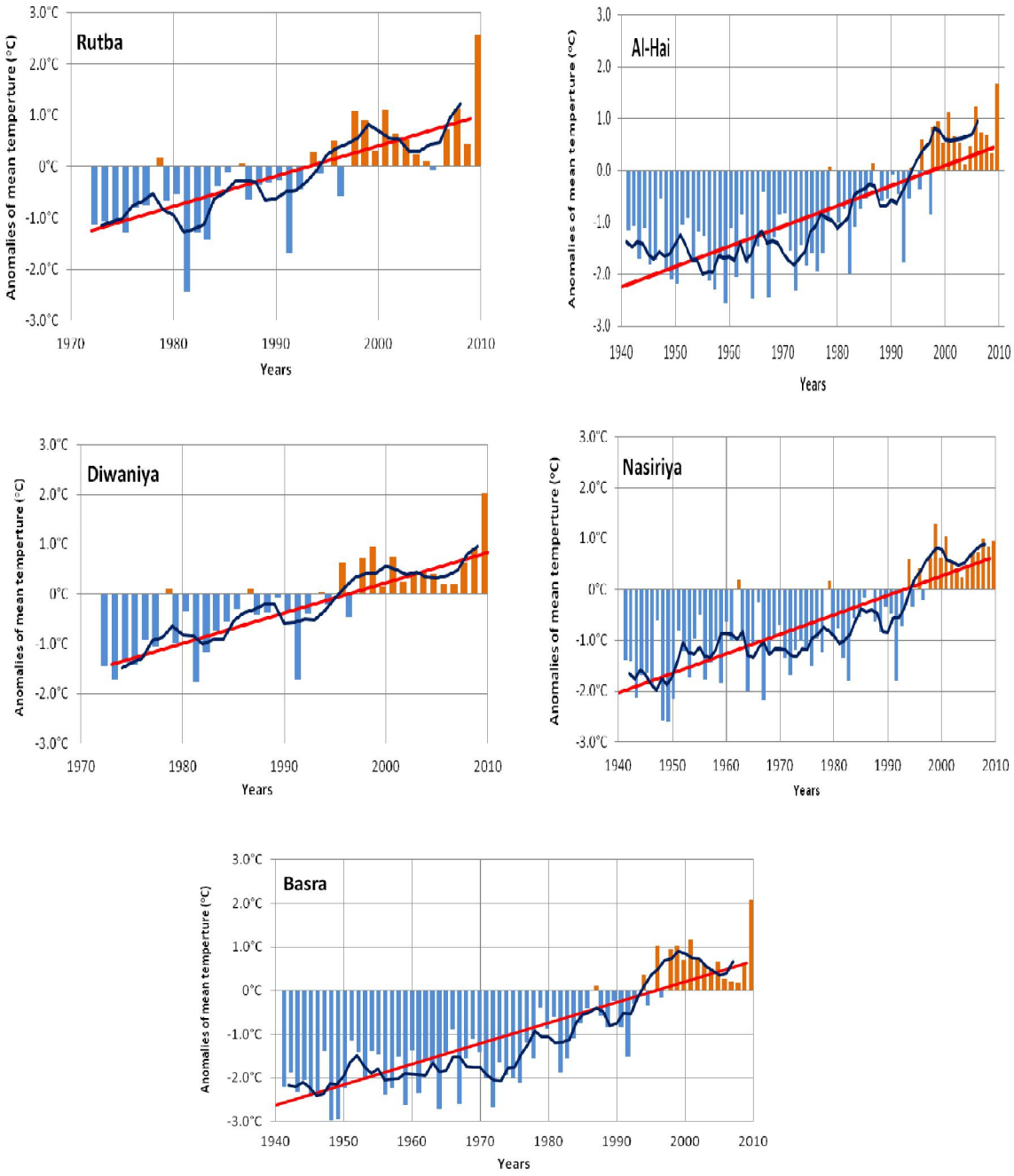

Figure2b. The same as Fig. 2a. But for Rutba, Al-Hai, Diwaniya,Nasiriya and Basra

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Distribution of *Schistosome* intermediate hosts in relation to aquatic plants and physico-chemical characteristics in different watercourses among Kafr El-Sheikh centers, Egypt

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Abstract: This study aimed to record the present status of schistosomiasis transmission in different watercourses among Kafr El-Sheikh Governorate. Snail survey incorporating 80 sites was conducted to determine the presence of intermediate host snails and ascertain whether active transmission was occurring within these areas. Aquatic plants at these sites were recorded, and the physico-chemical characteristics of the water were determined as well. Results showed that *Biomphlaria alexandrina* snails were found to be established in all types of watercourses (canals, drains and El-Borollos Lake) and in all centers with infestation percentage of 63.8% and density of 34.684 snails/site. Naturally infected *B. alexandrina* was observed in five centers exerting their highest infestation in Desouk, Baltim and El-Hamoul centers (37.5%). In the meantime *Bullinus truncatus* was observed in all types of watercourses and in all centers with infestation percentage of 25% and density of 4.10 snails/site. Naturally infected *B. truncatus* was observed only in Mottobis and Baltim centers with infestation percentage of 12.5 and 25%, respectively. *B. alexandrina* density was about 7 times that of *B. truncatus* and 3.3% of *B. alexandrina* and 0.9 % of *B. truncatus* were naturally infected. Results of physico-chemical parameters showed significant difference between centers in most parameters especially for conductivity, copper, sodium and iron levels. Baltim center showed the highest values in most of the examined parameters. In spite of the increased level of all Cu, Cd, K& Na means there was spreading of *B. alexandrina* and *B. truncatus* in all centers and those naturally infected in certain centers suggesting that chemical water pollution was not a limiting factor in these snails distribution. Highly significant correlation was observed between the moderate density of all the recorded plants and *B. alexandrina* infestation percentage while no significant correlation between the infestation pattern of *B. truncatus* and each of the examined plant densities.
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Key words: Schistosomiasis, *B. alexandrina*, *B. truncatus*, natural infection, rice fields, fish aquacultures.

1.Introduction

Schistosomiasis is endemic in 74 countries in Africa, South America and Asia. Worldwide, an estimated 200 million people are infected, of which 20 million is assumed to suffer from more or less a severe form of the disease **(WHO, 2002 and 2012).** In Egypt, there are two types of schistosomiasis *Schistosoma mansoni* and *Schistosoma heamatobium* which transmitted through the intermediate hosts, *Biomphalaria alexandrina* and *Bulinus truncatus*, respectively. Numerous factors act to determine the rate of schistosomiasis transmission in a given location. These include biotic and abiotic features, such as climatic, physical and chemical factors, which affect the survival and development of schistosome parasites and snail host populations **(Sturrock, 1993)**, as well as socio-economic and behavioral characteristics of the human community, such as water contact behavior and the adequacy of water and sanitation, which affect the frequency and intensity of exposure to infected water **(Bundy and Blumenthal, 1990).**

The Egyptian freshwater habitat has been deteriorating primarily due to the discharge of municipal waste water, industrial and agricultural into various water bodies across the country. The correlation between the distribution and population density of *B. alexandrina* and *B. truncatus* in Egypt was studied in one hand and several environmental parameters on the other hand. **Abdel kader *et al.*, (2005)** compared the levels of soluble nitrogen (NO3+ NH4), soluble phosphate, iron, manganese, zinc, copper, boron, cobalt, lead, cadmium and nickel in 22 selected canal and drainages resembling Delta region and the River Nile. Data showed that *B. alexandrina* was more distributed in canal and drains in spite of showing significant elevations in most of the measured parameters, compared to the River Nile. Also, **Ibrahim *et al.*, (2005)** found that the water temperature was ranged from 13ºC in January to 29.1ºC in July, pH ranged from 8.1-8.5, conductivity ranged from 280-460 in the River Nile at Greater Cairo for one year from April 2001- March 2002.

The correlation of *Schistosomes* prevalence in snails and human was studied by **Sayed *et al.*, (2004)** who examined the relationship between epidemiology of *S. mansoni* infection and snail distribution. A systemic human random sample (704 persons) was checked for schistosome infection at a village, related to Giza Governorate. Also, snail collection was done from 35 sites along the water bodies related to the village. The results showed that prevalence of *S. mansoni* was 25% and *S. haematobium* was 0%. *B. alexandrina* infested 12 sites with density of 12.4 snails/site, while *B. 147runcates* infested 7 sites with density of 0.5 snails/site. Natural infection rates among collected snails were 3.7 among *B. alexandrina* and 0 for *B. 147runcates*. So, the pattern of *S. mansoni* human infection was closely related to snail distribution and infection. *S. haematobium* infection was absent from human, and was also absent in snails. Additionally, **Mostafa *et al.*, (2005)** found no infection with *S. haematobium* among 150 people participating in rice farming practices in El Gharbiya and Kafr El-Sheikh Governorates and at the same time found no natural infection among the *B. 147runcates* snails collected from canals and drainages localized in the same areas.

Therefore, the present work aimed to study the distribution of the snail intermediate hosts of schistosomiasis in different watercourses among the 10 centers of Kafr El-Sheikh Governorate as a measurable indicators for transmission. In addition, the correlation between snail distribution and a aquatic plants type and density and water physicochemical parameters were determined.

2.Materials and Methods

Study area:

This study was conducted in the ten centers of Kafr El-Sheikh Governorate (Fuwwah, Desouk, Mottobis, Baltim, Kafr El-Sheikh, El-Hamoul, Sidi Salem, Biyala, El-Reyad and Qullin). Eight sites in each center were involved in the study. The watercourses included irrigation canals, agricultural drains and the Borolos Lake.

Snail survey:

Snail sampling was carried out from May to September 2010, in 80 sites located within the ten center of Kafr El-Sheikh Governorate. Snail sampling was performed through three visits per site using a standard dip net **(Jobin, 1970;** Yousif ***et al.*, 1992).** The collected *Biomphalaria* and *Bulinus* snails from each sampling site were placed in ice boxes and transferred to the laboratory. Non target snails were sorted and recorded in field survey sheet **(El-Emam and Roushdy, 1981)** and **(Yousif *et al.*, 1998a & b).** In the laboratory, snails were examined individually at weakly intervals for one month for their natural trermatode infection. Thus, snails were distributed in test tubes containing de-chlorinated tap water, placed under artificial light for about two hours, then were examined for cercarial shedding **(Favre *et al.*, 1995 and Yousif *et al.*, 1998a).** The snail aquaria of the collected snails were inspected daily to detect snails with signs of distress or mortality, and then the detected ones were crushed between 2 slides and inspected microscopically for schistosome sporocysts **(Olivier, 1973).** The natural schistosome infection rate was estimated for each host snail species in each study site, to be equal to the sum of cercariae shedding snails plus those have schistosome sporocyst divided by the total of collected snails.

Physico-chemical parameters of water:

Water temperature and conductivity were measured directly in the selected watercourses to the nearest o C and µs/cm, respectively using temperature conductivity meter (HANNA instrument, HI 9635). Also, Hydrogen ion concentration (pH) was measured by pH meter electrode (HI 9124 and HI 9125). All the physical parameters were measured between 11: 00 am to 3: 00 pm and were recorded in the field survey sheets. Water samples were collected from the watercourses 5 cm below the water surface immediately afterwards, the samples were filtrated, and kept at 4 o C till analysis. Samples were analyzed for concentrations of sodium (Na), potassium (K), calcium (Ca), cadmium (Cd), lead (Pb), copper Cu, iron (Fe), manganese (Mn) and nickel (Ni), using atomic absorption spectrophotometer (AVANTA)

Aquatic plants:

Existed aquatic plants in selected watercourses were sorted and their densities (low, moderate and high) were recorded in the field survey sheet **(El-Emam and Roushdy, 1981; Yousif *et al.*, 1998a & b).**

Statistical analysis:

All analyses were performed using SPSS version 18 (SPSS, Inc., Chicago, IL) using test for difference between two groups proportion. P values < 0.05 were considered statistically significant.

3.Results

Snail distribution and density:

Snail survey was done during the period from May-September /2010 in 80 sites representing the ten centers of Kafr El-Sheikh Governorate (8 sites/center). Results of *B. alexandrina* and *B. truncatus* distribution and density are summarized in Tables (1&2). They showed that *B. alexandrina* snails were found established in all types of watercourses and in all centers with infestation percentage of 64%. The highest percentage of infestation was in Fuwwah and Baltim centers, recording100%, while the lowest was in Qullin, representing 12.5%. Desouk, Baltim and Fuwwah centers were characterized by the highest snail density (92.13, 75.5, and 58.25 snails/site,

respectively). Test for difference between proportions was used to compare the infestation pattern of *B. alexandrina* between centers. Fuwwah center was very highly significant with Qullin center, highly significant with El-Reyad and significant with each of Mutubis & Biyala. Also, Desouk center was highly significant with Qullin center while significantly different from Mutubis and El-Hamoul centers. In addition, Mutubis was highly significant with Baltim center and Baltim was highly significant with each of El-Reyad, Kafr El-Sheikh and Qullin centers. Desouk, Baltim and El-Hamoul centers were the mostly infested with naturally infected *B. alexandrina* (37.5 %) while Biyala and Baltim showed the highest density of naturally infected snail (5.88 and 4.0 snails/site, respectively). Biyala center showed the highest percentage of natural infection (26.6%). Desouk center showed least percentage of natural infection (0.4%) in spite of characterizing by the highest snail density. On the other hand, Mutubes, Kafr El-Sheikh, Sidi Salem and El-Reyad centers were free of naturally infected snails. In the case of *B. truncatus*, snails were found in all centers in canals, drains and the lake with infestation percentage 25%. Mutubes center showed the highest infestation percentage as well as the highest snail density (percentage of infestation was 62.5% and the snail density was 23.5 snail/site). Statistical analysis showed that *B. truncatus* infestation pattern was significantly different only between Fuwwah & Mutubes centers. Naturally infected *B. truncatus* snails were observed only in Baltim and Mottobis centers (The percentage of infestation of naturally infected snails was 25% and 12.5%, respectively and their density were 0.125 and 0.286 snail/site, respectively). Baltim center showed the highest percentage of naturally infected snails (33.3%). Comparing both snail species in Kafr El –Sheikh Governorate revealed that the density of *Biomphalaria* was about 7 times that of *Bulinus* and the total percentage of naturally infected snails was higher among *B. alexandrina* snails (3.3%) than *B. truncatus* snails (0.9 %). The study of non-target snails' distribution observed eight snail species that naturally associated with *B. alexandrina* and *B. truncatus*. Results indicated that *Cleopatra bulimoide* and *Bellamya unicolor* were the mostly infested and found in all centers with infestation percentage 77.5% and 57.5%, respectively while *Planorbis planorbis* and *Helisoma duryi* showed the least infestation percentage (6.3% and 5%, respectively). *P. planorbis* was recorded in three centers and *H. duryi* was recorded in two centers. The other four species, *Lanistes carinatus*, *Lymnaea natalensis*, *Physa acuta*, and *Melanoides tuberculata* were found in most centers showing approximately the same infestation percentages (36.3, 35.0, 32.5 & 28.8%, respectively as shown in Table (3).

The association pattern between the snail intermediate hosts and non-target snails is presented in Table (4). The highest association percentage of each of *B. alexandrina* and *B. truncatus* were with *C. bulimoide* (percentage of association was 50% and 21.3%, respectively) followed by *L. carinatus*, *L. natalensis*, *P. acuta*, and *B. unicolor*. In the meantime, both snails showed the lowest association with *M. tuberculata*, *P. planorbis* 1.3% then *H. duryi*, 3.8%.

Physical and chemical parameters:

Results of physico-chemical parameters were recorded and their means in each center were presented in Tables (5&6). Statistical comparison between centers using T-test showed that significant difference in most of chemical parameters. All Pb, Mn, Ni & Ca fluctuated around the same levels and didn't exceed the maximum of low able concentration (MAC) recommended by National Recommended Water Quality Criteria. All Cu, Cd, K& Na means exceeded the level of concern, Cu ranged between 14.7 in Desouk – 47.4 ppb in Kafr El-Sheikh, Cd ranged between 6.8 in Mottobis – 225.9 ppb in Biyala, K ranged between 8.2 in Hamoul – 27.3 ppm in Baltim and Na ranged between 63.9 in Fuwwah – 834.6 ppm in Baltim. In the meantime Fe concentration ranged between 24.55 in Hamoul – 49.39 ppb in Mottobis.

The studied sites were divided into four categories, the first category included sites that harbored *Biomphalaria* snails, the second category harbored *Bulinus* snails, the third category harbored the non-target snails and the latest category includes sites that free from all snail types. The comparison of the physico-chemical parameters in these habitats are presented in table (7). Statistical analysis using ANOVA analysis revealed very highly significant in temperature between habitats of *B. alexandrina* and that of *B. truncatus* ($F= 7.415$ & $P <0.001$), and highly significant difference between habitat of *B. truncatus* and that of non-target snails ($F= 2.86$ & $P <0.003$). Habitats free from snails showed non-significant higher recordings of field observations and certain chemical concentrations Cd, Na and K than other categories while habitats harboring *B. alexandrina* and *B. truncatus* were more tolerant than non-target snails and *B. alexandrina* was more tolerant than *B. truncatus* to some of the examined parameters. Also, site category of *B. alexandrina* and that of *B. truncatus* was subdivided to habitat of negative snails and those naturally infected ones to compare the same physiochemical parameters. Statistical comparison between habitat characteristics of naturally infected *B. alexandrina* and *B. truncatus* and those negative ones using ANOVA test revealed significant difference

between naturally *B. alexandrina* and those negative in Na and Ca levels ($P < 0.01$, T=2.56 & d.f=30 and $P < 0.05$, T= -2.03& d.f = 37, respectively) while no significant difference was observed between the two habitats of *B. truncatus*. Also, habitats of naturally infected *B. alexandrina* and negative *B. truncatus* were very highly significant in Cu level ($P < 0.001$, T= 2.53 & d.f = 47 (Table, 8).

Aquatic plants:

The survey study observed four aquatic plant species, *Eichhornia crassipes*, *Lemna gibba*, *Ceratophyllum demersum* and *Jussiae sp*. *E. crassipes* and *L. gibba* were the mostly infested and found in the all centers with infestation percentage ranged between 50 – 100%, 12.5 –75%, respectively. The association pattern between the snail intermediate hosts and the observed aquatic plants is presented in Table (9). Results showed that *B. alexandrina* and *B. truncatus* were mostly associated with *E. crassipes* with percentages of 51.3% and 21.3, respectively. On the other hand, was mostly associated with *E. crassipes* and *C. demersum*16.3%, respectively. The correlation between two densities of the recorded aquatic plants, moderate and high densities, and the infestation percentage of *B. alexandrina* and *B. truncatus* in different centers was examined using test for difference between proportions (two proportion groups) SPSS Program (18). Results showed that significant correlation between *B. alexandrina* infestation and high density of *E. crassipes* in Fuwwah, Baltim & Sidi salem centers and high density *L. gibba* in Fuwwah & Baltim. Also, highly significant correlation was observed between the moderate density of all the recorded plants and *B. alexandrina* infestation percentage in Fuwwah center, *E. crassipes*, *L. gibba* and *C. demersum* in Blatim center, *E. crassipes* and *L. gibba* in Desouk and Sidi Salem center. On the other hand, no significant correlation between the infestations pattern of *B. truncatus* and each of the two examined plant densities were observed.

Table (1): The distribution and population density of *Biomphalaria alexandrina in* the examined watercourses among the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

Centers (8 sites) Items	Fuwwah	Desouk	Mottobis	Baltim	Kafr El-Sheikh	El-Hamoul#	Sidi Salem	Biyala	El-Reyad	Qullin	Total
No. of infested sites	8	7	3	8	3	6	6	5	4	1	51
% of infestation	100%	87.5%	37.5%	100%	37.5%	75%	75%	62.5%	50%	12.5%	63.8%
Total no. of snails	466	737	59	604	21	274	153	177	183	94	2759
% of center sample	16 %	6.7%	2.1%	21.9%	0.76	9.9%	5.5%	6.4%	6.6%	3.4%	79.3%
Snail density	58.25	92.125	7.375	75.5	2.625	34.25	19.125	22.125	22.875	11.750	34.684
No. of transmission sites	1	3	0	3	0	3	0	2	0	0	18
% of infestation of transmission sites	12.5%	37.5%	0	37.5%	0	37.5%	0	25%	0	0	22.5%
No. of naturally infected	3	3	0	32	0	5	0	47	0	0	90
Naturally infected snail density (infected snails/site ±SD)	0.375 ±1.061	0.429 ±0.535	0 ±0	4.00 ±6.990	0 ±0	0.625 ±1.061	0 ±0	5.875 ±16.217	0 ±0	0 ±0	1.139 ±5.670
% of Naturally infected snails	0.64%	0.4%	0	5.3%	0	1.8%	0	26.6%	0	0	3.3%

Table (2): The distribution and population density of *Bulinus truncatus in* the examined watercourses among the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

Centers Items	Fuwwah	Desouk	Mottobis	Baltim	Kafr El-Sheikh	El-Hamoul	Sidi Salem	Biyala	El-Reyad	Qullin	Total
No. of infested sites	1	2	5	2	1	2	3	4	1	4	20
% of infestation	12.5%	25%	62.5%	25%	12.5%	25%	37%	50%	12.5%	50%	25%
No. Of transmission sites	0	0	1	2	0	0	0	0	0	0	3
% of infestation of transmission sites	0	0	12.5%	25%	0	0	0	0	0	0	3.8%
Total no. of snails	2	10	188	3	8	6	6	46	2	57	328
Snail density (snail/site ±SD)	0.250	1.250	23.500	0.375	1.00	0.750	0.750	5.750	0.250	7.125	4.100
No. of infected snails	0	0	2	1	0	0	0	0	0	0	3
Infected snail density (infected snails/site ±SD)	0 ±0	0 ±0	0.286 ±0.756	0.125 ±0.354	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0.038 ±0.252
% of Naturally infected snails	0	0	1.1%	33.3%	0	0	0	0	0	0	0.9 %

Table (3): Infestation percentage of non target snails species in the examined watercourses among the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

	Percentage of infestation										
Centers/Non target snails	**Fuwwah**	**Desouk**	**Mottobis**	**Baltim**	**Kafr El-Sheikh**	**El-Hamoul**	**Sidi Salem**	**Biyala**	**El-Reyad**	**Qullin**	**Total**
Lymnaea natalensis	**75**	**25**	**37.5**	**25**	**25**	**50**	**50**	**37.5**	**0**	**0**	**35**
Lanistes carinatus	**0**	**37.5**	**25**	**0**	**12.5**	**75**	**62.5**	**37.5**	**75**	**75**	**36.3**
Physa acuta	**25**	**37.5**	**50**	**0**	**62.5**	**12.5**	**25**	**12.5**	**25**	**25**	**32.5**
Cleopatra bulimoide	**62.5**	**62.5**	**87.5**	**100**	**100**	**87.5**	**87.5**	**62.5**	**62.5**	**62.5**	**77.5**
Bellamya unicolor	**62.5**	**62.5**	**50**	**50**	**37.5**	**25**	**75**	**75**	**75**	**75**	**57.5**
Helisoma duryi	**0**	**12.5**	**0**	**12.5**	**0**	**0**	**0**	**0**	**0**	**0**	**5**
Melanoides tuberculata	**37.5**	**25**	**12.5**	**62.5**	**25**	**25**	**25**	**0**	**25**	**25**	**28.8**
Planorbis planorbis	**0**	**12.5**	**12.5**	**37**	**0**	**0**	**0**	**0**	**0**	**0**	**6.3**

Table (4): The association percentage of Biomphalaria alexandrina and Bulinus truncatus with non target snails species in the examined watercourses among the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

Non target snails	*Biomphalaria alexandrina*	*Bulinus truncates*
Bellamya unicolor	**26.3%**	**12.5%**
Cleopatra bulimoide	**50%**	**21.3%**
Lanistes carinatus	**22.5%**	**15%**
Lymnaea natalensis	**21.3%**	**17.5%**
Helisoma duryi	**1.3%**	**3.8%**
Melanoides tuberculata	**16.3%**	**3.8%**
Physa acuta	**11.3%**	**13.8%**
Planorbis planorbis	**3.8%**	**2.5%**

Table (5): The Physicochemical characteristics of the examined watercourses among the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

Centers Parameters (MAC)	Fuwwah	Desouk	Mottobis	Baltim	Kafr El-Sheikh	El-Hamoul	Sidi Salem	Biyala	El-Reyad	Qullin
Temperature (>25ºC)	26.38 ±0.52	26.38 ±0.92	25.63 ±6.61	29.04 ±0.74	28.33 ±0.85	27.54 ±0.82	28.08 ±0.53	26.33 ±4.68	27.95 ±0.79	24.50 ±3.88
Conductivity (≥ 800 µmoh/cm)	531.58 D ±141.08	846.71 ABC ±272.95	626.88 CD ±143.69	705.25 BCD ±145.53	944.88 AB ±334.22	856.42 ABC ±240.44	967.79 AB ±371.03	720.63 BCD ±254.36	1003.67 A ± 115.78	634.71 CD ±232.92
pH (6.5-9)	7.41 C ±0.18	7.49 C ±0.11	7.55 BC ±0.05	7.78 A ±0.33	7.56 BC ±0.15	7.48 C ±0.16	7.55 BC ±0.15	7.49 C ±0.15	7.69 AB ±0.12	7.46 C ±0.07
Cd (2 ppb)	7.43 B ±241	10.16 B ±4.30	6.84 B ±1.48	225.91 A ±116.09	11.69 B ±5.26	11.78 B ±5.84	12.95 B ±5.01	9.74 B ±4.35	14.13 B ±4.83	9.77 B ±4.67
Pb (65ppb)	18.74 abc d ±11.86	14.12 abc d ±8.27	8.78 d ±6.80	6.85 d ±4.46	29.35 a ±25.02	19.67 abc d ±10.21	27.13 ab ±22.63	10.04 c d ±8.24	25.17 abc ±18.92	12.15 bc d ±8.41
Fe (1000 ppb)	38.88 a b ±13.32	25.53 b ±11.30	49.39 a ±10.92	45.44 a ±26.37	36.57 a b ±16.14	24.55 b ±10.14	25.89 b ±6.51	28.22 b ±14.78	36.89 a b ±19.57	38.75 a b ±13.35
Cu (13 ppb)	29.51 B ±10.68	14.70 B ±11.76	26.33 B ±15.29	26.88 B ±4.71	47.41 A ±16.67	29.79 B ±3.29	24.87 B ±14.03	26.99 B ±15.88	28.92 B ±21.97	28.38 B ±11.54
Mn (1400 ppb)	7.71 D ±4.60	8.13 D ±3.88	58.48 B C ±11.92	114.40 A ±68.66	38.04 B C D ±37.75	59.32 B C ±36.400	27.33 C D ±10.92	13.69 D ±7.69	16.98 D ±9.57	65.44 B ±43.20
Ni (450 ppb)	11.27 B ±7.20	20.47 B ±4.88	356.03 A ±269.21	262.22 A ±190.19	10.88 B ±9.94	10.70 B ±10.02	14.69 B ±9.29	750 B ±5.88	6.54 B ±2.89	8.65 B ±6.94
Na (25 ppm)	63.94 B ±35.07	93.28 B ±39.96	64.46 B ±29.58	834.63 A ±427.88	166.73 B ±98.50	90.45 B ±86.91	217.74 B ±184.27	92.96 B ±66.17	131.14 B ±57.14	84.51 B ±61.12
K (6 ppm)	10.44 B C ±4.13	10.08 B C ±2.73	8.37 C ±1.1	27.32 A ±10.57	9.12 B C ±2.37	8.21 C ±1.66	13.54 B C ±6.40	10.84 B C ±5.36	14.95 B ±7.21	8.44 C ±1.21
Ca (100 ppm)	11.31 C D ±3.09	14.03 B C ±1.29	10.97 C D ±2.05	22.66 A ±4.48	11.59 C D ±3.61	13.60 B C ±4.70	15.89 B ±5.16	10.21 C D ±2.62	13.98 B C ±2.83	9.53 D ±2.86

a, b Means having different letter exponents among rows are significantly different ($P \leq 0.05$).
A, B Means having different letter exponents among rows are significantly different ($P \leq 0.01$).
*The maximum of low able concentration: (MAC).

Table (6): The Physicochemical characteristics of different habitats of the intermediate hosts of schistosomiasis, *Biomphalaria alexandrina* and *Bulinus truncatus*, non-target snails and that free from snails among watercourses in the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

Items (MAC)	*B. alexandrina* *	*B. truncatus* *	Non-target *	Free
Temperature (>25ºC)	27.24±2.57	24.72±1.8 ******	27.63±1.1** **	28.5±0.7
Conductivity (≥ 800 µmoh/cm)	815.42±262.60	677.64±72.9	781.188±64.0	1012.333±48.7
pH (6.5-9)	7.52±0.15	6.727±0.50	7.423±0.3	7.767±0.1
Cd (2 ppb)	54.418±13.80	45.042±15.40	11.423±1.2	82.876±71.4
Pb (65ppb)	23.54±3.50	19.757±3.60	23.337±5.7	13.920±1.3
Fe (1000 ppb)	32.98±18.02	35.16±17.79	32.38±17.88	24.33±0.3
Cu (13 ppb)	28.09±2.00	27.720±2.80	30.238±5.5	30.500±2.2
Mn (1400 ppb)	69.43±19.10	108.382±41.50 **	18.641±8.3	34.170±25.3
Ni (450 ppb)	45.70±6.90	55.420±10.80	35.205±7.9	50.017±16.6
Na (25 ppm)	240.97±46.40	179.383±43.20	103.390±13.3	451.123±32.57
K (6 ppm)	13.38±1.20	10.794±1.20	9.527±0.5	20.204±12.4
Ca (100 ppm)	15.35±1.10	12.891±1.00	17.713±6.1	17.072±3.4

*= Significant (< 0.05), **: highly significant (< 0.01), ***: very highly significant (< 0.001).
*The maximum of low able concentration: (MAC).

Table (7): The Physicochemical characteristics of habitat *Biomphalaria alexandrina* (negative & naturally infected) and *Bulinus truncatus* (negative & naturally infected) among watercourses in the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010. The maxi

Items (MAC).	*B. alexandrina*		*B. truncatus*	
	Negative *	Naturally infected *	Negative *	Naturally infected
Temperature (>25ºC)	26.2±6.817	25.6±7.987	25.1±8.565	28.5±1.378
Conductivity (≥ 800 µmoh/cm)	804.709±351.462	704.111±317.191	699.357±378.089	537.167±57.770
pH (6.5-9)	7.070±1.800	6.886 ±2.111	6.656±2.398	7.567±0.121
Cd (2 ppb)	9.54±4.74	26.71±16.82	9.69±4.68	7.65±2.45
Pb (65ppb)	18.83±16.54	19.70±12.83	13.09±8.22	1.524±0.74
Fe (1000 ppb)	36.30±19.019	22.50±10.15	33.93±18.05	46.23±14.25
Cu (13 ppb)	30.93±13.77	19.52±12.83 **	28.38±14.53 * ***	19.778±6.48
Mn (1400 ppb)	9.13±6.55	25.29±23.88	18.86±209.352	25.85±22.53
Ni (450 ppb)	38.49±38.83	63.50±57.44	50.28±49.87	116.99±104.94
Na (25 ppm)	69.82±42.06 ****	126.24±81.96 ****	83.77±58.38	35.72±14.71
K (6 ppm)	13.23±8.82	14.170±7.46	10.42±5.54	15.251±11.91
Ca (100 ppm)	14.96±8.36 **	16.83±5.69	12.61±4.97	16.227±9.41

*= Significant (< 0.05), **: highly significant (< 0.01), ***: very highly significant (< 0.001). * The maximum of low able concentration: (MAC).

Table (8): The distribution of aquatic plants expressed by infestation percentage and densities among watercourses in the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

Centers (10 examined sites) Aquatic plants			Fuwwah	Desouk	Mottobis	Baltim	Kafr El-Sheikh	El-Hamoul	Sidi Salem	Biyala	El-Reyad	Qullin
Ceratophyllum demersum	No. of infested sites		1	0	3	1	0	0	1	2	3	3
	% of infestation		12.5%	0	37.5%	12.5%	0	0	12.5%	25%	37.5%	37.5%
	Densities	+	0%	0	12.5%	12.5%	0	0	12.5%	12.5%		0%
		++	12.5%	0	12.5%	0%	0	0	0%	12.5%		25%
		+++	0%	0	12.5%	0%	0	0	0%	-		12.5%
Eichhornia crassipes	No. of infested sites		8	6	4	6	7	7	5	6	5	5
	% of infestation		100%	75%	50%	75%	87.5%	87.5%	62.5%	75%	62%	62%
	Densities	+	37.5%	0%	12.5%	12.5%	50%	25%	37.5%	37.5%	25%	50%
		++	12.5%	25%	25%	25%	0%	37.5%	12.5%	25%	25%	12.5%
		+++	50%	50%	12.5%	37.5%	37.7%	25%	12.5%	12.5%	12.5%	0%
Jussiae sp	No. of infested sites		1	0	0	0	1	1	1	1	3	0
	% of infestation		12.5%	0	0	0	12.5%	12.5%	12.5%	12.5%	37.5%	0
	Densities	+	12.5%	0	0	0	12.5%	12.5%	12.5%	12.5%	37.5%	0
		++	0%	0	0	0	0	0	0	0	0	0
		+++	0%	0	0	0	0	0	0	0	0	0
Lemna gibba	No. of infested sites		4	4	3	3	1	1	3	2	6	5
	% of infestation		50%	50%	37.5%	37.5%	12.5%	12.5%	37.5%	25%	75%	62%
	Densities	+	12.5%	0%	12.5%	25%	12.5%	12.5%	37.5%	12.5%	0%	25%
		++	25%	25%	12.5%	0%	0%	0%	0%	12.5%	25%	12.5
		+++	12.5%	25%	12.5%	12.5%	0%	0%	0%	0%	50%	25%

*** (+: low, ++: intermediate & +++: high)**

Table (9): The association percentage of *Biomphalaria alexandrina* and *Bulinus truncatus* with different species of aquatic plants among watercourses of the ten centers of Kafr El-Sheikh Governorate during the period from May-September 2010.

Aquatic plants	The snail intermediate hosts	
	Biomphalaria alexandrina	*Bulinus truncatus*
Ceratophyllum deresum	7.5%	16.3%
Eichhornia crassipes	51.3%	21.3 %
Jussiae sp.	5%	1.3%
Limma gibba	27.5%	8.8%

4.Discussion

The pattern of *Schistosomes* snail intermediate hosts distribution and their prevalence of infection are among the measurable indicators that reflect the magnitude of transmission **(Sayed *et al.*, 2004). Mostafa *et al.*, (2005)** studied the status of distribution of snail vectors of schistosomiasis and the transmission of the disease in 240 sites in Kafr El-Sheikh. The authors found that *B. alexandrina* was found in 5% of the examined sites, showing density of 11.25 snails/site while *B. truncatus* was found in 17.95% of the examined sites. Our findings of snail distribution and percentage of natural infection are higher than that observed by other authors. **Habib (2010)** studied the effect of geographical distribution of *B. alexandrina* snails on their susceptibility to *Schistosoma mansoni* infection in some localities in Egypt and found very low natural *S. mansoni* infection, 0.38% among the collected snails**.** Also, **El-Homossany (2006)** revealed that natural snail infection can occur in Nile especially in sites where fishing and agricultural activities, but mostly by low ratio, for *B. alexandrina* and *B. truncatus* snails was (0.71% and 0.29, respectively). In addition**, El-Khayat *et al.* (2005)** indicated that the natural infection among field *B. alexandrina* snails collected from different localities was low (1.1). Furthermore, **Haristone (1973)** concluded that low percentage of natural infection is the rule (1-2%), however high percentage (3.3-7.5) may be recorded in summer period suggestion that the proportion of snails infected with schistosome at any time depends upon complex interaction of different factors. The present survey observed association between *B. alexandrina* and *B. truncatus* and the most distributed non-target snails, *C. bulimoid* and *L. carinatus*. Also, many authors studied the association pattern between *B. alexandrina* and *B. truncatus* snails and the other non-target snails. **Yousef *et al.* (1998a)** found that *B. alexandrina* snails were positively associated with *L. carinatus*, and *C. bulimoides* while *B. truncatus* snails were found to be positively associated with *p. acuta* snails. Also, **Abdel Kader *et al*. (2001&2005)** reported that *B. alexandrina* snails mostly existed with *L. carinatus* and *Physa acuta* snails. However, **Frandsen (1976), Frandsen & Christensen (1977), Madsen & Frandsen (1979) and Madsen (1985)** found that the presence of *H. duryi* caused an important reduction in the growth of infected *Biomphalaria pfeifferi*, *B. glabrata, B. camerunensis* and *B. alexandrina* when they were in a direct competition situation. The present determined data of physiochemical parameters were processed to compare water quality in different examined centers and characteristics for different snail habitats. Results showed significant difference between centers in most parameters especially for conductivity, copper, sodium and iron levels. Baltim center showed the highest values in most of the examined parameters and this may be attributed to the include of this center to all types of watercourses; Borollos Lake, Kotshenar drains and El-Ayash, El-Sagheir and El-Gadida canals. All the means of Cd, Cu, Na and K exceeded the MAC that recommended by National Water Quality Criteria and represented risks for health. In spite of the increased level of these chemicals there was spreading of *B. alexandrina* and *B. truncatus* in all centers and those naturally infected in most centers suggesting that chemical water pollution was not a limiting factor in these snail distribution. This suggestion was reinforced by **Khairy (1998)** who studied the distribution of *B. truncatus* and *B. alexandrina* in two villages, El-Garda and Salamoniya, in Menoufia Governorate that differed greatly in the degree of chemical and faecal pollution of the watercourses. This was probably due to the existence of the sewage disposal system in El-Garda village and its absence in Salamoniya. In spite of the high pollution of watercourses in Salamoniya, both *Bulinus* and *Biomphalaria* snails were found and were often infected. On the other hand, in El-Garda, in spite of the lower pollution of its water courses, which would have been expected to be associated with higher snail counts, particularly in Kafr Tambidy canal which was less chemically polluted, *B. truncatus* was the only snail found and in very low counts. Also, **El-Hawary (1990) and Abdalla *et al.* (1997)** reported that snails exposed to Pb, Cd and Hg continued to be reproductively active and no significant effect of heavy metal exposure was demonstrated under all tested concentrations. **El-Emam and Roushdy (1981)** revealed that optimum temperature for these mollusca lies between 22-26 o C. Whereas in the present study,

it was found that the snails tolerated to a wider range of temperature, 16- 35 o C. Also, **Kariuki *et al*.(2004)** did not find a significant link between snail abundance and water temperature. In addition, **Mahmoud (2001)** reported that in general, adverse effects of water pollution on snail biology were modified by biotic factors including food supplies, aquatic plants, behavioral and physiological adaptation. The present comparison of habitat characteristics for *B. alexandrina*, *B. truncatus***,** non-target snails and that free from snails showed very highly significant in temperature between habitats of *B. alexandrina* and that of *B. truncatus*, and highly significant difference between habitat of *B. truncatus* and that of non-target snails. Habitats free from snails showed non-significant higher recordings of field observations of certain elements concentrations, Cd, Na and K than other categories while habitats harboring *B. alexandrina* and *B. truncatus* were more tolerant than non-target snails and *B. alexandrina* was more tolerant than *B. truncatus* to some of the examined parameters. The suggestion of more *B. alexandrina* tolerance may explain why it is more distributed than *B. truncatus* in the present study (by about 7 times). **El-Khayat *et al.* (2011a)** found that *B. alexandrina* was significantly found to live under the highest level of Pb, Cd,Cu, Na, K and Ca concentrations than the other twelve snail species (p<0.01). Also, **El-Khayat *et al.* (2011b)** studied the habitat characteristics for different freshwater snail species biologically through macroinvertebrate information and concluded that *B. alexandrina* was more distributed than *B. truncatus* in sites that evaluated as very poor sites (23% and 9.4%, respectively). In addition, **Mahmoud (1994)** showed that habitat preferred by *B. alexandrina* snails contains higher concentration of various common ions (Na, K, Ca) and tolerated higher water conductivity as compared with habitat preferred by *B. truncatus*. **Didonato *et al.* (2003)** commented that snails can live in a wide range of mineral content in water till they are approached by certain limiting values**. El-Khayat *et al.* (2009**) found *B. alexandrina* and *B. truncatus* in habitats with Cd ranging from 4.298 -13.761 PPb; Pb around 28 PPb, Cu ranging from 59.847–1881.17 ppb. These ranges were around that determined in the present work; Cd ranging from 45.042-54.418 ppb, Pb ranging from 19.757-23.535 ppb and Cu around 28.0 ppb. In the same consequence, studies on the distribution and population density of *B. alexandrina* and *B. truncatus* in Egypt revealed no significant differences in various parameters such as water PH, conductivity, oxygen concentration and salinity between habitats harboring snails and those free of them **(Abdel Kaer, 2001; Abdel Kader *et al.*, 2005 and Ragab and Bakry, 2006).** The survey study observed four aquatic plant species, *Eichhornia crassipes* and *Lemna gibba* were the mostly infested aquatic plants and found in all centers, both *Biomphalaria* and *Bulinus* were mostly associated with *E. crassipes* with percentages of 51.3 and 21.3%, respectively. This high infestation of aquatic plants may be related to high infestation of *B. alexandrina* and *B. truncatus*. Also, **El-Khayat *et al.* (2009)** planned a study to elucidate the association between macrophytes, snails and some water quality parameters. Results showed that sites in which snails associated with macrophytes (64%) were characterized with higher ranges of chemicals, dissolved oxygen and conductivity than that observed in sites with snails only indicating the helpful role of macrophytes for increasing snail tolerance to unfavorable conditions. **Dazo *et al.* (1966)** reported that *B. truncatus* was most abundant in large canals while *B. alexandrina* was most abundant in drains. Both species were most abundant in the presence of aquatic vegetation, but they differed in their respective associations with the and *E. crassipes*. Similar observations were made by **Kader (2001)** for *B. alexandrina* and *B. truncatus* in Egypt, who reported different associations in relation to aquatic plants**. Kloos *et al.* (2004)** found a significant association between vegetation density and snail occurrence. However, they also found large populations of *B. glabrata* in calcium-rich limestone springs and wells with little or no macro vegetation. Also, the present study observed significant correlation between moderate density of all the recorded plants and *B. alexandrina* infestation in most centers. These findings were reinforced by **El-Homossany (2006)** who reported that when the density of aquatic plants were high, the number of collected snails was fewer, indicating that snails prefer low- medium density of aquatic plants. Also, **Appleton (1978) and Madsen (1981)** found that schistosome vector snails prefer water bodies with a moderate growth of aquatic plants.

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Prevalence of *Mycoplasma* species in Urine Samples Collected from Female Patients attending University of Abuja Teaching Hospital, Gwagwalada, FCT-Nigeria

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Abstract: Prevalence of *Mycoplasma* species among female patients attending University of Abuja Teaching Hospital, Gwagwalada-Abuja was conducted. A total of one hundred urine samples were collected from females aged 15-40 years. 0.5 ml of each urine sample was poured into a universal bottle containing 5 ml of freshly prepared Brain- Heart Infusion broth and seeded with 1 ml suspension of sterilized yeast, 1 ml suspension of penicillin, 5 ml of phenol red and 5 ml of L-argentine and was incubated at 37^{o}C for 24 hours. Furthermore, 0.3 ml of the mixture was inoculated onto freshly prepared Brain-Heart Infusion Agar and incubated at 37^{o}C for 3days. The result obtained showed that the overall prevalence rate of *Mycoplasma* species was 14%. Younger age group (15-20 years) had higher prevalence rate (42.90%) of *Mycoplasma* species infection than the older female subjects. However, out of 14 positive samples identified, nine (64.30%) were infected by *Ureaplasma urealyticum* and five (35.70%) were infected by *Mycoplasma hominis*. This study has highlighted the need to raise awareness of colonization of the urinary tracts by *Mycoplasma* species, hence there is need to expand services for prevention and treatment of this infection among females.

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Keywords: Prevalence, *Mycoplasma*, urinary tracts, females

1. Introduction

Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. Urinary tract consists of the various organs that produce, store and get rid of urine and it include the kidneys, the ureters, the bladder and the urethra (Ugoh *et al*., 2013). The urinary tract can be infected from above (by bacteria entering the kidneys from the blood stream and travelling downward) or from below by bacteria entering the urethra and travelling upward (Zahoor *et al*., 2005). Urinary tract may be inhibited by a relatively consistent normal flora consisting of *Staphylococcus epidermitis*, *Enterococcus faecalis* and some alpha-hemolytic Streptococci. *Chlamydia* and *Mycoplasma* have also been implicated to be among the organisms found in the urinary tract (NIDDK, 2005). From a microbiological perspective, urinary tract infection exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidneys or prostrate.

Microorganisms such as *Mycoplasma* can also cause urinary tract infection in both human and animals. Genital *Mycoplasma* (*Ureaplasma urealyticum* and *Mycoplasma hominis*) are frequently isolated in the genitourinary tract, particularly in sexually active women. It is generally difficult to determine whether these agents cause colonization or infection. The incidence of infection is affected by the menstrual cycle and pregnancy, and the use of vaginal contraceptives. The prevalence of these organisms is significantly associated with socio-economic conditions, i.e. poverty, and large numbers of sexual partners (Kreiger *et al*., 1996).

Colonization of the urinary tract by *M. hominis* and *U. urealyticum* can occur during birth but in most cases, the infection will be cleared. Only in a small number of cases does colonization persist. However, when individuals become sexually active, colonization rates increase. Approximately 15% are colonized with *M. hominis* and 45% - 75% with *U. urealyticum*. The carriers are asymptomatic but the organisms can be opportunistic pathogens.

Ureaplasma species have been isolated from cervico-vaginal specimens in 40-80% of women who are asymptomatic and sexually active (Razin *et al*., 1998) while *M hominis* has been isolated from cervico-vaginal specimens in 21-53% of women who are asymptomatic and sexually active. These rates are somewhat lower in males. Only subgroups of adults who are colonized in the lower uro-genital tract develop symptomatic illness from these organisms. Non gonococcal urethritis is the most common sexually transmitted infection. *Ureaplasma* species and *M. genitalium* may account for a significant portion of cases that are not due to *Chlamydiae*. More than 20% of live born infants may be colonized by *Ureaplasma* species and infants born preterm most likely harbor the organisms. Colonization declines after 3 months of age. Less than 5% of children and 10% of adults who are

not sexually active are colonized with genital mycoplasmal microorganisms (Tsunoe *et al*., 2000).

Immunosuppression (e.g, from antibody deficiency or prematurity) increases the likelihood of developing disseminated disease. Much less is known about the epidemiology of species such as *M genitalium* and *M fermentans*. Some organisms, such as *M. pirum* and *M. penetrans,* have been primarily isolated from persons with HIV infection but their significance as pathogens in this population has not been established (Tsunoe *et al*., 2000).

However, the aim of this work is to determine the prevalence of *Mycoplasma* species among females attending University of Abuja Teaching Hospital, Gwagwalada, Federal Capital Territory, Abuja, Nigeria.

2. Materials and Methods

2.1 Study Area

Gwagwalada is located in Abuja, the Federal Capital Territory of Nigeria. It lies between latitude 8^0 – 9^0N and between longitude 7^0 – 8^0E, with a total land area of 1, 043km^2 and annual humidity of 20% - 30%, and average temperature of between 27^0C – 32^0C. It has an estimated population of 157, 770 at the 2006 census.

2.2 Study Population

One hundred (100) early morning clean-catch urine samples were collected randomly from female patients (between the ages of 15-40 years) with symptoms of vaginitis who were referred to the Microbiology Laboratory of University of Abuja Teaching Hospital, Abuja.

2.3 Collection and storage of the urine samples

Early morning mid-stream "clean catch" urine samples were collected from female patients, using universal sterile containers with screw caps. The patients were instructed on how to collect the samples observing all aseptic conditions such as washing the vagina with sterile water and avoiding the vagina from making contact with the container. After collection, the samples were stored in the refrigerator at temperature of 4^{o}C until when needed.

2.4 Preparation and sterilization of media

The media used were Brain- Heart Infusion Broth and Brain-Heart Infusion Agar. The media were prepared based on manufacturer's instructions and sterilized by autoclaving for 15 minutes at 121^0C.

2.5 Microbiological analysis of the urine samples

This was carried out according to the modified method by Chessbrough (2006). 0.5ml of each urine sample was poured into the smaller bottles containing 5 ml of freshly prepared Brain heart infusion broth. About1 ml of the sterilized yeast suspension, 1 ml of penicillin suspension, 5 ml of phenol red and 5 ml of L-argentine were added to it to serve as an inoculated transport media. The bottles were labeled accordingly for easy identification. It was then incubated at 37^{o}C for 24 hours. Color change was taken as a criterion for growth i.e. a change from pink color to golden yellow or yellow color.

Furthermore, 0.3 ml of medium sample was inoculated unto a freshly prepared Brain-Heart Infusion agar using spread plate technique. The plates were incubated at 37^{o}C for 3 days. "Fried egg" appearance and growth of colony into the agar was observed and are typical characteristics of *Mycoplasma* species. The colonies were further characterized by their hemolytic and glucose fermentation properties.

2.6 Statistical analysis

The data were subjected to Chi-square test at 5% level of significance ($P \leq 0.05$).

3. Results

The identification of genital mycoplasmas was based on colonial morphology and growth properties. The colonies of a fried–egg appearance represented a prominent feature that started as a central dense growth in agar.

Table 1 shows the overall prevalence rate of *Mycoplasma* species infection among female patients visiting University of Abuja Teaching Hospital, Abuja. Out of one hundred urine samples collected from the female patients that were examined, 14 (14%) were infected with *Mycoplasma* spp.

Table 1: Rate of *Mycoplasma* species infection among female patients

Number of samples examined	Number infected by *Mycoplasma* species	Percentage Rate of Infection
100	14	14.00%

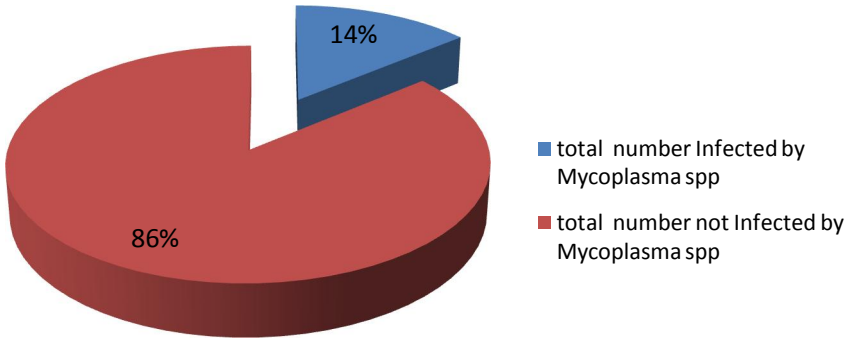

Fig 1: Rate of *Mycoplasma* spp Infection among female patients

Table 2 shows the age specific prevalence rate of *Mycoplasma* spp Infection among female patients visiting University of Abuja Teaching Hospital, Abuja. No *Mycoplasma* spp was isolated in the urine samples of patients between the ages of 15-20 years. Seven per cent infection rate was recorded for the ages of 21-25 years. 14.30% infection rate was recorded for the ages of 26-30 years. 35.7% infection rate was recorded for the ages of 31-35 years. 42.9% infection rate was recorded for the ages of 36-40 years.

Table 2: Sex Specific Prevalence Rate of *Mycoplasma* spp Infection among Female Patients Visiting University of Abuja Teaching Hospital, Abuja

Age Group (Years)	Total Number infected by *Mycoplasma* spp	Rate of Infection (%)
15-20	6	42.90%
21-25	5	35.70%
26-30	2	14.30%
31-35	1	7.0%
36-40	0	0%
Total	14	14

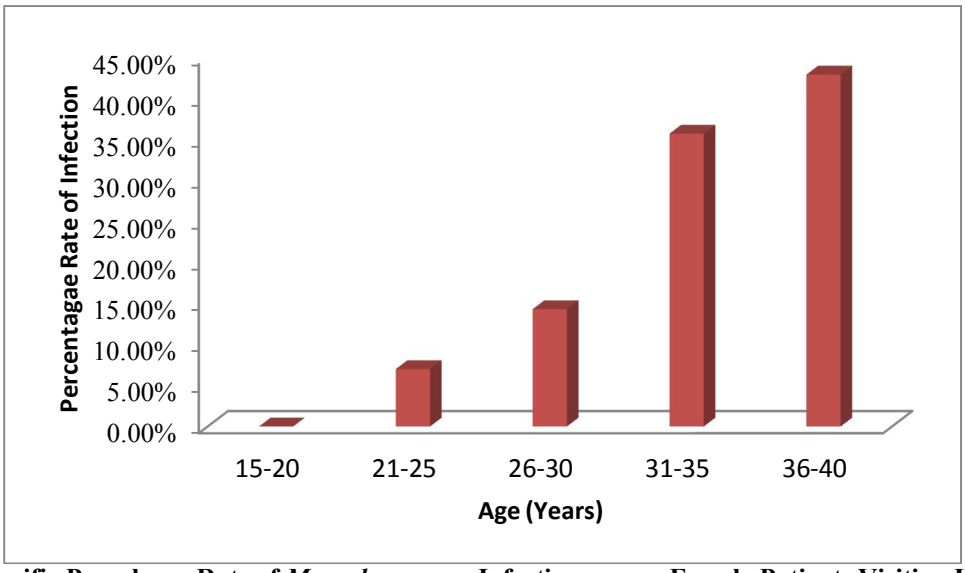

Fig 2: Sex Specific Prevalence Rate of *Mycoplasma* spp Infection among Female Patients Visiting University of Abuja Teaching Hospital, Abuja

Table 3 shows the species specific prevalence rate of *Mycoplasma* spp Infection among female patients visiting University of Abuja Teaching Hospital, Abuja.Out of 14 positive samples identified, 9 (64.30%) were infected by *Ureaplasma urealyticum* and 5 (35.70%) were infected by *Mycoplasma hominis.*

Table 3: Species- Specific Prevalence Rate of *Mycoplasma* Infection among Female Patients Visiting University of Abuja Teaching Hospital, Abuja

Species	Total Infected	Percentage Rate of Infection (%)
Ureaplasma urealyticum	9	64.30
Mycoplasma hominis	5	35.70
Total	14	100

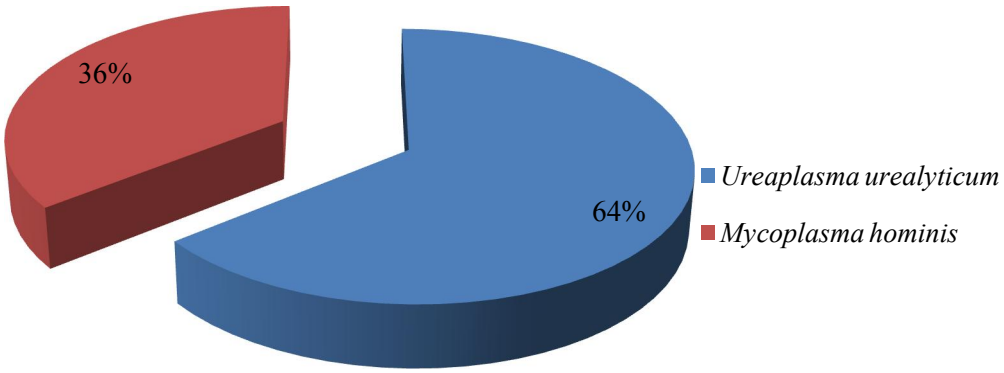

Fig 3: Species-Specific Prevalence Rate of *Mycoplasma* Infection among Female Patients Visiting University of Abuja Teaching Hospital, Abuja

4. Discussion

The overall incidence rate of *Mycoplasma* species in this study population was 14% (Table 1). The incidence of *Mycoplasma* spp reported in this study may also be attributed to such factors as poor housing conditions of some rural areas in Gwagwalada, lack of proper personal and environmental hygiene among the female patients. These can serve as a contributory factor in increasing the rate of *Mycoplasma* spp infection among the female patients.

The age specific prevalence rate of *Mycoplasma* spp infection among female patients attending University of Abuja Teaching Hospital, Abuja showed that younger age groups had higher prevalence rate of *Mycoplasma* spp infection than older years, hence the prevalence rate of *Mycoplasma* spp infection decreases as age increases. No *Mycoplasma* spp was isolated in the urine samples of patients between the ages of 35-40 years. This confirms the report of Gonzalez *et al*. (2003) that the risk of UTIs decreases with age.

The species specific prevalence rate of *Mycoplasma* spp infection among female patients visiting University of Abuja Teaching Hospital, Abuja showed that out of 14 positive samples identified, 9 (64.30%) were infected by *Ureaplasma urealyticum* and 5 (35.70%) were infected by *Mycoplasma hominis* (Table 3), hence *Ureaplasma urealyticum* had higher prevalence rate than *M. hominis.* This is in accordance with the findings of Chua *et al*. (1998) on maternal cervical colonization rates where *U. urealyticum* had higher prevalence rate (58%) than *M. hominis* (16%).

The findings of this study revealed that *Mycoplasma* species were found to be the commensals of peri-anal and vaginal regions and this call for increase in personal hygiene. This study has highlighted the need to raise awareness of UTI and to expand services for prevention and treatment among females. To do this effectively, however, it may be necessary to improve the quality of health care provided at the community level. Since UTI may be symptomatic and asymptomatic in most cases, it is therefore suggested that routine screening of patients with unexplained sources of fever be done and the appropriate chemotherapy administered in order to prevent the cases becoming symptomatic later with resultant renal damage.

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11/12/2013

Spermidine as modulator of growth, some metabolic activities and reproductive development of *Helianthus tuberosus plants* grown in two types of soil

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Abstract: Growth, yield and some metabolic activities of *Helianthus tuberosus* plants were studied in response to the treatment with spermidine under normal and saline soil. The obtained results revealed that, under saline conditions, treatments with spermidine (50 & 100 ppm) generally enhanced most of the growth and yield characteristics (shoot length, root length, fresh and dry weights of shoots and roots/ plant, number of tubers / plant and tubers weights) of *Helianthus tuberosus* plants. Plants which planted in saline soil showed significant decreases in contents of chlorophyll (a), (b) & (a + b), carbohydrates, soluble proteins, while phenols, carotenoids and free proline contents were increased throughout the experimental period. At saline conditions, catalase activities (CAT) and peroxidase activities (POX) were increased. Treatment with spermidine caused significantly increases in CAT and POX activities in plants grown in saline or non-saline soil.
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Keywords: spermidine; saline soil; *Helianthus tuberosus*; metabolic activities

1. Introduction

Soil salinity considered the major factors that reduce plant growth in many regions in the world. Two important limitations for crop production in arid and semi–arid regions are water shortage and salinity **(Sepaskhah and Yousofi-Falakdehi, 2010).** In salt–affected soil, there are many salt contaminants, especially NaCl which readily dissolves in water to yield the toxic ions, sodium ion (Na^+) and chloride ion (Cl^-). Also, the water available in the salt–contaminated soil is restricted, inducing osmotic stress **(Pagter *et al.*, 2009; Siringam *et al.*, 2011).**

Jerusalem artichoke (*Helianthus tuberosus*), which is also called the sunchoke, sunroot or earth apple, is an Angiosperm plant species of Asteraceae family **(Tassoni *et al*., 2010)**. More importantly, as *Helianthus tuberosus* possesses prophylactic and medicinal properties for sufferers of sugar diabetes, and act as a biogenetic factor for the development of natural intestinal microflora after disbacteriosis **(Rakhimov *et al.*, 2003),** it has been becoming an interesting research topic and it is even labeled as a new cultivated crop **(Terzic and Atlagic, 2009).**

Approaches have been carried out to minimize the adverse effect of salinity by using polyamines a foliar spray **(Amri and Shahsavar, 2010).** Polyamines influence plant development in many ways and the response of plants to abiotic stress involves complex physiological and biochemical responses, including changes in the concentration and ratio of different metabolites **(El-Bassiouny *et al.*, 2008).** Although most of the known polyamines are regulators of growth, maximum plant growth may depend on the ratio rather than the absolute levels of these substances in plants. These ratios may change dramatically in response to salinity stress, thereby leading to physiological disturbances associated with a general reduction in growth **(Sood and Nagar, 2003).** Also pretreating seed with optimal concentrations of polyamines can effectively improve germination as well as growth and yield of crops under both normal and stress conditions (**Abd El Monem, 2007; Karimi and Rahemi, 2012).** So, the aim of the present work was to evaluate the use of polyamines for improving growth and productivity as well as increasing salt tolerance of *Helianthus tuberosus* plant, and also to study the oxidative defense mechanism of *Helianthus tuberosus* plants against salt stress. Via determination of the growth characters and some essential metabolites throughout the different stages of the growth.

2. Material and Methods

2.1. Methods of planting, treatments and collection of samples

Uniform *buds Helianthus tuberosus L.* tubers were planted in two types of soils in Kafr El_Shikh one represented non-saline soil (Table 1) and the other place is saline soil (Table 2) in terraces. The developed plants were irrigated whenever required. Plants grown in either saline or non-saline soil were divided into 3 groups representing the following treatments:

- Treated with distilled water (control).
- Spermidine (50 ppm spd as spermidine).

- Spermidine (100 ppm spd as spermidine).

The plants of *Helianthus tuberosus L.* were treated twice with the above mentioned treatments (as foliage spraying). The first treatment was made when the age of plants was 60 days, while the second treatment was made when the age of plants was 90 days of sowing. The plant samples were collected for analysis when the plants were 70 (Stage I) and 100 (Stage II) days old. At the end of the growth season (180 days), analyses of the tubers yielded from the different treatments as well as the control were done.

Table 1. Chemical properties of the used soil (Non-saline soil)

TSS ppm	pH	E.C. mmhos/cm	Cations meq/L				Anion meq/L			
768	7.9	1.24	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	$SO4^{--}$	$HCO3^-$	$CO3^{--}$
			2.5	0.5	2	1	4	1	1	Zero

Table 2. Chemical properties of the used soil (Saline soil)

TSS ppm	pH	E.C. mmhos/cm	Cations meq/L				Anion meq/L			
4760	8.3	7.25	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	$SO4^{--}$	$HCO3^-$	$CO3^{--}$
			4.75	1.5	5	2.5	7	5.25	1.5	zero

2.2. Measurement of growth parameters

Plant height (m),root length (cm), number of leaves per / plant, fresh and dry weights of shoots (Kg/plant), fresh and dry weights of roots (g/plant), number of tubers, weight of tuber were determined at different growth stages.

2.3. Chemical analysis

Three plant samples were taken during the growing season, at vegetative growth stage, flowering stage and yield stage.

Photosynthetic pigments were estimated using the method of **Vernon and Selly (1966)**. Contents of soluble carbohydrates were measured according to the method of **Umbriet *et al.* (1969).** Contents of soluble proteins were estimated according to the methods of **Lowery *et al.* (1951).** Phenolic compounds were estimated according to the methods of **Daniel and George (1972).** Proline contents tubers were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000g at 4°C for 10 min. The supernatants were used for proline estimation according to the method of **Bates *et al.* (1973).** Enzymes catalase (CAT) and peroxidese (POX) were estimated according to the methods of **Kong *et al.* (1999)**.

2.4. Statistical analysis

The data were subjected to the proper statistical analysis of variance of a randomized complete block design For comparison between treatments means, using t test, (PASW® Advanced Statistics 20, 2010) at 5%level was used.The values recorded in the values of the biochemical analysis are means of three replicates. Discriminant analysis is used to classify several observations, into these known groups **(Härdle and Simar, 2007).**

3. Results and Discussion

3.1. Growth responses

The obtained results (Figs. 1-8) showed a retarded growth in salt-stressed plants. Plant height, fresh and dry weight of both shoots and roots and the number of leaves/plant were significantly decreased under saline condition. The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the plants competitors between Na^+ and Cl^- and further anions and cations may result in a reduced plant growth and yield **(keutgen and Pawelizik, 2008)**. Many studies have shown that biomass partitioning between roots and shoots is strongly influenced by the most limiting resource under stress growth conditions, and resource deficiency is often ameliorated by increasing the biomass allocation to the part of the plant responsible for acquiring the most limiting resource **(Jamil *et al.*, 2006).**

Results of the present study (Fig. 8) showed that, with a few exceptions application of spd was of stimulatory effects regarding the growth characters as well as the yield of the tested plants. This was the case in plants grown under both saline and non-saline conditions. These results are in agreement with the previous data obtained by **El-Bassiouny *et al.* (2008); Mostafa *et al.* (2010)**. The use of polyamine to improve the yield and yield components was recommended by other investigators. In this regard, **Abd El-Wahed and Gamal El-Din (2005)** on chamomile indicated that the sprayed plants with polyamine showed a marked increase in the number of pods/ plant, weight of pods/ plant and seed index.

The improving of plant growth under salt stress conditions by spraying with PAs may be due to the role of PAs on enhancing cell division activity, increasing of proline accumulation **(Hussein *et al.*, 2006).** Polyamines are currently considered to be regulators of plant growth and development owing to their effects on cell division and differentiation **(Verma and Mishra, 2005). Alsokari (2011)** investigate the control of physiological stress resulted from salinity on cowpea plants using foliar spray with certain growth regulators (spermine). He found that, growth regulation treatments alleviated, with variable degrees, the harmful effects of salt stress on plant growth measurements of different criteria during different periods of growth.

Hussein *et al.* (2006) also reported that PAs improved all metabolic activities in pea plants under salinity stress conditions and make plants more tolerant to stress, thereby increase growth and yield of pea plants. In addition, **Suleiman *et al.* (2002)** stated that PAs associated in the regulation of cellular ionic environments, maintenance of membrane integrity and prevention of chlorophyll loss and stimulation of synthesis of proteins and nucleic acids. Furthermore, PAs were effective in improving grain yield in wheat under saline conditions **(Iqbal *et al.*, 2006).**

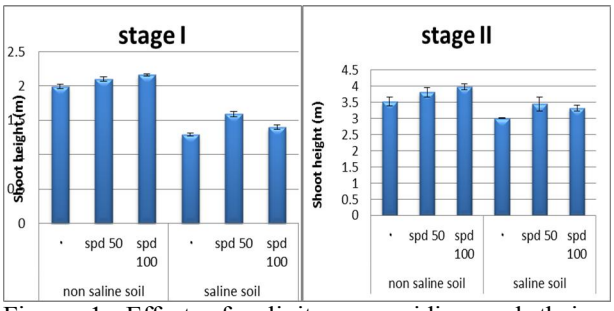

Figure 1. Effect of salinity, spermidine and their interactions on shoot height of *Helianthus tuberosus* plants

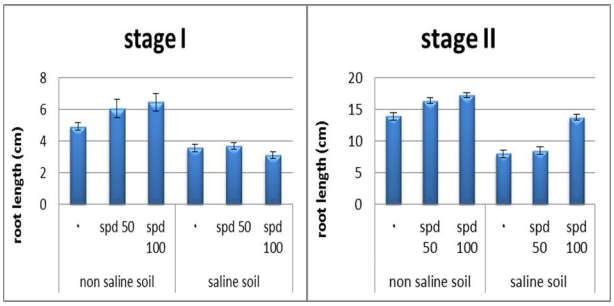

Figure 2. Effect of salinity, spermidine and their interactions on root length of *Helianthus tuberosus* plants

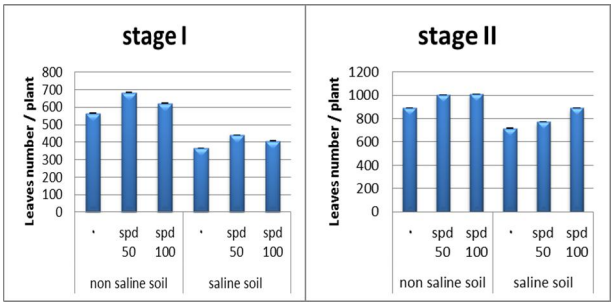

Figure 3. Effect of salinity, spermidine and their interactions on no. of leaves of *Helianthus tuberosus* plants

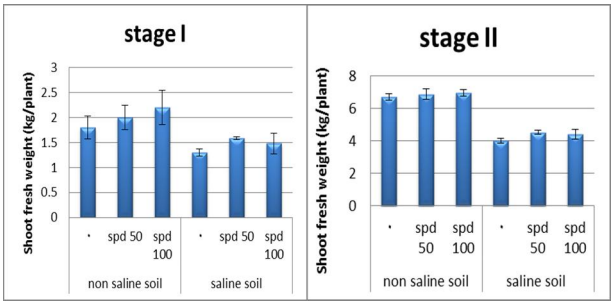

Figure 4. Effect of salinity, spermidine and their interactions on fresh weight of shoot (kg) of *Helianthus tuberosus* plants

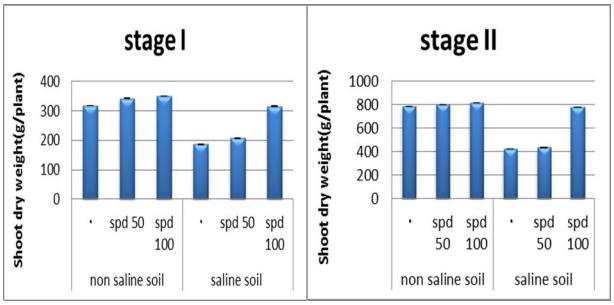

Figure 5. Effect of salinity, spermidine and their interactions on dry weight of shoot (g) of *Helianthus tuberosus* plants

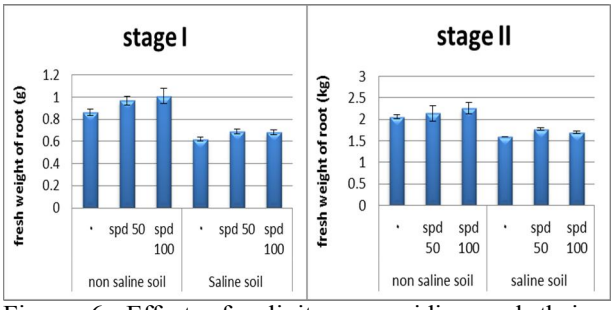

Figure 6. Effect of salinity, spermidine and their interactions on fresh weight of root (g) of Helianthus tuberosus plants

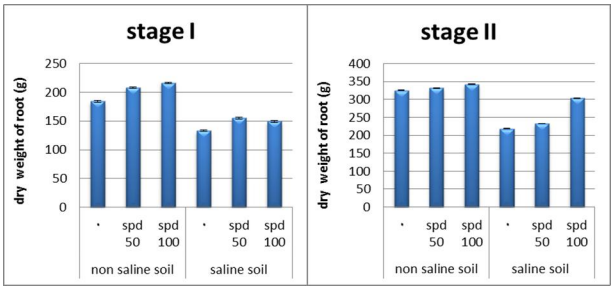

Figure 7. Effect of salinity, spermidine and their interactions on dry weight of root (g) of *Helianthus tuberosus* plants

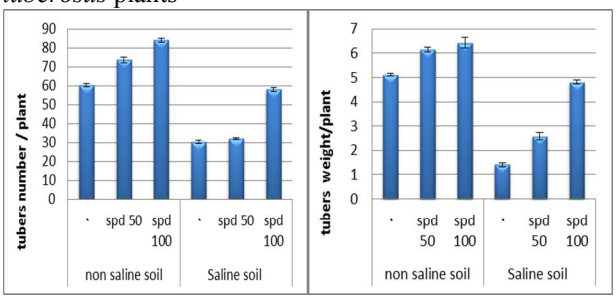

Figure 8. Effect of salinity, spermidine and their interactions on No.of tubers and tubers weight of *Helianthus tuberosus* plants

3.2. Photosynthetic pigments

Photosynthesises is essential for the normal growth and metabolism of plants. It depends on the normal availability of chlorophylls in the chloroplasts and on several enzymes which function during the light and dark phases. A normal functioning of the dark phase results in the production of monosaccharide, disaccharides and polysaccharide by the plant.

In the present investigation, results (Figs. 9-12) clearly reveal a reduction in the photosynthetic pigment levels (chlorophyll a, chlorophyll b and carotenoids) in the leaves of helianthus plants due to saline soil. This finding is supported by the study of **Almodares *et al.* (2008)** on sorghum, and **Sofy (2011)** on lentil. The reduction in chlorophyll contents due to salinity is probably due to the inhibitory effect of the accumulated ions (Na^+ and Cl) on the biosynthesis of the different chlorophyll fractions (**Mohammed, 2007**).

Results of the present work (Figs. 9, 10, 11) showed that, at different soils, contents of chlorophyll (a) and (b) as well as total chlorophyll (a + b) were, mostly, significantly increased due to the application of spd. Also, contents of cartenoids were significantly increased throughout the two stages of growth spesialy in plants grown under saline condition. In this regard, **El-Bassiouny and Beckheta (2001)** reported that PAs mitigate stress through modulating stomatal movement, providing a link between stress condition, PAs levels and stomatal regulation. The addition if PAs in nutritive solution reduced rice growth in the absence of NaCl and did not offered protection in the presence of salt **(Ndayiragijc and Lutts, 2006).**

Chattopadhayay *et al.*, (2002) added that PAs prevented chlorophyll loss, inhibition of photochemical reactions of photosynthesis. Also **Huiguo *et al.* (2006)** found that exogenous application of PAs protect PSll against water stress at both transcriptional and translational levels and allow PSll to retain a higher activity level during stress in wheat seedling resulting in the increase in chlorophyll contents.

Gupta *et al.* (2003) found that PAs increased the total chlorophyll contents of wheat plants. Also **El-Bassiouny and Bekheta (2001)** reported that exogenous application of putrescine (1.25 mM) induced significant increase in chlorophyll, Mg contents and total soluble sugars.

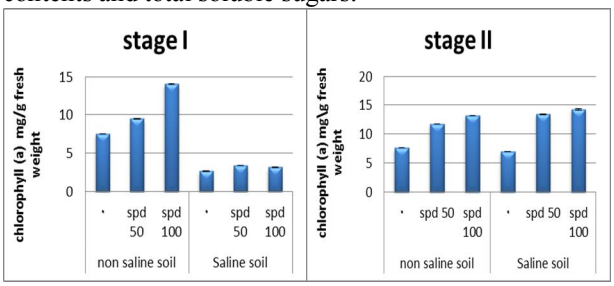

Figure 9. Effect of salinity, spermidine and their interactions on chlorophyll a content of *Helianthus tuberosus* plants

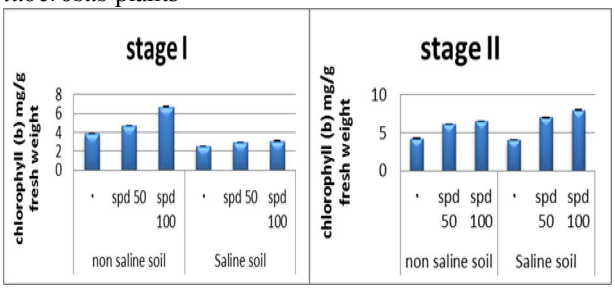

Figure 10. Effect of salinity, spermidine and their interactions on chlorophyll b content of *Helianthus tuberosus* plants

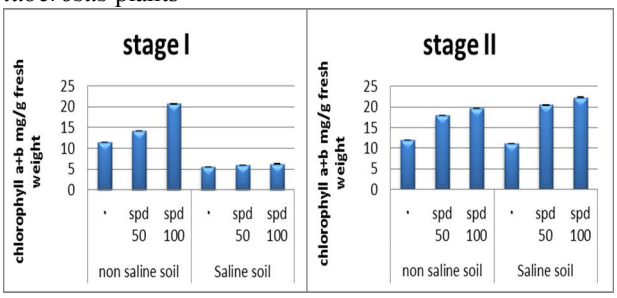

Figure 11. Effect of salinity, spermidine and their interactions on chlorophyll a+ b content of *Helianthus tuberosus* plants

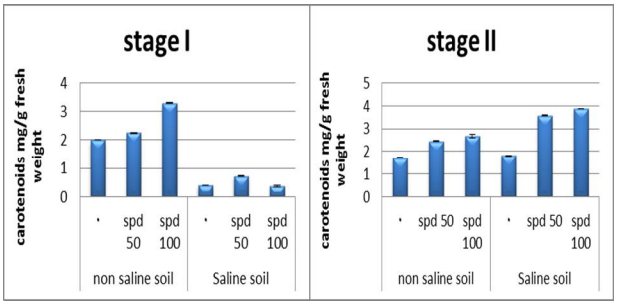

Figure 12. Effect of salinity, spermidine and their interactions on Carotenoids content of *Helianthus tuberosus* plants

3.3. Carbohydrates

In the present study, results show that total soluble carbohydrates decreased in helianthus plants grown in saline soil. **Rejeskova *et al.* (2007)** indicated that salinity caused a decrease in total carbohydrates in *Olea europaea* L. shoots. The decrease was probably not due only to osmotic stress but also to the presence of toxic Na^+ and Cl^- ions.

Results of the present work (Figs. 13, 14, 15) showed that contents of total carbohydrates (total soluble sugars) were, generally, significantly increased in shoots, roots and in yielded tubers in response to the treatment with spd. In this regard, several investigators proved that polyamine plays an important role in carbohydrate metabolism **El-Bassiouny*et al.*, (2008)** found that application of PAs treatment had favourable effect on the synthesis and accumulation of carbohydrates in leaves of wheat plants. In most cases, plants sprayed with PAs at the different concentrations had higher contents of total carbohydrates in their leaves, compared to the untreated control. Moreover, **Liu *et al.* (2002)** found that spraying Brassica plants with PAs gave the highest value of total soluble sugar percentage. PAs alleviated the adverse effects of salt stress on total soluble sugars concentration and this is at least partially due to enhanced amylase activity and chlorophyll content, reported as a response **(Sood and Nagar, 2003).**

Data of the present investigation (Figs. 13, 14, 15) reveal that the reduction in the total carbohydrate levels induced by high salinity counteracted by low and high applied concentrations of spd. These effects were observed at all growth stages but with different degrees. Application of spd generally increased the total soluble carbohydrates in helianthus plants in saline soil. It suggested that in the presence of spd, the leaves accumulated more compatible osmolytes as soluble sugar, glucose and fructose, a sugar alcohol, sorbitol and proline **(Szepesi, 2006).**

Hussein *et al.* (2006) reported that increasing the sugars concentration by putrescine application may be due to the role of putrescine on improving plant growth; carbohydrate content resulted in increasing plant tolerance to salinity stress.

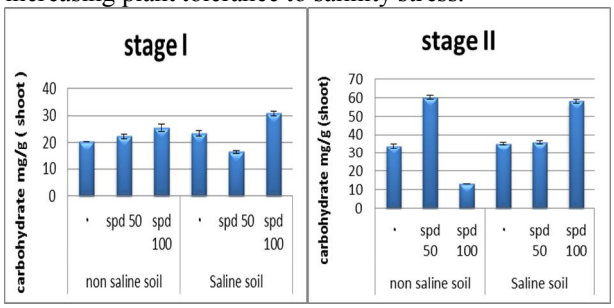

Figure 13. Effect of salinity, spermidine and their interactions on carbohydrate content (shoot) of *Helianthus tuberosus* plants

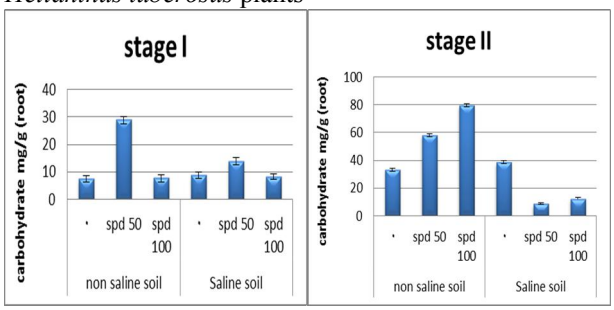

Figure 14. Effect of salinity, spermidine and their interactions on carbohydrate content (root) of *Helianthus tuberosus* plants

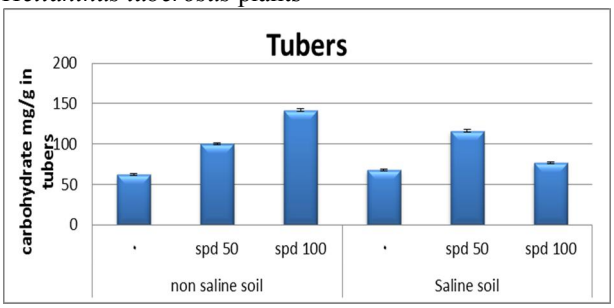

Figure 15. Effect of salinity, spermidine and their interactions on carbohydrate content (tubers) of *Helianthus tuberosus* plants

3.4. Protein

Our results (Figs. 16, 17, 18) showed that the total soluble protein decreased significantly in shoots roots and yielded with saline soil. In *phyllanthus amarus* plants, NaCl reduced the protein content **(Jaleel *et al.*, 2008**); these results were in agreement with **Sofy (2011)** in lentil.

Results of the present study (Figs. 16, 17, 18) indicated that protein contents in shoots, roots as well as in the yielded tubers of helianthus plants were, generally, increased in response to the all used concentrations of spd. The obtained results are in

agreement with those of **Jaleel *et al.* (2008)** on *Phyllanthus amarus* plant, indicated that foliar application of PAs markedly increased the protein contents in the yielded seeds. **El Tayeb (2005**) found that PAs treatment significantly increased the contents of soluble protein in the shoots of barley plants. **Sood and Nagar (2003)** mentioned that spraying wheat plants with PAs gave the greatest values of crude protein present per rose.

Results of the present work(Figs. 16, 17, 18) revealed that, helianthus plants, treatment with spd, in saline soil, resulted in, mostly, significant increases in protein contents in shoots, roots and also, in the yielded tubers. These results are in harmony with those obtained by **Amira Abdul Qados (2010)** on mung bean, where they found that, contents of proteins increased as a result of PAs application with saline soil.

El-Bassiouny *et al.* (2008) who indicated that PAs was the most effective compound in increasing soluble carbohydrate, poly saccharides, total carbohydrates, proline, total amino acid and protein contents of wheat plants and grains under normal or stressed condition.

3.5. Total phenols

Phenols act as free radical scavengers as well as substrates for many antioxidant enzymes (Martin-Tanguy, 2001). In the recent study of phenol levels (Figs. 19, 20, 21) were significantly increased in the shoot, root as well as yield tuber of helianthus plants which grown in saline compared with those of unstressed control plant through the experimental period.

Results of the present study revealed that contents of total phenol in shoots of spd-treated plants were significantly increased throughout the stages of growth. These increases were found to be in response to the applied concentrations of spd. The potent effect of spd as regards the contents of total phenols was recorded by other investigators. In this regard, **Agastian *et al.* (2000)** deduced that application of various concentrations of PAs induced high significant increases in the total phenol content of cowpea shoots. This increase in total phenol contents in response to PAs treatments concurrently with increase in IAA contents in shoots of cowpea plants led to the suggestion that most of phenol compounds are diphenols and polyphenols which may inhibit IAA oxidase activity resulting in auxin accumulation, which reflected in stimulated growth and yield of the treated plants.

The exogenous application of putrescine significantly increase the accumulation of phenols in stressed cowpea shoots and roots as being compared with their reference controls (grown at 100 and 200 mM NaCl). The increase in the accumulation of the phenolic compounds in stressed cowpea shoots and roots might be due to their increase in their biosynthesis. The increase in phenol levels have been reported in a number of plants grown under stress condition (**Farag Abeer, 2009).**

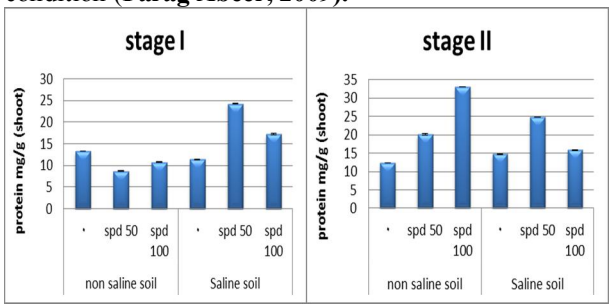

Figure 16. Effect of salinity, spermidine and their interactions on protein content (shoot) of *Helianthus tuberosus* plants

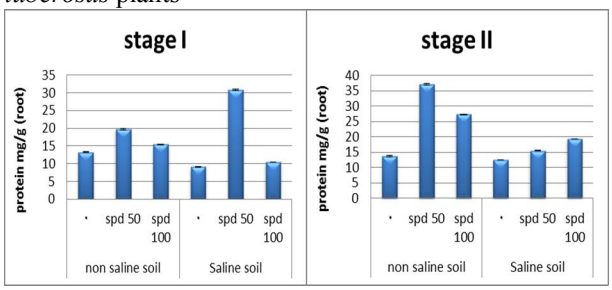

Figure 17. Effect of salinity, spermidine and their interactions on protein content (root) of *Helianthus tuberosus* plants

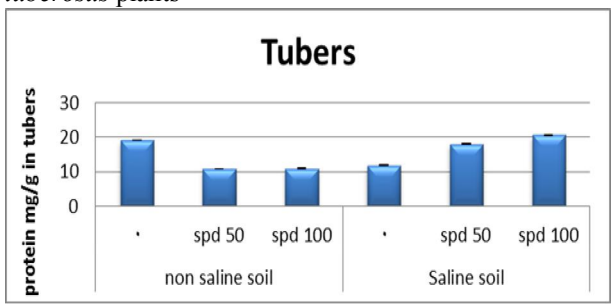

Figure 18. Effect of salinity, spermidine and their interactions on protein content (tubers) of *Helianthus tuberosus* plants

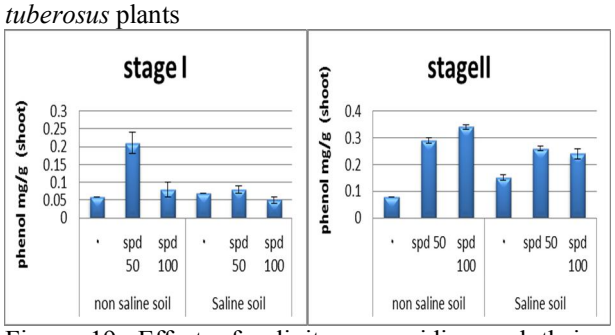

Figure 19. Effect of salinity, spermidine and their interactions on phenol content (shoot) of *Helianthus tuberosus* plants

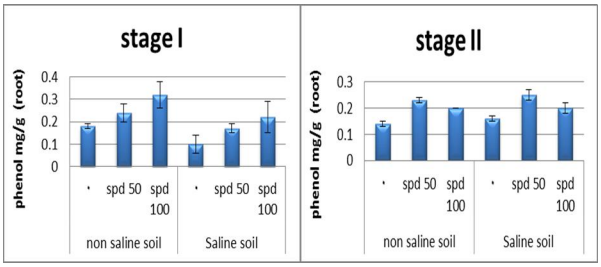

Figure 20. Effect of salinity, spermidine and their interactions on phenol content (root) of *Helianthus tuberosus* plants

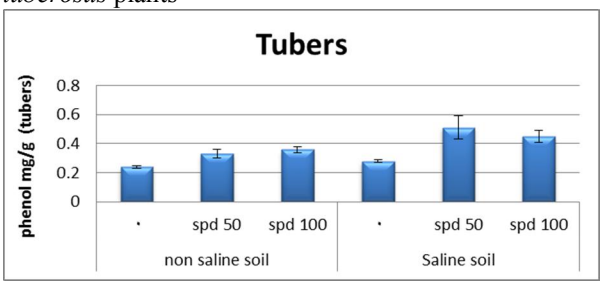

Figure 21. Effect of salinity, spermidine and their interactions on phenol content (tubers) of *Helianthus tuberosus* plants

3.6. Total proline

In organisms ranging from bacteria to higher plants, there is strong correlation between increased cellular proline levels and the capacity to survive under salt stress. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub cellular structure (membrane and proteins) scavenging free radicals and buffering cellular redox potential under stress conditions (**Ashraf and foolad, 2007).**

Our results (Figs. 22, 23, 24) showed that the proline contents increased significantly in plants which grown in saline soil. These results are in agreement with **Lobato *et al.* (2008)** in *Vigna unguiculata L.* and **Sofy (2011**) in lentil.

Many plant species naturally accumulate proline and glycine betaine as major organic osmolytes when subjected to different a biotic stress such as drought, salinity, extreme temperature and heavy metals. Proline is thought to play adaptive roles in mediating osmotic adjustment and protecting sub-cellular structure in stressed plants **(Ashraf and Foolad, 2007).**

Furthermore, **Kavi Kishor *et al.* (2005**) suggested that proline which is accumulated under stress conditions might serve as a sink for excess reductants providing the NAD^+ and $NADP^+$ necessary for maintenance of respiratory and photothetic processes. When proline synthesis generates $NADP^+$, its degradation produces NADPH which is essential for buffering cellular redox potential in the cytosol as well as plastids.

Proline was markedly increased in salt-stressed plants. Used polyamine affects the proline level of these plants. The interaction was significant spd, in control conditions, significantly increased proline concentration in *Vigna* plants as compared with corresponding untreated plants. The data presented showed that proline concentration was greatly increased in the salt-stressed plants and this is compatible with the fact that many higher plants accumulate free proline in response to salt stress **(Kavi Kishore *et al.*, 2005**).

Hussein *et al.* (2006) found that salinity stress increased proline content in pea shoots, whereas foliar application of putrescine caused a highly significant increase of proline contents over the untreated plants (control) but still lower than that of the salinized ones. They reported that the increase in proline contents in pea shoots may be during the catabolic processes of polyamines.

Accumulation of some compatible solutes (proline and free amino acids) in stressed plants produced lower solute potential, which allows plant cell to maintain a higher water content than the corresponding control. These solutes play an important role in plants under stress conditions, where major functions of sugars are osmoprotection and/or osmotic adjustment as reported by **Parida *et al.* (2002).**

Duan *et al.* (2008) reported that exogenous spermidine substantially increased the level of proline in cucumber roots under salinity stress.Although the proline level was increased by NaCl in salinized cowpea plants, yet putrescine reduced this measure below them. Despite of putrescine induced reduction in proline level, it was still significantly greater than the control ones (0.0 mM NaCl). Similar results were obtained by **Mansour *et al.* (2002)** who found that PAs treatment decreased ethylene production and proline accumulation which were increased by NaCl. They postulated that PAs inhibits proline accumulation under salt stress via the change in the properties of P-5-C.

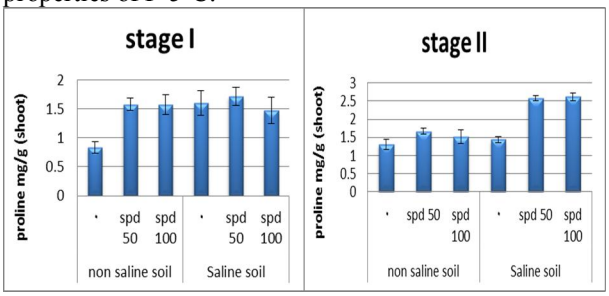

Figure 22. Effect of salinity, spermidine and their interactions on proline content (shoot) of *Helianthus tuberosus* plants

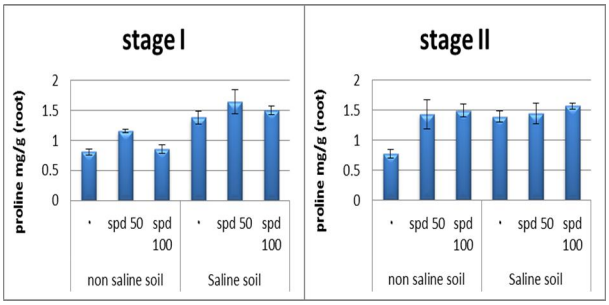

Figure 23. Effect of salinity, spermidine and their interactions on proline content (root) of *Helianthus tuberosus* plants

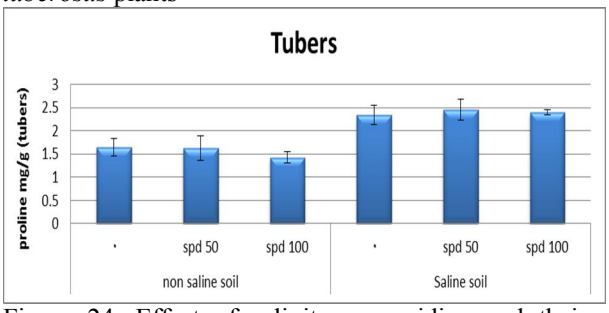

Figure 24. Effect of salinity, spermidine and their interactions on proline content (tubers) of *Helianthus tuberosus* plants

3.7. Antioxidant enzymes

Environmental stresses result in the generation of reactive oxygen species (ROS) in plants. ROS accumulate in cells and lead to the oxidation of proteins, chlorophyll, lipids, nucleic acids, carbohydrates etc. Cells have evolved intricate defence systems including enzymatic (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), non-enzymatic systems such as ascorbic acid (ASH), phenolic compounds, alkaloids, non-protein amino acids and α-tocopherol, which can scavenge the indigenously generated ROS. Plant stress tolerance mediated by antioxidants has been shown by many workers. Antioxidant resistance mechanisms may provide a strategy to enhance plant stress tolerance. Various enzymes involved in ROS-scavenging have been manipulated, over-expressed or down-regulated to add to the present knowledge and understanding of the role of antioxidant system. ROS induce the synthesis of several plant hormones, such as ethylene, salicylic acid (SA), jasmonic acid, brassinosteroids, abscisic acid (ABA) etc. These Phytohormones are required for growth and development of plants and deafens responses during environmental stresses. The present review throws light on the enzymatic and non-enzymatic antioxidants in plants to enhance stress tolerance in plants and also in particular the role of brassinosteroids and ethylene during a biotic stress tolerance in plants (**Parvaiz *et al*., 2010**).

ROS are scavenged by plant antioxidant defense systems, comprising both enzymatic and non-enzymatic components **(Ashraf, 2009).** Environmental stresses, such as salt stress, may lead to an imbalance between antioxidant defenses and ROS levels, resulting in oxidative stress **(Foyer and Noctor, 2003).**

Soil salinization caused osmotic stress which stimulates the production of ROS such as anion radical (O_2^-) hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) **(Azevedo Neto *et al.*, 2006**) the resistance to environmental stress may therefore depend at least partially on the inhibition of ROS production by enhancing the antioxidant defense system.

In this investigation, CAT activity was significantly increased in Jerusalem plants shoots which grown in saline soil compared to unsalinized control plants. These results are in accordance with results obtained by **Vardhini and Rao (2003)** observed that CAT activity decreased in susceptible sorghum and maize varieties but increased in resistance varieties as compared to unstressed control.

In addition, the inhibition of CAT activity is a phenomenon that occurs in many plant species exposed to oxidative stress and is related to the accumulation of SA **(Shim *et al*., 2003**). However, **Cai-Hong *et al.* (2005**) observed that the activity of CAT increased significantly in halophyte *suaeda salsa* L. grown under high concentration of NaCl (200 m M). They deduced that the increase in Cat activity might be related to the result of lowered H_2O_2 level.

An increase in peroxidase (POX) activity is considered as a second line of defence for scavenging the H_2O_2 under salinity (**Sharma *et al.*, 2005).** Data presented in this study showed that POX activity increased during salt stress and this increase was positively related to NaCl concentration. The increase in POX activity by salinity stress is established by **(Cavalcanti *et al*., 2004).**

The increase in POX activity appears to be caused either by by activation of existing enzymes isoforms. High reduction of plant growth (**Lin and Kao, 2002**) and this reduction has been attributed to POX catalysis of feruloylation of hemicelluloses and insolublization of hydroxyproline rich glycoprotein causing cell wall stiffening (**Dionisio-Sese and Tobita, 1998**) resulted in the inhibition of growth elongation (**Sudhaker *et al.*, 2001).**

Our results showed that (Figs. 25, 26), spd increase the activities of key enzymes involved in oxidative stress such as CAT, POX as being compared with their corresponding controls (NaCl), such effect of spermdine 100 ppm was greater at NaCl. Similar results are obtained by **Farag abeer (2009)** observed that putrescine increase the activities of key enzymes

involved in oxidative stress such as CATand POX, as being compared with their corresponding controls (100 and 200 mM NaCl), such effect of putrescine was greater at 100 mM NaCl.. Similar results are obtained by **Tang and Newton (2005)** on *Virginia pine*. However, **Kubis (2008)** showed that exogenous application of spermidine differentially influenced enzymes of the antioxidative system under stress conditions. He observed an increase of POX activity and to a lesser degree, and reduction of SOD and CAT in spermidine treated cucumber exposed to water stress. It was proposed that the protective effect of polyamine against the damage of the superoxide depended on their conversion to conjugated forms (**Langerbartels *et al.*, 1991).**

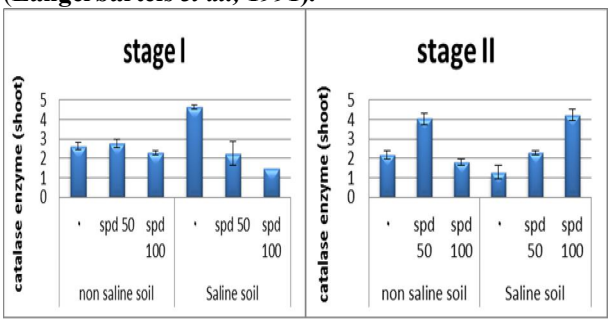

Figure 25. Effect of salinity, spermidine and their interactions on catalase enzyme (shoot) of *Helianthus tuberosus* plants

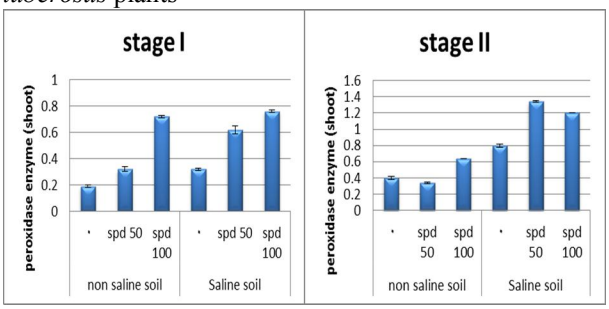

Figure 26. Effect of salinity, spermidine and their interactions on peroxidase enzyme (shoot) of *Helianthus tuberosus* plants

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Response of Maize Crop to Cyanobacteria Applied Under Different Nitrogen Rates

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Abstract: The present investigation was conducted in sandy soil at Ismailia Agricultural Research Station, Agric. Res. Center (ARC) (Latitude 30° 35′ 41.901″ N and Longitude 32° 16′ 45.843″ E), Egypt during two successive summer seasons of 2011 and 2012.Maize hybrid SC10 was used to study the effect of cyanobacteria (Cyano) and nitrogen fertilizer on maize growth, grain yield, yield components, and their effects on the biological activity of the soil around the rhizosphere of maize plants. Four treatments of cyanobacteria: 1) soaking grains in Cyano filtrate for 24 h then sprayed with Cyano filtrate after 30 d from planting, 2) side dressing along the row (dry) then sprayed at 30 d from planting, 3) soaking grains for 24 h + dry Cyano + Cyano spray, 4) and control (untreated); and three rates of nitrogen: 107 ($^1/_3$ N), 214 ($^2/_3$ N), and 321 kg ha^{-1}(full N rate) were used. Experimental design was split-plot with four replications, where Cyano treatments were assigned to main plots and nitrogen rates in the sub plots. Results showed significant differences among Cyano treatments for days to 50% tasseling and days to 50% silking in 2012 season. Early days to 50% tasseling and days to 50% silking and were associated with the application of Cyano treatment (dry + spray) in 2012 season. However, Cyano did not affect days to 50% tasseling and days to 50% silking in 2011 season. Treatment of Cyano (soaking + dry + spray) was accompanied with the tallest plants and the highest values of ear heights. While, the shortest plants and the lowest values of ear heights were associated with using Cyano (soaking + spray). Effect of Cyano inoculation on grain yield was significant in both years.The highest grain yield was associated with Cyano treatment (soaking + dry + spray).The highest values for ear length and ear diameter were recorded as a result of using Cyano treatment (soaking + spray).Number of kernels row^{-1} was significantly affected by Cyano inoculation in the two years. Nitrogen application hastened the time of tasseling and silking. The increase of nitrogen rate from $^1/_3$ N to full N increased significantly the plant height. Application of $^1/_3$ N ha^{-1} was accompanied with the shortest plants and the lowest values of ear height. Grain yield increased as N increased up to the highest level. Regarding yield components, ear length, ear diameter, and number of kernels row^{-1} were significantly affected by N application. Increasing N levels up to full N rate (the highest N rate) was associated with the tallest ears and the highest values for number of kernel row^{-1} and ear diameter. Nitrogen x Cyano interaction had significant effect on days to 50% tasseling and days to 50% silking in the second season only. Applying full N ha^{-1} with Dry Cyano + Cyano spray accelerated days to 50% tasseling and days to 50% silking. Using Cyano (soaking+ spray) with $^1/_3$ N ha^{-1} gave the shortest plants. The highest grain yield was achieved as a result of applying (full N + soaking in Cyano + dry Cyano and Cyano spray) in the two seasons. Furthermore, the use of cyanobacteria in combination with different nitrogen rates increased the rhizosphere soil biological activity of the maize rhizosphere soil.

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1.Introduction

Maize is considered among the most important cereal crops in terms of grain production in Egypt.The local production is not sufficient to meet the exponential increase in population and to cover the gap between production and local consumption. Therefore, any attempt for increasing maize production is considered a matter of at most importance to face human and animal demands **(Gouda *et al.*, 2009).**Maize has a great nutritional value as it contains about 66.7% starch, 10% protein, 4.8% oil, 8.5% fiber, 3% sugar, and 7% ash **(Chaudhary, 1983)**.Intensive farming practices that aims to produce higher yield, require extensive use of agro-chemicals, which are costly and create environmental pollutions **(Kozdro *et al.*, 2004)**. Farmers are used to consume substantial quantities of chemical fertilizers. Nitrogen fertilizer application is one of the major factors that affect maize production and seed quality. It is required in large quantities for plants to grow and is mainly provided in the form of synthetic chemical fertilizers. Such products pose a health hazard, besides making the production costly and expensive **(Badran and Safwat, 2004).** Recently,

a real challenge faces the workers in the agricultural research field to stop using high rates of agro-chemicals, which adverse negatively human health and environment. Many attempts have been tried to replace a part of those harmful chemical fertilizers by biofertilizers to get yield of a high quality without loss in its quantity**.** The use of the biological nitrogen fixation through cyanobacteria ensures saving entirely or partially the mineral nitrogen required in crop production. Recently, there is a great deal of interest in creating novel association between agronimically important plants, particularly cereals such as wheat, maize and N_2-fixing microorganisms including cyanobacteria **(Spiller *et al.*, 1993).** Biofertilizers are able to fix atmospheric nitrogen in the available form for plants **(Chen, 2006)**.Positive response of maize to nitrogen fertilizer has been reported by **Aflakpui *et al.*(1997)**. Many attempts have been tried to replace a part of those harmful fertilizers by biofertilizers in maize to get yield of a good quality without loss in its quantity **(El-Kholy *et al.*, 2005)**.Diazotrophs such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas* and cyanobacteria frequently colonize the important cereal crops including wheat, rice and maize and promote plant growth by producing certain PGPR **(Malik *et al.*, 1994 and Rashid *et al.*, 2007)**.

The objective of the current work is to study the impact of cyanobacteria inoculation under different nitrogen fertilizer rates on maize yield grown in sandy soil and its components, as well as, their effect on the biological activity of the soil in the rhizosphere maize plants.

2.Materials and Methods

A field trial was conducted in sandy soil at Ismailia Agricultural Research Station, (ARC) (Latitude 30° 35′ 41.901″ N and Longitude 32° 16′ 45.843″ E) in 2011 and 2012, to study the impact of cyanobacteria inoculation under different nitrogen fertilizer rates on maize yield (hybrid SC10) grown in sandy soil and its components, as well as, their effect on the biological activity of the soil in the rhizosphere maize plants.

This study was practiced in sandy soil.Soil physical and chemical properties are shown in Table (1) according to **Page *et al.*(1982).**

Table (1): Some chemical and physical analyses of the experimental soil

pH (1:2.5) Soil suspension	EC dSm^{-1} (Soil paste)	Soluble cations meq L^{-1}				Soluble anions meq L^{-1}			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
8.10	0.30	0.30	0.50	1.90	0.30	0.00	0.80	1.10	1.10

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	Texture class
83.18	11.17	3.35	2.50	1.30	Sandy

Available N (mg Kg^{-1})	Available P (mg Kg^{-1})	Available K (mg Kg^{-1})
15	2.80	110

Cyanobacteria were provided by Agric.Microbiol.Dept., Soils, Water & Environ.Res.Inst., ARC, Giza, Egypt.Cyanobacteria (Cyano) were applied as culture filtrate that contains a mixture of different Cyanobacteria strains, i.e., *Nostoc calcicola*, *Anabaena oryzae*, *Tolopothrix tenius and Anabaena oryza*.To obtain the cyanobacteria culture filtrate, each cyanobacterium strain was grown and propagated for 5 weeks on the free nitrogen BG 11_0 medium described by **Allen and Stanier (1968)**.The developed cyanobacteria cultures were centrifuged (3000 rpm min^{-1}) and the supernatant were used as cyanobacteria filtrate by mixing the supernatant for each strain together to have the cyanobacteria culture filtrate (**Aref *et al.*, 2009)**.The filtrate was used in soaking treatment for maize grains before planting and to be also used as foliar spray at the rate of 40 L fed^{-1}.As well as, these cyanobacteria strains were prepared as soil based inoculum as described by **Venkataraman (1972)** to be used for maize as seed side dressing (dry inoculum) along the rows.Cyanobacteria are introduced in four treatments, i.e., 1) Soaking grains in Cyano filtrate for 24 h then sprayed with Cyano filtrate at 30 d from planting, 2) Side dressing along the row (dry) then sprayed at 30 d from planting, 3) soaking grains for 24 h + Side dressing along the row (dry) then sprayed at 30 d from planting, and 4) Control (untreated); while nitrogen was introduced in three rates of 107 ($^1/_3$ N), 214 ($^2/_3$ N), and 321 kg ha^{-1} (full N).Experimental design was split-plot with four replications, where Cyano treatments were assigned to main plots and nitrogen rates in the sub plots.Maize hybrid SC 10 was used.Plot size was 5 rows, 6 m in length, 80 cm in width, and 20 cm between hills.One blank row was left between treatments.Nitrogen was added in the form of ammonium nitrate (33.5% N) and split into eight equal doses, the first was added at germination, and the rest were added weekly up to 60 days after

planting.Phosphorus at a rate of 71 kg P_2O_5 ha^{-1} in the form of superphosphate (15% P_2O_5) and potassium at a rate of 57 kg K_2O ha^{-1} in the form of potassium sulphate 48% K_2O were added at soil preparation.Soil samples (0.5 kg) were taken from the experimental site before planting for chemical, physical, and biological analysis.Cultural practices were applied as recommended.Data recorded for maize for both tested seasons were number of days from planting to 50% tasseling (DTT) and number of days from planting to 50% silking (DTS), plant height (PHT) and ear heights (EHT) (cm), number of kernels row^{-1} (KPR), ear length (EL) (cm), ear diameter (ED) (cm), and grain yield (t ha^{-1}).Grain yield was adjusted to 15.5% moisture.Statistical analysis of the data was performed according to **Steel and Torrie (1980).**After 70 days from planting, a maize rhizosphere soil samples were collected from maize rhizosphere to determinate total *Azotobacter* and *Azospirillum* counts (**Cochran, 1950**), total cyanobacteria count (**Allen and Stanier, 1968**)**,** total fungi count (**Martin, 1950**), total *Actinomycetes* count (**Williams and Davis, 1965**), total bacterial count (**Allen, 1959**), dehydrogenase activity (**Casida *et al*., 1964**), nitrogenase activity **(Hardy *et al*., 1973)** and CO_2 evolution amount (**Pramer and Schmidt, 1964**).

3.Results and Discussion

a) Cyanobacteria effect:

Data presented in Tables (2 and 3) indicate that the effect of cyanobacteria inoculation was significant on maize growth attributes and grain yield in 2011 and 2012 seasons.No significant differences among cyanobacteria treatments for days to 50% tasseling and days to 50% silking in 2011 season.But this effect was significant in 2012 season.Early days to 50 % tasseling and days to 50% silking were associated with application of Cyano treatment (dry + spray) in the second season.Application of Cyano (soaking + dry + spray) was accompanied with the tallest plants and the highest values of ear heights in both seasons.While, the shortest plants and the lowest ear heights were associated with using Cyano (soaking + spray) in the two seasons.Effect of Cyano inoculation on grain yield was significant in the two seasons.The highest maize grain yield was achieved when treatments of Cyano (soaking + dry + spray) were used in the first and second seasons, respectively.On the other hand, the untreated plants with Cyano inoculation had the lowest grain yield in both seasons.

Ear length and ear diameter were not affected by Cyano inoculation in 2011 season, but they were significantly affected by Cyano in the second season (Table 3). The highest values for ear length and ear diameter were recorded as a result of using of Cyano (Soaking + spray) in the second season.Number of kernels row^{-1} was significantly affected by Cyano inoculation in the two years and the highest values were associated with the treatment of soaking in Cyano + dry Cyano + Cyano spray in the two seasons.

Table (2): Effect of cyanobacteria on days to 50% tasseling (DTT), days to 50% silking (DTS), plant height (PHT), ear height (EHT), and grain yield (GY) in 2011 and 2012

	DTT	DTS	PHT (cm)	EHT (cm)	GY (t ha^{-1})
Cyano treatments:					
---------- 2011 ----------					
Soaking + spray	60.8	62.3	264	137	8.58
Dry + spray	60.4	62.2	268	135	7.67
Soaking + dry + spray	60.0	61.6	278	145	8.77
Control	61.0	62.6	275	139	7.11
$LSD_{0.05}$	NS	NS	4.00	5.00	1.13
---------- 2012 ----------					
Soaking + spray	64.4	66.2	276	144	8.19
Dry + spray	63.3	65.1	282	150	8.04
Soaking + dry + spray	64.1	65.8	285	153	9.09
Control	63.7	65.6	280	153	7.80
$LSD_{0.05}$	0.70	0.60	6.00	6.00	1.08

NS= not significant at 0.05 level.

B) Nitrogen effect:

Effect of nitrogen fertilization on maize growth, grain yield, and yield components was significant in both years, except for ear diameter in 2011 season (Tables 4 and 5).Nitrogen fertilizer significantly affected DTT and DTS in both years.Increasing Nitrogen rates up to full N dose ha^{-1} hastened the time of tasseling and silking in 2011 and 2012 seasons.However, in the first season, there was no significant differences between $^1/_3$ and $^2/_3$ N ha^{-1} for DTT and between $^2/_3$ N and full N ha^{-1} for DTS.Whereas, significant differences among the three nitrogen rates for DTT and DTS were detected in the second season.Generally, the earliest DTT and

DTS were associated with application of full N ha^{-1} in the two seasons.

Effect of nitrogen on plant height was significant in 2011 and 2012.Increasing nitrogen rates up to $^2/_3$ N ha^{-1} was accompanied with the tallest plants in 2011.However, increasing N rates from $^2/_3$ to full N ha^{-1} was not associated with a corresponding increase in plant height in the first season.In the second season, plant height increased as N increased up to the highest rate (full N ha^{-1}).On the other hand, the shortest plants were associated with application of $^1/_3$ N ha^{-1} in the two seasons.Concerning ear height, the effect of nitrogen on ear height was significant in 2011 and 2012.Increasing N up to $^2/_3$ N ha^{-1} was associated with significant increase in ear height in 2011 season.But no significant difference was detected between $^2/_3$ and full N ha^{-1} regarding their effect on ear height in the first year.However, increasing N levels up to the highest rate (full N ha^{-1}) gave the highest value for ear height in the second season.Moreover, the lowest values of ear height (136 and 134 cm) were accompanied with application of $^1/_3$ N ha^{-1} in both years, respectively.Grain yield increased as N increased up to the highest level (full N rate) in both growing seasons (Table 4).

Table (3): Effect of cyanobacteria on ear length (EL), ear diameter (ED) and number of kernels row^{-1} (KPR) in 2011 and 2012

	EL (cm)	ED (cm)	KPR^{-1}
Cyano treatments:			
---------- 2011 ----------			
Soaking + spray	19.0	4.57	43.6
Dry + spray	19.3	4.55	45.1
Soaking + dry + spray	19.5	4.57	45.7
Control	19.1	4.60	43.2
$LSD_{0.05}$	NS	NS	1.10
---------- 2012 ----------			
Soaking + spray	18.1	4.52	44.2
Dry + spray	17.9	4.42	44.5
Soaking + dry + spray	18.3	4.47	45.7
Control	17.1	4.25	42.8
$LSD_{0.05}$	0.40	0.08	0.90

Table (4): Effect of nitrogen fertilizer on DTT, DTS, PHT, EHT, and GY in 2011 and 2012

	DTT	DTS	PHT (cm)	EHT (cm)	GY (t ha^{-1})
N rates(ha^{-1})					
---------- 2011 ----------					
$^1/_3$ N	61.1	62.8	264	136	6.38
$^2/_3$ N	60.7	62.0	276	141	7.89
Full N	59.9	61.7	275	140	9.83
$LSD_{0.05}$	0.50	0.40	4.00	4.00	0.98
---------- 2012 ----------					
$^1/_3$ N	64.8	66.7	249	134	6.54
$^2/_3$ N	63.8	65.6	288	148	8.75
Full N	62.9	64.7	306	168	9.56
$LSD_{0.05}$	0.03	0.40	5.00	5.00	0.94

DTT=days to 50% tasseling, DTS=days to 50% silking, PHT= plant height, EHT= ear height, GY= grain yield.

This increase in grain yield was more pronounced when full N ha^{-1} was applied in 2011.But the difference between $^2/_3$ and full N treatments was not significant in 2012 season.This result revealed that application of $^1/_3$ N ha^{-1} was linked to the lowest grain yield (6.38 and 6.54 t ha^{-1}) in the two seasons, respectively.Regarding yield components, ear length, ear diameter, and number of kernels row^{-1} were significantly affected by N fertilizer treatments in both seasons, except for ear diameter in 2011 season (Table 5).Increasing N up to the highest rate (full N ha^{-1}) was associated with the tallest ears and the highest number of KPR in both seasons as well as the highest value for ED in the second season.But no significant difference was detected between $^2/_3$ and full N treatments for EL in 2011 season.In this respect, **Gouda *et al.*(2009)** found that increasing nitrogen rates up to full N produced the highest values of grain yield per unit area. **Dahmardeh (2011)** confirmed that increasing N up to 300 kg ha^{-1} significantly increased all the studied parameters of maize yield.**Hokmalipour and Darbandi (2011)**

showed that in maize field trail, increasing nitrogen levels up to 180 kg ha^{-1} increased the harvest index, kernels yield, 1000 kernels weight, number of kernels per ear, and number of rows per ear.They reported that increasing nitrogen fertilization rates led to significant increase in 100 grain weight and grain yield of maize compared with control treatment.They explained that the variation in grain yield due to different levels of nitrogen is related to the differences in size of photosynthetic surface and to the relative efficiency of total sink activity.**Ghazal *et al.*(2013)** mentioned that elevating nitrogen level from $^2/_3$ N to full N rate enhanced grain yield of maize.

Table (5): Effect of nitrogen on ear length (EL), ear diameter (ED), and number of kernels row^{-1} (KPR) in 2011 and 2012

	EL (cm)	ED (cm)	KPR
N rates (ha^{-1})			
---------- 2011 ----------			
$^1/_3$ N	18.5	4.53	43.1
$^2/_3$ N	19.4	4.57	44.1
Full N	19.8	4.61	46.0
$LSD_{0.05}$	0.50	NS	1.00
---------- 2011 ----------			
$^1/_3$ N	16.2	4.28	41.3
$^2/_3$ N	17.8	4.40	44.9
Full N	19.6	4.56	46.8
$LSD_{0.05}$	0.30	0.07	0.80

EL= Ear length, ED= Ear diameter, and KPR= Number of kernels row^{-1}.

C) Cyanobacteria × nitrogen interaction effect:

Effect of Cyano × N interaction on DTS and DTS was not significant in 2011, but this effect was positively significant in 2012 (Table 6).Application of full N ha^{-1} combined with dry Cyano + Cyano spray was associated with the earliest DTT and DTS in 2012 season.In contrast, application of $^1/_3$ N ha^{-1} with soaking seeds in Cyano filtrate + Cyano spray was accompanied with the latest DTT and DTS in 2012 season.Effect of Cyano × N interaction on plant height was significant in 2011, but this effect was not significant in 2012 season.Application of $^2/_3$ N ha^{-1} plus soaking in Cyano filtrate + Dry Cyano + Cyano spray was associated with the tallest plants (285 cm), but with no significant difference with increasing N up to full N ha^{-1} without Cyano inoculation (control) in the same season.In contrast, the shortest plants (254 cm) were attained by application of $^1/_3$ N ha^{-1} (the lowest rate of N fertilizer) + soaking in Cyano Filtrate + Cyano spray in the first season.No significant difference was detected between the tallest plants (285cm), which received ($^2/_3$ N ha^{-1} + soaking in Cyano filtrate + dry Cyano + Cyano spray) and the untreated plants with Cyano that received the highest rate of N (full N ha^{-1}).Effect of Cyano × N interaction on grain yield was positively significant in both seasons (Table 7).There was no significant difference between $^2/_3$ N and full N ha^{-1} (without Cyano) for grain yield in both tested years.The highest grain yield (11.11 and 10.03 t ha^{-1}) was associated with application of full N ha^{-1} + soaking in Cyano + dry Cyano and Cyano spray in 2011 and 2012 seasons, respectively.However, no significant difference was detected between the rate of $^2/_3$ N and full N ha^{-1} + seed soaking in Cyano filtrate and the treatment of full N + Dry Cyano + Cyano spray and between the rate of $^1/_3$ and $^2/_3$ N ha^{-1} combined with Dry Cyano + Cyano spray in 2011 season.In contrast, significant differences amongst the three nitrogen levels were detected with seed soaking in Cyano + Cyano dry +Cyano spray in 2011 season.While, in the second season, there were no significant differences between $^2/_3$ N and full N ha^{-1} for all treatments of Cyano, and between $^1/_3$ N and $^2/_3$ N ha^{-1} with Dry Cyano + Cyano spray.

D) Effect of nitrogen and cyanobacteria on rhizosphere soil biological activity:

Data in Tables (8, 9 and 10) indicate the rhizosphere soil biological activity of maize rhizosphere soil samples for 2012 seasons under the effect of different nitrogen rates and cyanobacteria.The soil biological activity was expressed in terms of the total count of *Azotobacter*, *Azospirillum*, cyanobacteria, fungi, actinomycetes and bacteria.Dehydrogenase activity, nitrogenase activity and carbon dioxide evolution were also considered.Results noted that increasing nitrogen rate from $^1/_3$ N to full N rate increased significantly the biological activity due to the soil maize rhizosphere area with priority to $^2/_3$ N rate.This nitrogen rate gave significantly the highest significant mean total counts numbers of *Azotobacter* (10 x 10^4 cfu dry rhizosphere $soil^{-1}$), *Azospirillum* (8.25 X 10^4 cfu dry rhizosphere $soil^{-1}$), cyanobacteria (8.78 X 10^3 cfu dry rhizosphere

soil^{-1}), fungi (44.90 X 10^3 cfu g dry rhizosphere soil^{-1}), actinomycetes (52.03 X 10^3 cfu g dry rhizosphere soil^{-1}) and bacteria (79.90 X 10^4 cfu dry rhizosphere soil^{-1}).Similar trend was true for dehydrogenase activity, nitrogenase activity and CO_2 evolution for the same nitrogen rate of $^2/_3$ N.The corresponding mean values were 465.45 mg TPF dry rhizosphere soil^{-1} day^{-1}, 196.45 mmole C_2H_4 dry rhizosphere soil^{-1} day^{-1} and 853.90 mg CO_2 100 g dry rhizosphere soil^{-1} day^{-1}, respectively.Moreover, results also revealed that the use of cyanobacteria (Cyano) as seed soaking, dry and\or spray increased significantly all the tested terms of the biological activity due to the soil maize rhizosphere area.Nevertheless, the use of cyanobacteria as seed soaking + dry + spray gave significantly the highest significant mean values for biological activity of the maize rhizosphere soil compared to the other applied cyanobacteria treatments.The corresponding significant mean values were *Azotobacter* (7.97 x 10^4 cfu dry rhizosphere soil^{-1}), *Azospirillum* (8.67 X 10^4 cfu dry rhizosphere soil^{-1}), cyanobacteria (10.73 X 10^3 cfu dry rhizosphere soil^{-1}), fungi (43.25 X 10^3 cfu g dry rhizosphere soil^{-1}), actinomycetes (43.25 X 10^3 cfu g dry rhizosphere soil^{-1}) and bacteria (79.40 X 10^4 cfu dry rhizosphere soil^{-1}).Similar trend was observed for dehydrogenase activity, nitrogenase activity and CO_2 evolution in response to the same cyano treatment.The corresponding mean values were 652.35 mg TPF dry rhizosphere soil^{-1} day^{-1}, 287.17 mmole C_2H_4 dry rhizosphere soil^{-1} day^{-1} and 1152.47 mg CO_2 100 g dry rhizosphere soil^{-1} day^{-1}, respectively.

Table (6): Effect of Cyanobacteria x nitrogen interaction on DTT, DTS, and PHT in 2011 and 2012 seasons

Treatments	N rate (ha^{-1})	DTT	DTS	PHT (cm)
		---------- 2011 ----------		
Soaking seeds in Cyano filtrate + CYANO spray	$^1/_3$ N	61.0	63.0	254
	$^2/_3$ N	61.0	62.0	275
	Full N	60.3	62.0	264
Dry Cyano + Cyano spray	$^1/_3$ N	61.0	62.5	261
	$^2/_3$ N	60.5	62.0	273
	Full N	59.8	62.0	271
Soaking in Cyano filtrate + dry Cyano + Cyano spray	$^1/_3$ N	60.5	62.3	268
	$^2/_3$ N	60.3	61.5	285
	Full N	59.3	61.0	283
Control	$^1/_3$ N	61.8	63.5	274
	$^2/_3$ N	61.0	62.5	271
	Full N	60.3	61.8	281
$LSD_{0.05}$		NS	NS	7.60
		---------- 2012 ----------		
Soaking seeds in Cyano filtrate + Cyano spray	$^1/_3$ N	65.8	68.0	243
	$^2/_3$ N	64.5	65.8	285
	Full N	63.0	64.8	300
Dry Cyano + Cyano spray	$^1/_3$ N	64.5	66.3	254
	$^2/_3$ N	63.0	65.0	285
	Full N	62.3	64.0	308
Soaking in Cyano filtrate + dry Cyano + Cyano spray	$^1/_3$ N	64.5	66.0	251
	$^2/_3$ N	64.3	66.0	289
	Full N	63.5	65.3	316
Control	$^1/_3$ N	64.5	66.5	249
	$^2/_3$ N	63.5	65.5	291
	Full N	63.0	64.8	301
$LSD_{0.05}$		0.60	0.90	NS

Cyano = Cyanobacteria, DTT= Days to 50% tasseling, DTS= days to 50% Silking, PHT= Plant height.

Due to the interaction effect of both cyanobacteria and nitrogen fertilizer rate on the biological activity of maize rhizosphere soil, results revealed that all the treatments received any rate of nitrogen combined with any cyanobacteria treatments gave significantly higher values of the biological activity of maize rhizosphere soil compared to those received any of the nitrogen rates only.Moreover, the treatment of $^2/_3$ N rate + Cyano seed soaking + Cyano dry + Cyano spray gave significantly the highest values for the terms of the biological activity of maize rhizosphere soil compared with the other treatments received the

other nitrogen rates combined with cyanobacteria treatments.The corresponding biological activity of maize rhizosphere soil were *Azotobacter* (9.30 x 10^4 cfu dry rhizosphere soil^{-1}), *Azospirillum* (11.80 X 10^4 cfu dry rhizosphere soil^{-1}), cyanobacteria (12.30 X 10^3 cfu dry rhizosphere soil^{-1}), fungi (67.3 X 10^3 cfu g dry rhizosphere soil^{-1}), actinomycetes (84.10 X 10^3 cfu g dry rhizosphere soil^{-1}) and bacteria (121.40 X 10^4 cfu dry rhizosphere soil^{-1}).In addition, similar trend was observed for dehydrogenase activity, nitrogenase activity and CO_2 evolution in response to the same interaction cyano x N treatment.The corresponding mean values were 830.86 mg TPF dry rhizosphere soil^{-1} day^{-1}, 460.61 mmole C_2H_4 dry rhizosphere soil^{-1} day^{-1} and 1708.67 mg CO_2 100 g dry rhizosphere soil^{-1} day^{-1}, respectively.

Table (7): Effect of Cyanobacteria x nitrogen interaction on maize grain yield in 2011 and 2012 seasons

Treatments	N rate ha^{-1}	Grain yield (t ha^{-1})	
		2011	2012
Seeds Soaking in Cyano filtrate + Cyano spray	$^1/_3$ N	6.71	6.08
	$^2/_3$ N	8.69	8.54
	Full N	10.35	9.96
Dry Cyano + Cyano spray	$^1/_3$ N	6.58	6.69
	$^2/_3$ N	7.27	8.17
	Full N	9.17	9.26
Seed soaking in Cyano + dry Cyano + cyano spray	$^1/_3$ N	6.47	7.24
	$^2/_3$ N	8.73	10.00
	Full N	11.11	10.03
Control	$^1/_3$ N	5.75	6.14
	$^2/_3$ N	6.89	8.28
	Full N	8.69	8.98
$LSD_{0.05}$		1.96	1.87

Table (8): Effect of cyanobacteria inoculation and N-fertilization on N_2 fixers (*Azotobacter*, *Azospirillum* and total cyanobacteria) counts in maize rhizosphere soil (Data are a mean of two seasons)

N- rate (ha^{-1})	Treatments				
	Control	Cyanobacteria			Means
		Soaking + spray	Dry + spray	Soaking + dry + spray	
	Azotobacter x10^4 cfu g dry rhizosphere soil^{-1}				
$^1/_3$ N	2.60	4.40	5.80	7.50	6.77
$^2/_3$ N	5.40	6.70	8.60	9.30	10.00
Full N	5.00	5.30	6.30	7.10	7.90
Means	4.33	5.47	6.90	7.97	
L.S.D.at 5%					
N:	2.22				
Cyano:	1.23				
N X Cyano:	0.80				
	Azospirillum x 10^4 cfu g dry rhizosphere soil^{-1}				
$^1/_3$ N	1.40	2.20	4.30	5.70	3.40
$^2/_3$ N	5.20	6.80	9.20	11.80	8.25
Full N	4.60	5.30	7.20	8.50	6.40
Means	3.73	4.77	6.90	8.67	
L.S.D.at 5%					
N:	1.88				
Cyano:	1.78				
N X Cyano:	2.63				
	Total cyanobacteria x 10^3 cfu g dry rhizosphere soil^{-1}				
$^1/_3$ N	2.70	4.70	6.20	9.70	2.83
$^2/_3$ N	6.00	8.20	9.00	12.30	8.87
Full N	5.43	6.30	7.60	10.20	7.38
Means	4.71	6.40	7.60	10.73	
L.S.D.at 5%					
N:	1.19				
Cyano:	2.14				
N X Cyano:	2.24				

Table (9): Effect of cyanobacteria inoculation and N-fertilization on total fungi, actinomycetes and total bacterial counts in maize rhizosphere soil (Data are a mean of two seasons)

N-rate (ha^{-1})	Treatments				
	Control	Cyanobacteria			Means
		Soaking + spray	Dry + spray	Soaking + dry + spray	
	Total fungi $x10^3$ cfu g dry rhizosphere $soil^{-1}$				
$^1/_3$ N	7.20	10.10	15.70	28.90	15.48
$^2/_3$ N	22.60	36.00	53.70	67.30	44.90
Full N	15.60	18.50	33.20	42.80	27.53
Means	14.93	21.53	24.20	46.33	
L.S.D.at 5%					
N:	17.52				
Cyano:	23.10				
N X Cyano:	13.19				
	Actinomycetes x 10^3 cfu g dry rhizosphere $soil^{-1}$				
$^1/_3$ N	8.00	15.20	20.10	31.80	18.78
$^2/_3$ N	18.70	46.70	58.60	84.10	52.03
Full N	11.30	31.60	35.80	57.10	33.95
Means	12.67	31.17	38.17	43.25	
L.S.D.at 5%					
N:	17.13				
Cyano:	5.02				
N X Cyano:	23.20				
	Total bacteria $x10^4$ cfu g dry rhizosphere $soil^{-1}$				
$^1/_3$ N	16.10	18.70	40.20	65.10	35.03
$^2/_3$ N	38.20	77.60	82.00	121.40	79.80
Full N	28.50	34.70	39.80	51.70	38.78
Means	27.60	43.67	54.00	79.40	
L.S.D.at 5%					
N:	41.00				
Cyano:	24.80				
N X Cyano:	38.40				

In the present work the use of cyanobacteria combined with different nitrogen rates ($^1/_3$, $^2/_3$ and full N recommended rates) enhanced maize soil rhizosphere biological activity in terms of total count of *Azotobacter*, *Azospirillum*, cyanobacteria, fungi, actinomycetes and bacteria, dehydrogenase activity, nitrogenase activity and carbon dioxide evolution.In this concern, **Zulpa *et al.*(2008)** found that the the biomass and extracellular products of *Tolypothrix tenuis* and *Nostoc muscorum* increased significantly the soil microbial activity and its nutrients availability.*Nostoc muscorum* and *T.tenuis* biomasses increased the soil oxidizable C (15%; 14%), total N (10%; 12%) and available P (22%; 32%), respectively.In addition, *Tolypothrix tenuis* extracellular products increased oxidizable carbon by 28% and *N.muscorum* extracellular products increased the available phosphorus by 15%.These increases caused the soil biological activity to be increased also because they are a continuously renewable carbon source.Production of bioactive substances, which accelerate the decomposition process in the soil due to the increase of microbial activity and because they are a continuously renewable organic matter source (**Caire *et al.*, 2000).**They also added that cyanobacteria can increase the soil enzymatic activity.Besides, exopolysaccharide secreted by cyanobacteria are a source of organic carbon for the soil microflora increasing microbial activity **(Storni de Cano *et al.*, 2002)**.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 biofertilization with cyanobacteria led to increase microorganisms' community and in turn soil biological activity in soil through increasing the organic matter and microbial activity.**Ghazal *et al.*(2013)** stated that the use of cyanobacteria to maize increased the soil biological activity of the maize plants rhizosphere in terms of total count bacteria, carbon dioxide evolution, dehydrogenase activity and nitrogenase activity.

Table (10): Effect of cyanobacteria inoculation and N-fertilization on nitrogenase, nitrogenase and CO_2 evolution in maize rhizosphere soil (Data are a mean of two seasons)

N- rate (ha^{-1})	Treatments				
	Control	Cyanobacteria			Means
		Soaking + spray	Dry + spray	Soaking + dry + spray	
	Dehydrogenase activity (mg TPF* g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})				
$^1/_3$ N	75.10	125.23	420.08	510.75	282.79
$^2/_3$ N	110.12	230.55	690.28	830.86	465.45
Full N	91.27	145.42	520.74	615.43	343.22
Means	92,16	167.07	543.67	652.35	
L.S.D.at 5%					
N:	120.23				
Cyano:	138.58				
N X Cyano:	106.68				
	Nitrogenase activity (mmole C_2H_4 g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})				
$^1/_3$ N	30.45	42.56	65.78	170.16	77.24
$^2/_3$ N	52.15	82.18	190.85	460.61	196.45
Full N	41.62	50.12	81.36	230.74	100.96
Means	41.41	58.29	112.66	287.17	
L.S.D.at 5%					
N:	93.49				
Cyano:	165.75				
N X Cyano:	170.50				
	CO_2 Evolution (mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1})				
$^1/_3$ N	130.12	268.15	660.25	835.33	473.46
$^2/_3$ N	180.58	510.67	1015.66	1708.67	853.90
Full N	145.06	318.43	730.45	913.40	526.84
Means	151.92	365.75	802.12	1152.47	
L.S.D.at 5%					
N:	125.06				
Cyano:	190.40				
N X Cyano:	145.25				

In conclusion, results from the present study indicate that the application of cyanobacteria and nitrogen fertilizer rate can positively affect the maize yield and its components, especially for the treatment received $^2/_3$ N (214 kg N ha^{-1}) + Cyano seed soaking + dry Cyano + Cyano spray, which recorded a maize yield that was not significantly differed from that recorded by the use of full N dose alone (321 kg N ha^{-1}).In general, application of cyanobacteria along with nitrogen can reduce the demands for chemical fertilizers and subsequently reduce environmental pollution.However, further studies are required to determine economically feasible application cyanobacteria under different field conditions.

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Biochemical effects of caffeine on bone of growing rats

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Abstract: This study investigated the biochemical effects of caffeine on bone of growing rats. The safety of caffeine use among children is understudied and poorly understood. Given that some caffeine-containing beverages are marketed directly to children and that caffeine use is growing among children, it is important to understand the potential effects of caffeine use within this population. Caffeine; 1, 3, 7 trimethylxanthine, a purine alkaloid, is a key component of many popular drinks, mainly tea and coffee, but most phytochemists know little about its biochemistry and molecular biology. A total of 25 rats (8 weeks old) were divided randomly into three groups: Group 1 (n =10), caffeine-high dose; group 2 (n = 10), caffeine-low dose; group 3 (n = 5), serve as the control group. The caffeine was fed via the animals' dietary water and the high dose of caffeine=10 mg/100 g body weight/day, and the low dose of caffeine=2.5 mg/100g body weight/day. Body weight was measured weekly. After sacrifice, blood samples were collected in tubes, and separated the serum for the determination of Ca, ALP, Zn and Mn. The results showed that serum Ca level for high dose group is significantly lower than the low dose than the control group, serum Zn level for high dose is significantly lower than the low dose than control group, and serum Mn level of high dose group is significantly lower than the low dose group than the control group. The Alkaline phosphatase in low dose group is not significant smaller than the control group, but the high dose group has significantly elevation value than the control group. It is reported that the oral administration of caffeine lead to significant reduction in serum Ca, Zn and Mn and a significant elevation in serum ALP according to the increase of caffeine dose.

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1. Introduction:

Caffeine is the most commonly used psychoactive substance throughout the world (Nehlig, 1999). The safety of caffeine use among children is understudied and poorly understood. Given that some caffeine-containing beverages are marketed directly to children (Bramstedt, 2007) and that caffeine use is growing among children (Frary *et al*., 2005), it is important to understand the potential effects of caffeine use within this population. There is also a strong belief that the people consuming high amounts of caffeine tend to carry a higher risk of developing bone problems, including osteoporosis, as well as problems in metal absorption, excretion and reabsorption processes in intestines and in kidney (Chen and Whitford, 1999; Massey, 2001; Borse *et al*., 2002; Pan *et al*., 2003), and iron deficiency anemia (Hallberg and Rossander, 1982). Thus caffeine is probably the most commonly consumed pharmacologically active compound in the world, certainly in Europe and North America. Probably it is partly for that reason that caffeine has often been a target of opportunity for investigators seeking to identify environmental factors that may contribute to the burden of chronic disease. Caffeine and the other methyl xanthines act in a variety of tissues, generally by interfering with the action of phosphodiesterase and there by potentiating the activity of agonists acting through the adenylate cyclase – cAMP pathway. At sufficient doses, therefore, they could theoretically exert effects directly on the cellular apparatus controlling bone remodeling. In high doses caffeine interferes with fetal rat skeletal development (Nakamoto *et al*., 1989; Schneider *et al*., 1990). The first publication showing a negative effect of caffeine on the calcium economy came from this author's laboratory (Heaney and Recker, 1982). Shortly thereafter, Massey and colleagues (Massey and Wise, 1984; Massey and Hollingbery, 1988; Bergman *et al*., 1990) showed that a caffeine-induced diuresis increased urinary calcium loss acutely. In the clinical setting, alkaline phosphatase (ALP) is frequently used as a biochemical marker for osteopenia due to its ease of measurement. ALP is a routine marker in the diagnosis of hepatic disorders and metabolic bone diseases.

There is ample evidence that zinc plays an important role in bone metabolism and is required for normal bone development (Follis and Mccollum, 1941; Calhoun, and Smith, 1968; Calhoun *et al.,* 1974;

Calhoun *et al*., 1975). Moreover, zinc deficiency has been shown to impair collagen biosynthesis (Fernandez *et al*., 1973; Yamaguchi *et al*., 1982; Yamaguchi and Yamaguchi, 1986; Yamaguchi and Ehara, 1995; Yamaguchi and Ehara, 1996) and the lower activity of zinc is dependent on enzymes in the bone (Prasad *et al*., 1967; Roth and Kirchgessner, 1974). Zinc deficiency has also been implicated to play a role as a risk factor in the development of human osteoporosis (Gaby and Wright, 1990; Fushimi *et al*., 1993; Saltman and Strause, 1993). Manganese is an essential trace nutrient in all forms of life. The human body contains about 12 mg of manganese, which is stored mainly in the bones; it is mostly concentrated in the liver and kidney (Emsley and John, 2001). In recent years there has been a rebirth of interest in studies concerning the role of trace elements in the development and maintenance of the skeleton (Asling and Hurley, 1963). A relationship among manganese, copper and skeletal growth has been observed in the abnormal fetal development of rats in deficient dams (Hurley, 1981).The dose of caffeine given to rats in the present study was equivalent to slightly more than four cups of coffee a day in the human based on metabolic body weight (Kleiber, 1961). Caffeine increases urinary calcium excretion by reducing renal reabsorption and, possibly, reducing calcium absorption, leading to a negative calcium balance (Bergman, 1990; Massey and Whiting, 1993; Ilich *et al*., 2002). Other study find that a high caffeine dose may influence vitamin D receptor protein (VDR) expression stimulated by vitamin 1,25(OH)2D3 and controlled by vitamin 1,25(OH)2D3 activity of human osteoblast cells by reducing alkaline phosphatase activity (Rapuri *et al.,* 2007).

Caffeine-containing beverage consumption has been reported to be associated with reduced bone mass and increased fracture risk in some, but not most, observational studies. Human physiological studies and controlled balance studies show a clear but only a very small depressant effect of caffeine itself on intestinal calcium absorption, and no effect on total 24-h urinary calcium excretion (Heaney, 2002). Liu *et al.,* (2011) reported that the calcium contents in tibia and femur of caffeine-treated rats were also lower than that in the control group. The osteoclastogenesis of bone marrow cells isolated from caffeine-treated rats was markedly enhanced as compared with the control group.

The aim of the present experiment was to investigate the biochemical effect of oral administration of caffeine on the serum level of Ca, Zn, Mn and ALP. The bad effect of caffeine on bone of growing rats was also studied.

2. Material and Methods

Animals

Twenty-five 7-week old Wistar rats with a mean body weight of 100 g at the beginning of the experiment, and were all kept under controlled conditions. After_1 week of adaptation; animals were fed with Purina Laboratory Rodent Diet (PMI; St. Louis, MO) (0.95% calcium) and distilled water *ad libitum*. This approach was used because many younger adults and more than 50% of older adults living in the United States, including those who use calcium-containing antacids or supplements, consume diets that are considered to be inadequate in calcium (Ervin and Kennedy, 2002), and thus may be at higher risk of loss of bone mass during weight loss (Riedt *et al*., 2005). The experiment began when the rats were 8- weeks old.

Experimental Design

The animals were randomly divided into three groups: Group1 (n =10), caffeine-high dose; group 2 (n = 10), caffeine-low dose; group 3 (n = 5), serve as the control group.

Caffeine Feeding

The dose of caffeine was delivered to each rat in its drinking water daily throughout the duration of the 8 week study.

We prepare 0.5% gm caffeine (Sigma Aldrich, St. Louis, Mo, USA) solution, the high dose of caffeine=10 mg/100g body weight/day, and the low dose of caffeine=2.5 mg/100g body weight/day.

Measurements

At the end of the experiment, 8-weaks after the start of the experiment, all animals were killed by decapitation under light anesthesia with diethyl ether. Blood samples were collected in tubes, and serum was separated for the determination of Ca, ALP, Zn and Mn. Zn and Mn were measured by atomic absorption spectrophotometry (Perkin-Elmer 560, Norwalk, CT). Ca was measured by Colorimetric method based on formation of color complex between calcium and o-cresolphtalein in alkaline medium (Young, 2001). ALP were measured by "Optimised standard method"according to the recommenations of the German Clinical Association (Deutsche Gesellschaft f ur Klinische Chemie) (Klin, 1972). Ca and ALP are measured by Biosystem BTS 330 spectrophotometer analyzer.

Statistical analysis

All values were expressed as means±SD. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Differences with a value of $P < 0.05$ were considered as statistically significant.

3. Results

At 8 weak after caffeine feeding, Calcium (Ca), Zinc (Zn), Manganese (Mn), and Alkaline phosphatase (ALP) serum levels are measured. Serum Ca level for low dose group (8.94 ± 0.17) mg/dl were significantly decreased compared with the control group (10.5 ± 0.34), and the high dose group (7.7 ± 0.23) were significantly decreased lower than the low dose group (table 1).

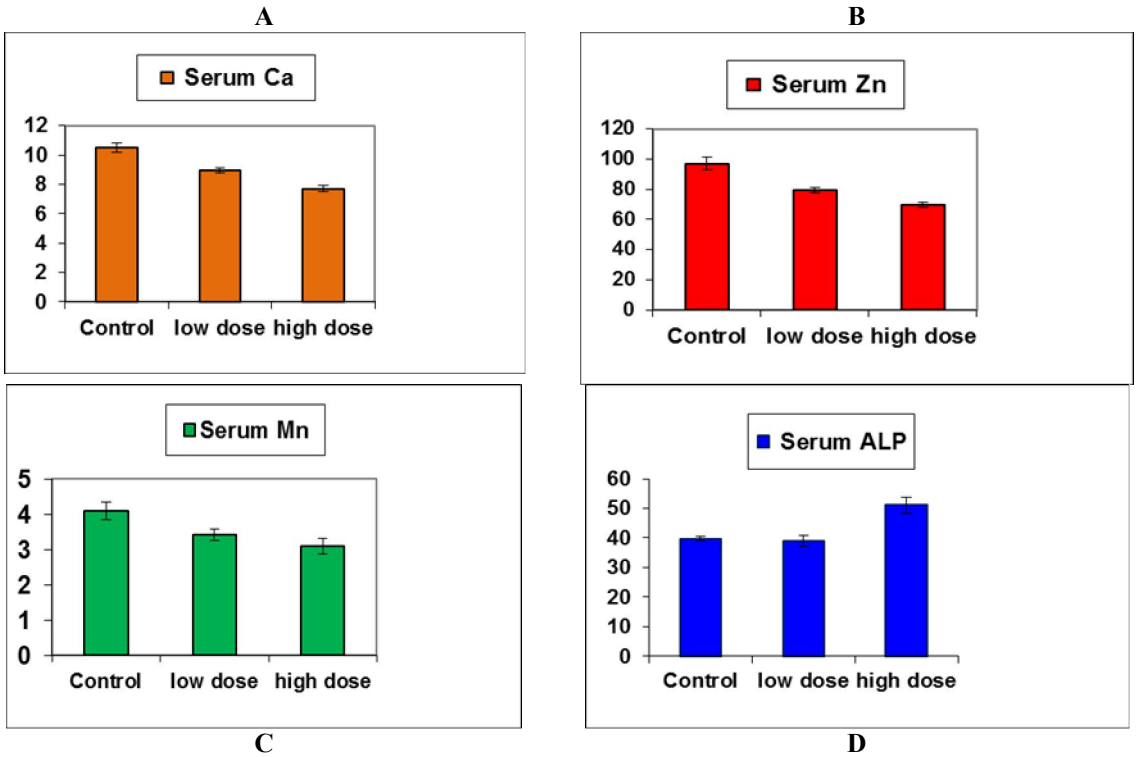

Figure 1. A) Serum Ca level of growing rats. **B)** Serum Mn level of growing rats. **C)** Serum Zn level of growing rats.
D) Serum ALP level of growing rats.
Data are expressed as the mean ±SD; *P < 0.05 versus control group.

Table 1. Significant decrease of serum Ca level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum Ca	10.5±0.34	8.94±0.17*	7.7±0.23*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

Serum Zn level for low dose group (79.14± 1.71) µg/dl were significantly decreased in compared with the control group (97± 4.2), and the high dose group (69.66 ± 1.87) were significantly decreased lower than the low dose group (table 2).

Table 2. Significant decrease of serum Zn level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum Zn	97 ± 4.2	79.14±1.71*	69.66±1.87*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

Serum Mn level for low dose group (3.42 ± 0.17) µg/dl were significantly decreased compared with the control group (4.1±0.24), and the high dose (3.1± 0.22) were significantly decreased lower than the low dose group (table 3) .

Table 3. Significant decrease of serum Mn level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum Mn	4.1±0.24	3.42±0.17*	3.1±0.22*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

The Alkaline phosphatase in low dose group (39 ± 1.93) UI/L is lower than the control group (39.75 ± 0.62) without significant difference, but the high dose group (51.16±2.72) is significantly increased compared with the control group (table 4).

Table 4. Change of serum ALP level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum ALP	39.75±0.62	39±1.93	51.16±2.72*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

4. Discussion

The dose of caffeine used in this study (10 mg/100 g body weight) was similar to those used in previous investigations (Yeh and Aloia, 1986; Glajchen *et al*., 1988). Yeh and Aloia, (1986) reported also that this dose was equivalent to 1360 mg/ 70 kg in humans. There is a belief among the public that those who drink too much tea or coffee are disposed to have mineral deficiency problems (Conlisk *et al*., 2000; Horie *et al*., 2002). There is some experimental evidence supporting a negative effect of caffeine on bone. Following caffeine administration, teratogenic effects on ossification of the fetus have been observed in some rodent studies (Nakamoto and Shaye, 1984). Whiting and Whitney (1987) reported that the administration of coffee or caffeine to rats was followed by a negative calcium balance, an effect that could possibly be explained by an increase in the excretion of urinary and faecal calcium.

Possible explanations for the injurious role of the caffeine on bone, observed in these studies, may be due to the action of this compound on calcium metabolism and on the proliferation of osteoblast-like cells. Caffeine increases urinary calcium excretion by reducing renal reabsorption and, possibly, reducing calcium absorption, leading to a negative calcium balance (Bergman, 1990; Massey *et al*., 1993; Ilich *et al*., 2002).

Currently, Rapuri *et al*. (2007) observed that the caffeine dose dependently decreases vitamin D receptor expression and alkaline phosphatase enzyme activity in human osteoblasts; constituting a possible mechanism by which caffeine may affect bone metabolism. The effect of caffeine on bone tissue is related to calcium metabolism. Caffeine slightly impairs calcium absorption from intestines; however it has no effect on calcium excretion with urine (Heaney, 2002). In addition, Kamagata *et al.* (1999) demonstrated that caffeine has an inhibitory effect on the proliferation of osteoblast-like cells *in vitro*. Tsuang *et al.* (2006) concluded that caffeine may induce apoptosis and decrease the viability of osteoblasts. In this work; alkaline phosphatase level does not differ at low dose of caffeine; this finding is in agreement with (Liu *et al*., 2011) who showed that the low concentration of caffeine (0.005-0.1 mM) did not affect the bone marrow cell viability and alkaline phosphatase activity during osteoblast differentiation from bone marrow stromal cells, but it effectively enhanced the osteoclastogenesis from bone marrow hematopoietic cells and the bone resorption activity by pit formation assay.

Rapuri *et al*. (2007) concluded that 1,25-Dihydroxyvitamin D3 (1,25(OH)2D3) performs a fundamental role in the regulation of bone metabolism. A receptor for this vitamin (VDR, Vitamin D Receptor) occurs in osteoblast cells. This means that a high caffeine dose may influence VDR expression stimulated by vitamin 1,25(OH)2D3 and controlled by vitamin 1,25(OH)2D3 activity of human osteoblast cells by reducing alkaline phosphatase activity.

Zinc plays an important role in the maintenance of cell membrane structure and function (Bettger and O'Dell, 1993). Zinc deficiency causes a reduction in osteoblastic activity, collagen and chondroitin sulfate synthesis, and alkaline phosphatase activity (Calhoun *et al*., 1974). The elements, such as zinc, copper, magnesium and calcium have relatively low intestinal absorption efficiency and are excreted primarily in feces (Avioli, 1980; Li and Vallee, 1980; Shils, 1980).

Manganese absorption is influenced by many factors such as chemical form, presence of chelating or complexing agents and interactions between different micronutrients. It has been suggested that manganese and iron share a common mechanism of absorption and transport in the digestive tract (Keen and Zidenberg, 1996). This finding is in consistent with Leach and Harris (1997) who found that Mn and Fe share mechanisms of transport and cell uptake.

Nowadays, it is impossible to avoid caffeine intake, and its effect on bone tissue is not fully understood. Researchers' opinions on the influence of caffeine on bone tissue differ. Further studies are in progress to elucidate cellular and biochemical mechanisms by which trace elements participate in bone metabolism.

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Inhibitory Effect of Different Probiotic Bacterial Strains on Salivary *Streptococcus mutans* and Identification of the most Suitable Dairy Product for Delivery of the most Potent One: An *In-vitro* Study

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Abstract: Aim: to evaluate the possible inhibitory effect of selected *Probiotic* bacterial strains against *Streptococcus mutans* (*SM*) and to identify the most suitable Dairy product in which most potent *Probiotic* strain will exhibit inhibitory activity against SM. **Material & Methods**: Six Probiotic strains including (*Lb reuteri* ATCC 23272, *Lb rhamnosus* ATCC7469, *Lb acidophilus* ATCC 4356, *Lb acidophilus* TISTR 450, *Lb plantarum* ATCC 14917 and *Bifi bifidium* DSM 20082) were tested against SM. Bioyoghurt, stirred fermented milk and kareish cheese were prepared and tested as delivery vehicle for most potent *Probiotic* strain. **Results:** All Probiotics in Group 1-6 significantly reduce % survival rate of SM at all ratio subgroups i.e. A- C (ratio of 3:1, 1:1 and 1:3 *SM*: Probiotic strain, respectively), with exception of Group 6 at ratio subgroup A. With exception of Groups 4 & 5 at ratio subgroup A, statistically significant difference between all *Probiotic*s in the inhibitory activity against SM at all tested ratio subgroups (A-C). *Lb reuteri* ATCC 23272 displayed strongest inhibitory activity followed by *Lb. rhamnosus* ATCC7469, *Bifi. bifidium* DSM 20082, *Lb. plantarum* ATCC 14917 then *Lb. acidophilus* ATCC 4356 and last *Lb. acidophilus* TISTR 450 displayed weakest inhibitory activity. *Lb reuteri* ATCC 23272 on stirred fermented milk showed strongest inhibitory activity against *SM*, followed by Bio-yoghurt then kareish cheese, with statistically significant difference between them. **Conclusion:** Different *Probiotic*s under study reduce the oral carriage of *SM* with varying degrees. Stirred fermented milk containing *Lb reuteri* ATCC 23272 is considered the best *Probiotic* delivery vehicle for dental caries prevention.

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Key words: Probiotics, *Lactobacilli*, *Bifidobacteria*, *Streptococcus mutans*, Caries prevention.

1- Introduction

Dental caries remains the most prevalent chronic disease in children. It can be controlled by several strategies used either alone or in combination. These strategies include approaches that involve altering the bacterial flora in the oral cavity, modifying the diet, increasing the resistance of tooth enamel to acid attack or reversing the demineralization process[(1)].

Despite the use of conventional physical and chemotherapeutic agents for caries management, dental caries still continues to be the most prevent oral infectious disease. Clearly, additional caries prevention approaches which can augment the existing ones (e.g.fluoride, brushing, flossing, etc.), are clearly desirable [(2)].

Probiotic bacteria, defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001), are suggested to play a role in the maintenance of oral health [(3,4)]. Such health promoting bacteria are added to different commercial dairy products such as milk, cheese and yogurt as well as chewing gums and fruit drinks. Possible actions of probiotic bacteria in the oral environment are competition of binding sites, production of antimicrobial substances and activation and regulation of the immune response [(5)].

For some decades now, bacteria known as *Probiotics* have been added to various foods because of their beneficial effects for human health. *Probiotics* are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt or as dietary supplements. The number of products containing *Probiotics* entering the market is increasing. These products usually contain *lactobacilli* or *bifidobacteria*. The application of *Probiotics* strategies may, in the near future, provide an end to many infections occurring in oral cavity [(6)].

The present study aimed to evaluate the possible inhibitory effect of some *Probiotic* bacterial strains against caries producing *SM* and to identify most suitable dairy product in which the most potent *Probiotic* strain will exhibit inhibitory activity against *SM*.

2- Materials and methods

Microorganisms and culture conditions:

SM isolated from Egyptian child saliva, identified using Biolog system [7] ,were used in the study. The isolate was grown in Trypticase soy broth supplemented with 0.5% yeast extract (TSBY) incubated at 37°C in anaerobic incubator with 5% CO_2.

Cells were harvested during the exponential growth phase by centrifugation at 1000 RPM, washed twice with Phosphate buffer saline (PBS), resuspended in the same buffer and subjected to a low-intensity ultrasonic treatment to disperse bacterial aggregates according to ***Nikawaa et al.,*** [8].

Six *Probiotic* strains provided by Cairo Microbiological Resources Centre, Ain -Shams University, were used in the study includes *Lb reuteri* ATCC 23272, *Lb rhamnosus* ATCC7469, *Lb acidophilus* ATCC 4356, *Lb acidophilus* TISTR 450, *Lb plantarum* ATCC 14917 and *Bifi bifidium* DSM 20082**.**

Each strain was grown in brain–heart infusion broth (BHI: Difco), harvested during the exponential growth phase by centrifugation at 1000 RPM, washed twice with PBS (pH 6.8) and resuspended in the same buffer. The optical densities of the bacterial suspensions were measured in a 1.0-ml cuvette with a 1 cm light path, and the suspensions were adjusted to a final concentration of 1.0×10^8 colony forming unit (CFU)/ml before use [8].

Inhibitory effect of *Probiotic* bacterial strains:

Bacteriological assay was conducted according to ***Nikawa et al.,*** [8]. The suspensions of *SM* and *Probiotic*s and PBS were mixed in sterile test tubes and divided to 7 groups; Group (1- 6): SM mixed with (*Lb. reuteri* ATCC-23272, *Lb. rhamnosus* ATCC- 7469, *Bifi. bifidium* DSM- 20082, *Lb. plantarum* ATCC- 14917, *Lb. acidophilus* ATCC-4356 and *Lb. acidophilus* TISTR- 450 respectively).

According to the ratio of mixing, previous groups were subgrouped to: Subgroup (A- C): ratio of mixing was 3:1, 1:1and 1:3 *SM*: tested *Probiotic*s respectively. Group 7(Control): SM was mixed with the same amount of PBS. Then 100 µl were added to 10 ml of BHI broth and vortex mixed for 10 s, followed by incubating for 90 min at 37° C with gentle shaking.

Afterwards each suspension was centrifuged at 1000 RPM, washed twice with PBS, and plated on Mitis Salivarius Agar Base supplemented with 1% Potassium Tellurite solution modified by adding 0.2 units/ml Bacitracin and 20% sucrose (MSB) [9] and sealed in anaerobic jar with Gas Generating Kit incubated in electric incubator at 37°C for 24 hrs to determine the number CFU of *SM*.

The % survival rate of SM was obtained using the formula mentioned by ***Nikawa et al.,*** [8]:

$$\%\ survival\ rate\ of\ SM = \frac{CFU\ of\ SM\ incubated\ with\ probiotic\ strain}{CFU\ of\ SM\ incubated\ with\ PBS} \times 100$$

The assays were carried out on two independent occasions, with quadruplicate samples on each occasion.

Production of dairy products containing *Lb. reuteri* ATCC 23272:

Kareish cheese was made according to ***Francois et al.,*** [10] with some modifications: reconstituted skim milk (14% w/v) was pasteurized at 65±1ºC for 30 mins, and then cooled to 32±1ºC. The heat treated milk was inoculated with *Lb. reuteri* ATCC 23272 (3% in milk at 32±1ºC) until curding. The formed curd was ladled into wooden frames lined with muslin cloth and 1% salt was dispersed. Resultant cheese was stored in refrigerator (5±1ºC) for 24 hrs.

Low-fat bio yoghurt was prepared using (14% w/v) reconstituted skim milk powder according to ***El-Batawy,*** [11] with some modifications: The reconstituted milk was heated at 90ºC for 10 min, cooled to 42ºC and inoculated with 3% mixed starter culture; (1.5% yoghurt starter culture *Strep thermophilus* & *Lb. delbrueckii* subsp. *bulgaricus*1:1) and 1.5% *Lb. reuteri* ATCC 23272. The inoculated milk were aseptically transferred into 100 ml plastic containers, and incubated at 42ºC till coagulation (pH 4.7), then cooled to 4ºC.

Stirred bio fermented milk was manufactured by the method of ***Farahat and El-Batawy,*** [12] with some modifications: reconstituted milk was prepared by reconstitute 14% skim milk powder in water. The mix was heated to 85°C for 10 min, and cooled to 45°C. *Lb. reuteri* ATCC 23272 was added at the rate of 3% (w/v). The mix was filled into 2 kg plastic cups and incubated at 43°C. Incubation was terminated till pH 4.5. At this point, the fermented milk was stirred, filled into 250g plastic cups and stored in a refrigerator (5±1°C) for 1 day. Three replicates were done for each product.

Anti- *SM* activity test:

The antimicrobial activity test for dairy product prepared with *Lb reuteri* ATCC 23272 was performed using an agar diffusion test [8] with some modifications: The *SM* was subcultured and grown in TSBY incubated in anaerobic jar and 100 µl of overnight *SM* were plated on MSB supplemented with 1% Potassium Tellurite modified by adding 0.2 units/ml Bacitracin and 20% sucrose. Plates were air dried for 15 min and filter disc (6mm in diameter) impregnated with 30 µl of each extract product. After incubation at 37°C for 24 hrs, zone of inhibition was measured.

As negative and positive controls sterile distilled water and penicillin (Benzathine Penicillin G) were used respectively. The diameter of inhibition zones was scored as mentioned by ***Pan et al.,*** [13]: 6 mm equals no inhibition (-), 0 - 3 mm

(weak, +), 3 - 6 mm (good, ++) and diameter > 6 mm (strong, +++).

Data were presented as mean and standard deviation (SD) values. One-way ANOVA followed by Tukey's post-hoc were used for comparisons between the groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

3.Results

Inhibition effect of different *Probiotics* on *SM* in different Groups (1-6) represented as decrease in the % survival rate of SM regarding: (I) Different ratio subgroups (A-C) of the same group and (II) Different groups at the same ratio subgroup.

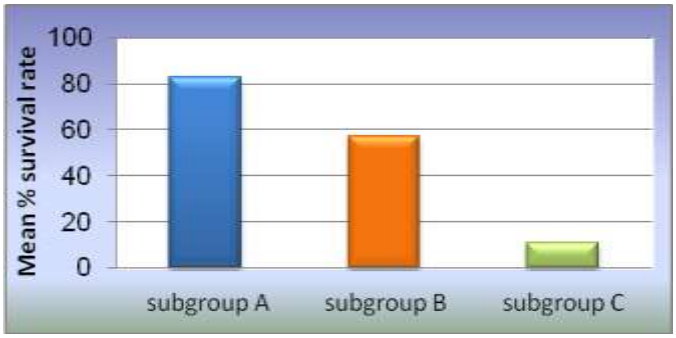
I. Comparison between % Survival rate of *SM* in different ratio subgroups (A- C) of the same Group:

Loss of viability of SM was noted via incubation with *Lb reuteri* ATCC 23272 in a ratio-dependent manner, i.e. highest inhibitory effect shown in subgroup C, followed by subgroup B, then subgroup A.% survival rate of SM in Group (1-6) at different ratio subgroups were presented in **(Table 1, Fig 1)**. It can be observed that, subgroup C showed the statistically significant lowest mean % survival rate, followed by subgroups B & A respectively .

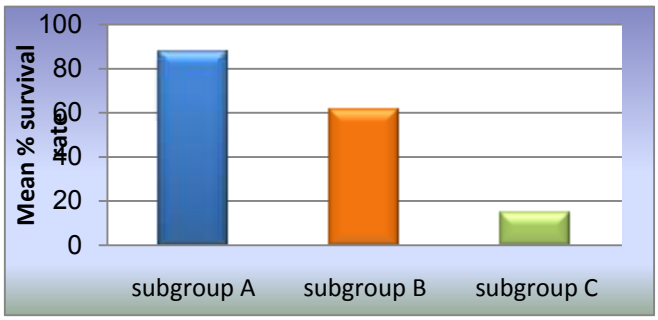
Table (1): % survival rate of *SM* in different Groups at different ratio subgroups:

Ratio subgroup / Group	Subgroup A (ratio of 3:1)		Subgroup B (ratio of 1:1)		Subgroup C (ratio of 1:3)	
	Mean %	±SD	Mean %	±SD	Mean %	±SD
Group 1	82.8 [e]	2.7	57 [g]	0.8	11 [g]	0.8
Group 2	87 [d]	0.8	60.5 [f]	1.9	14.5 [f]	0.3
Group 3	90.4 [c]	0.9	64.6 [e]	1	21.9 [e]	0.9
Group 4	94.3 [b]	2.5	75.2 [d]	1	45.5 [d]	0.9
Group 5	96.1 [b]	0.9	83.1 [c]	0.4	71.2 [c]	0.7
Group 6	98.5 [a]	0.6	89.2 [b]	2	77.7 [b]	1.2
Group 7 (control)	100 [a]	0	100 [a]	0	100 [a]	0
***P*-value**	<0.001*		<0.001*		<0.001*	

**: Significant at $P \leq 0.05$, Different letters in the same column are statistically significantly different*



Group (1)



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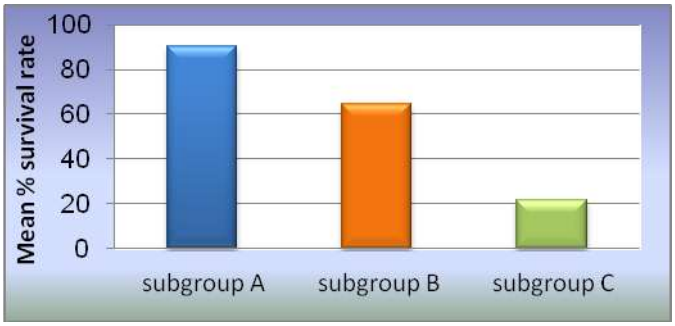
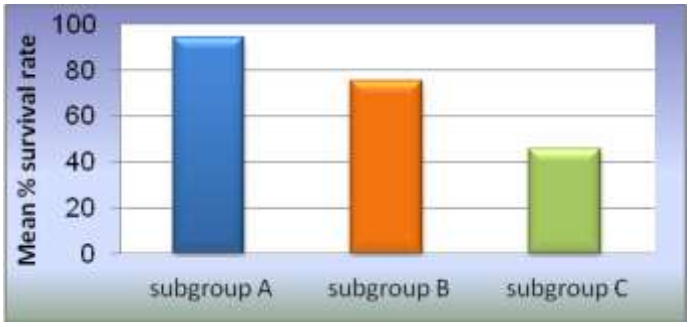
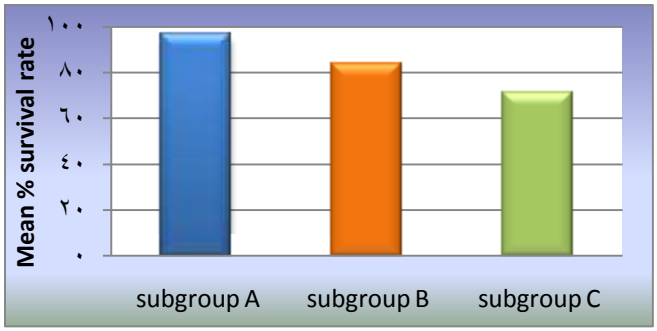


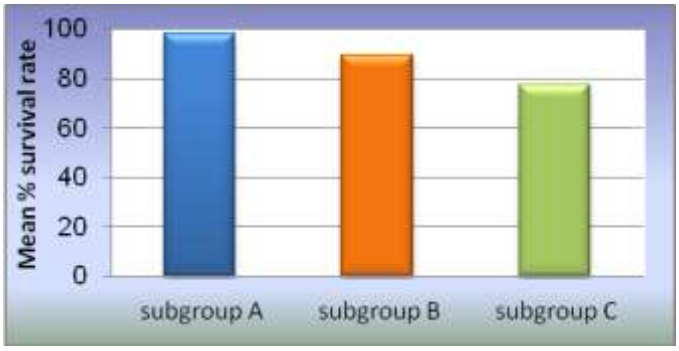
Group (3)



Group (4)



Group (5)

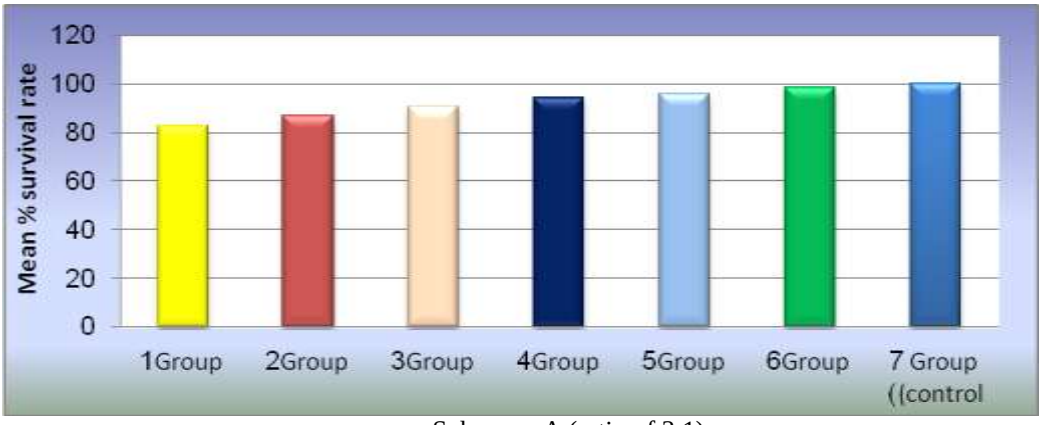


Group (6)

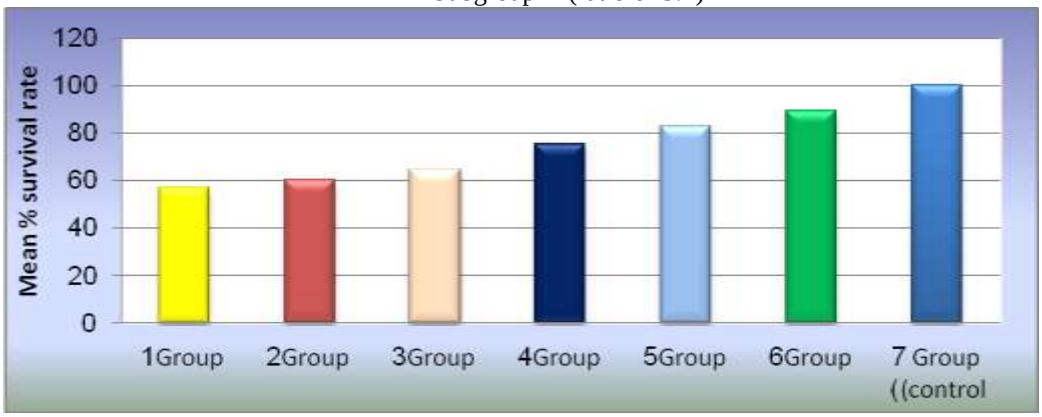
Figure (1): A bar chart representing mean % survival of SM in different ratio subgroups of different Groups.

II. Comparison between % survival rates of SM in different Groups at the same ratio subgroup:

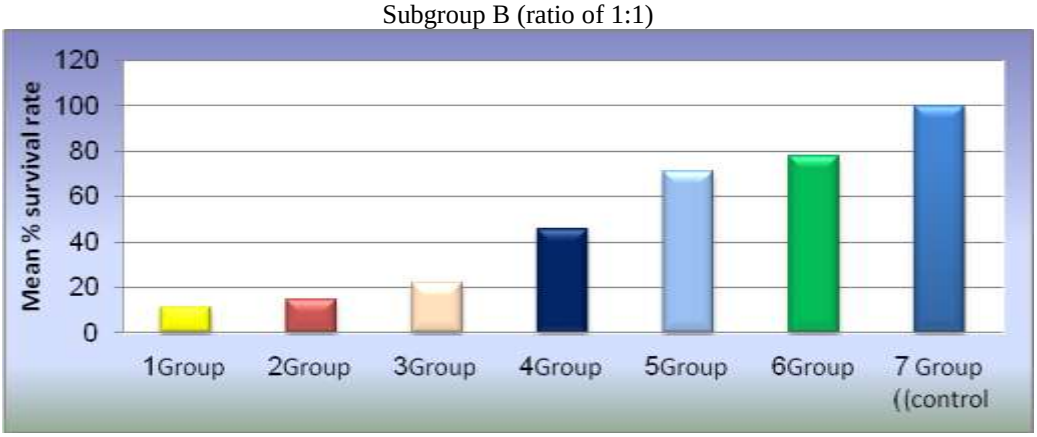
Table 1, Fig (2): Group 1, subgroup A, showed the statistically significant lowest mean % survival rate, followed by Groups 2 & 3 respectively. There was no statistically significant difference between Groups 4 & 5(higher mean % survival rates). There was no statistically significant difference between Groups 6 & 7 (highest mean % survival rates).



Subgroup A (ratio of 3:1)



Subgroup B (ratio of 1:1)



Subgroups C (ratio of 1:3)

Figure (2): A bar chart representing % survival rate of SM in different Groups at ratio different subgroups.

Group 1, subgroups (B & C) showed the statistically significant lowest mean % survival rate, followed by Groups 2, 3, 4, 5 & 6 respectively with a statistically significant difference between them. Group 7 showed 100% mean survival rate.

Evaluation of the inhibitory effect of different delivery vehicles containing *Probiotic Lb reuteri* on SM: were shown in **(Table2, Fig3).**

Table (2): Diameter of inhibition, mean values of inhibition zones of SM, score and description of control and different delivery vehicle groups:

Tested material	**Negative control**		**Positive control**		**Fermented milk**		**Bioyoghurt**		**Kareish Cheese**	
Diameter (mm)	6		16.3		13.3		11.3		9	
Inhibition zone (mm)	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
	0 ^{e}	0	10.3^{a}	0.5	7.3 ^{b}	0.5	5.3 ^{c}	0.5	3 ^{d}	0.8
Score	-		+++		+++		++		+	
Description	No inhibition		Strong		Strong		Good		Weak	

P-value <0.001, Significant at P ≤ 0.05, Different letters in the same row are statistically significantly different

Figure (3): Photograph showing inhibition zone of positive control, negative control and different dairy products containing *Lb reuteri* on SM

Positive control group showed the statistically significant highest mean inhibition zone, followed by fermented milk, then Bioyoghurt. Kareish cheese and negative control showed statistically significant lower and lowest mean inhibition zones respectively **(Table 2, Fig, 4)**.

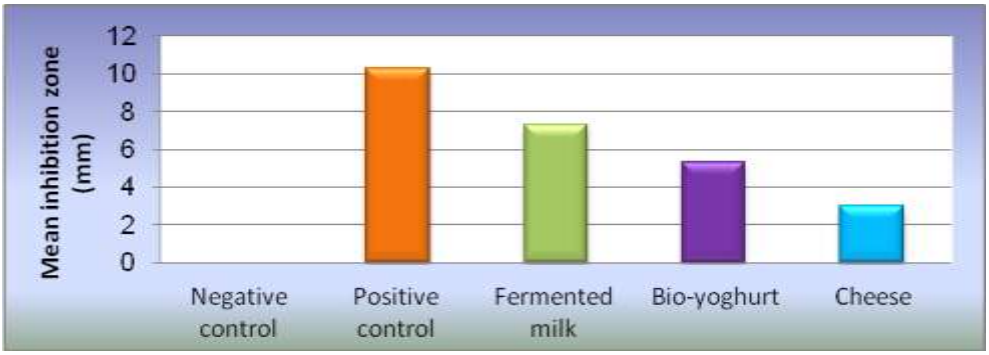


Figure (4): Bar chart representing mean inhibition zone of control and different delivery vehicle groups.

4- Discussion

As shown in **Figs (1, 2)**, loss of viability of SM was noted via incubation with all *probiotic*s (Group 1-6) in a ratio-dependent manner. These results are in agreement studies of ***Nikawa et al.,*** [8] who stated that *Lb reuteri* showed a significant growth inhibitory effect against SM, and ***Hasslöf et al.,*** [14] who studied strains of *Lb. reuteri*, *Lb. rhamnosus* ,*Lb. plantarum* and *Lb. acidophillus* La5 where the first three inhibited the growth of SM completely at concentration ranging from 10^9 to 10^5 CFU/ml, while a slight inhibition at concentration corresponding to 10^7 and 10^5 CFU/ml was observed in the last.

The previous results also agree with in vivo studies of ***Çaglar et al.,*** [15] who concluded that short-term daily ingestion of *Lb. reuteri* ATCC 55730 delivered by prepared straws or lozenges reduced the levels of salivary SM in young adults, ***Çaglar et al.,*** [16] who found that daily chewing gums containing probiotic *Lb. reuteri* ATCC 55730 reduced the levels of salivary SM significantly, and daily ingestion of *Lb reuteri* ATCC 55730 delivered via medical device reduced the levels of salivary SM. ***Näse et al.,*** [17]**, *Ahola et al.,*** [18] and ***Zaazou et al.,*** [19] concluded that *Lb. rhamnosus* GG reduce SM counts significantly.

The results are in concordance with ***Çaglar et al.,*** [20], ***Çaglar et al.,*** [21], ***Cildir et al.,*** [22] and ***Polka et al.,*** [23] who mentioned that, Bifidobacteria strains significantly inhibit SM. However, early administration of *Bifi. lactis* Bb-12 did not result in permanent oral colonization of this *Probiotic* or significantly affect SM colonization in the study of ***Taipale et al.,*** [24].

Fig (2) showed that ,with exception of Group 6 at ratio of subgroup A, all other tested *Probiotic*s were significantly lower the SM CFU and % survival rate of i.e. significantly inhibit the growth of SM at different tested ratio. These findings come in agreement with ***Simark-Mattsson et al.,*** [25] and ***Hasslöf et al.,*** [26]**.**

The previous results of *Probiotic*s under study were explained on the bases that, *Probiotics* secret active molecules (e.g. bacteriocins, antibiotics, free fatty acids, hydrogen peroxide) that control growth and/or survival of surrounding microorganisms [27]. Whether production of bacteriocin, or of other factors, was the main source of lactobacillus-mediated interference, remains to be determined [24].The final pH in the medium has been suggested to be an important factor for growth inhibition, either directly or due to the production of bacteriocins at low pH [28].

Similar strain-dependant differences have previously been observed concerning the metabolic capacity to form acids from dietary sugars that differs significantly between various *Probiotic*s [14, 29].However, also some bacteria with fairly weak acid production proved to be effective against SM. This indicates that other inhibitory substances also may be involved with hydrogen peroxide being among the primary metabolites with inhibitory capacity against microbial pathogens. The antimicrobial glycerol derivative reuterin is another example of a growth inhibitory substance produced by *Lb reuteri* [30, 31].

The most commonly consumed *Probiotics* are fermented dairy products such as yogurt and butter milk [32]. *Probiotic*s can currently be administered in the form of sachets or capsules, or can be added to the food supply. Some data show that adequate colonization may be achieved at a lower dose if *Probiotics* are administered in food [33].

Administration of *Lb reuteri* ATCC 23272 in three forms of dairy products to inhibit SM was tested in this study using and the results are shown in **Table 3, Figs 3-4;** positive control showed the statistically significantly highest mean inhibition zone, followed by fermented milk then Bio-yoghurt. kareish cheese and negative control showed statistically significantly lower and lowest mean inhibition zones respectively.

Significant difference between tested dairy products may be related to several factors as previously discussed by ***Vinderola et al.,*** [34] who stated that, mixed-strain cultures of lactic acid starter and *Probiotic*s are commonly used in the manufacture of *Probiotic* fermented milks and cheeses. In these bacterial combinations, interactions among different strains can result in stimulation, inhibition, or absence of effects on microbial growth rate and metabolic activity. The low pH values that *Probiotic*s are submitted to during the processing of dairy products, such as yogurts and fermented milk, is also a matter of concern. Since that with the exception of few Lactobacillus and Leuconostoc species, lactic acid bacteria are neutrophilic, that is, have optimum growth pH between 5 -9[35] .

Conclusion

In conclusion, it could be reported that, different *Probiotic*s under study displayed inhibitory effect against SM with varying degrees. *Lb. reuteri* ATCC 23272 is considered the *Probiotic* with the most promising results SM. Different dairy products could be used as *Probiotic* delivery vehicle for inhibition of caries producing bacteria and stirred fermented milk is the best *Probiotic* delivery vehicle for dental caries prevention.

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The Concept of Signifyin(g) Monkey in "*Beloved*" by Toni Morrison

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Abstract: Language has been an ideological and political tool in the hand of imperialists and colonists. An outstanding feature of African American literature has been proved to be a distinctively variety of English language used prevalently among black communities in the United States of America and specifically in Black Narratives which is an answer to the monolithic feature of Standard English. Toni Morrison is an author for whom language goes beyond a mere tool of communication and the present article is a study of her novel 'Beloved' and deals with the concept of black vernacular English through Henry Louis Gates, Jr.'s "Signifyin(g) Monkey" which reinforces the plurality and flexibility of Black vernacular English in contrast to Standard English.
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Keywords: Colonial Language; Dialogical Language; Signifyin(g) Monkey; Toni Morrison; Beloved

1. Introduction

In spite of its variations, Phenomenon of colonialism has expanded in different ways and has created the dominant discourse almost at any location all over the world. Nowadays one can rarely feel the existence of direct colonialism in any country due to the structure of modern life. However the cultural and social impact of imperialism and colonialism is more provoking and deeper than before. No doubt the West, the United States and many European countries, today, are acquiring more than what they would rob at gunpoint in poor nations years ago by means of the dominant discourse in terms of knowledge. Today, every nation that somehow suffered directly or indirectly from colonial domination requires reviewing and reconstructing the distorted history and identity that the West fabricated for them. Discussing African American community as a colonized nation is an ironic case mainly because firstly, it eradicates the notion of the West and the East as mere geographical concepts and secondly, colonizing can be occurred within one country. Perhaps those generations of black people who were forced to leave their homeland, that is African American slaves, suffered from the cruelty of colonization more than any other colonized nation since they had been robbed of their identity, language and history. Today African American people are forming their own discourse by challenging the long-term established Western discourse.

1.1. Black Discourse

Black social, cultural and literary movements such as black arts movement, black power movement, the Harlem Renaissance, and the civil rights movement were unknown until recent years while these movements constitute the main pillars of African American Criticism which includes a huge portion of the United States Literature. Although racial domination in America put cultural and literary works of African Americans into long-term isolation and, roughly from the sixties and seventies of twentieth century they have achieved many national and international rewarding reputations and are allocated many academic positions (Baker, Houston A., Jr. (1991). The dominant ideology of America's society has excluded all its minorities from the history and it is the reason that critics like Lois Tyson emphasizes that it will probably more significant if the history of America is renamed the history of white American due to the exclusion of minorities through prevailing hegemony. (Tyson, 2006: 360).

One of the most effective and natural means to naturalize the concept of racism in America was fields of art and literature. Therefore the Western philosophy and literature is based on Euro-center definitions which reflect the experiences of Westerners and are surprisingly labeled as universal. According to the same principles literature of African - American as well as other minorities was ignored until the mid-twentieth century. In other words, the literature and culture of America have been a mirror of society and an executor of white patriarchal hegemony. Writers and artists of African - Americans have played considerable role in the hegemonic process including Toni Morrison, Alice Walker, John Edgar Waldman, Maya Angela, Gloria Naylor, Nikki Giovani, Charles Johnson, Rita Dove among others have been able to challenge the white patriarchal monologue voice and they accounted for a significant portion of America's literature(Blake, Susan. 1984).

The naturalization of racism had a profound influence on America's communities so that even in the first half of the twentieth century black people were suffering from the concept of "internalized racism" that was much worse than racism. Victims of "Internalized racism "come to believe that white people are naturally master race and based on this,

white people are more capable, wiser, more attractive and generally are located a higher level (Denard, Carolyn. 1998). consequently during this period the most common community disease for African – Americans was psychological acceptance of inferiority of black and "superiority of white people. Whatever was white and related to the sovereign rule was considered superior and whatever was black was considered indecent and improper. The tragic thing was that this inferiority sense of the black population had been displaced as a natural phenomenon. Toni Morrison has illustrated this psychological complex in her novel "The Bluest Eye" successfully and skillfully. In this novel Pecola, the black girl who is unable to appreciate her beauty and this inability is rooted in internalized racism, considers "blue eyes" and white skin as the symbols of beauty and always thinks that if she had blue eyes she were charming and attractive.

Tyson believes that internalized racism may lead to "intra-racial racism"(362) that is a kind of discrimination among black people based on the degree of being darker or whiter.

Those who are lighter skinned, are considered to be better and closer to the dominant norms of society. In the novel "The Bluest Eye" Pecola is humiliated because she has darker skin comparing to other characters. On the other side Maureen Peal has the better position for her lighter skin (Holloway, Joseph E. (ed.) (1990).

1.2. Double consciousness is a term coined by Du Bois that refers to his famous theory of African American "double consciousness". The term originally referred to the psychological challenge of reconciling an African heritage with a European upbringing and education. African Americans belong to two completely different and sometimes contradictory cultures: European cultures of America whites imposed on them and African culture which is their heritage brought to America by previous generations (362). Language is an outstanding aspect of "double consciousness". People of African - Americans need to use different languages in different social situations. They use language of Black African - American with together and with family and in situations which is more dominated by Western culture Such as academic places or at work, they have to speak Standard English. One of the challenges for African - American writers is whether to use African - Americans language or Standard English in their writings. Is their audience black or white or both? This matter has influence on the text .For example, the famous poet of the Harlem, Countee Cullen, preferred to use 'pure' English in his poems without any impact of black English although many of the themes of his poetry are about race and political issues (363). But some other poets as Langston Hughes used Black English which is different from Standard English in many ways. Hughes's poetry is mixed with the rhythms and speech patterns of African - Americans and their blues music. In fact, language is for artists such as Hughes a means to introduce the culture and rich heritage of African – Americans.

In the early twentieth century and later African - American artists used unique artistic techniques to illustrate their identity in the form or content of their works to create equal opportunities and this attitude was developed and reinforced more in "Black Arts Movement". Amiri Baraka who was one of outstanding representatives of this movement believed that the main responsibility of African American author was to identify and provide some solutions to destroy the demon of racism. Criticizing the thought and philosophy which introduced the binary system of suppressing and suppressed, colonizing and colonized was the central topic for critics and writers in this period. This topic, criticisms of white supremacy, has remained to this day.

Another significant topic that was challenged by Black Arts Movement is the validity of Criteria of Western critical theories for interpretation of the black arts. They believed that According to the same Western criteria and Definitions the African - American literature has been marginalized thus those norms lose its validity and objectivity. White people create all contemporary literary theories based on what they considered to be true or false. Therefore, is it possible to criticize and evaluate the marginalized minority's literature based on these criteria?

On the other hand there are many critics who reject this idea as absolutism and believe that there are many western theories that are effective to interpreter African – American literary works. Gates who is one of them asserts that whatever contributes to a better understanding of African American works, should be used. This is also his strategy for producing his "Signifying Monkey "; his literary theory was influenced by Mikhail Bakhtin and Saussure.

1.3. African – American Language

The second half of the twentieth century was the golden era of black literature in America. Within this new discourse the used-to-be 'other' expresses 'self' rather than being expressed through the lenses of others. No doubt one of the new elements to be used was the very unique language of African American people. The history of black literature has shown that different writers have different reactions to language and almost no one has ignored it. The emergence of this language is sometimes demonstrated stereotypically but frequently it is used meticulously with precise Linguistic techniques. These features, whether in narrative form or content, contain

considerable messages that are very important to understand and interpret the works. The first African American novel, according to Greene, that employed Black English in the history of African American literature was "Clotel: Or the President's Daughter: A Narrative of Slave Life" by William Wells Brown in 1853.

This novel represented African- American language which was common at that time and the black character were left free to employ their common language. Greene believes that this novel is an important work because the author depicted events related to slavery laws and its consequences while many of the parameters of African- American language can be found in the narrative. (Greene, 2002: 169). Many African - American theorists believe that linguistic confusion experienced by black communities has definitely interfered the integrity of psychological "self" of black subject and therefore the subject of African - Americans are constantly searching for suitable tool for the expression of "self" which is one of the frequent themes in their art and literature (Gates, 1983: 239). Houston Baker, Henry Louis Gates and Barbara Johnson have studied African American narratives from the perspective of black discourse analysis. They believe that particular linguistic techniques and patterns which are used by African – American authors have made a text particularly 'black' or 'African-American'.

Since the language(s) of African slaves disappeared generally and except for a collection of words and sounds which remained till now, it/they dissolved in Standard English. However any African American text is hunted by its/their ghost-like presence. Consequently the new adopted language, that is African American English, do not provide a coherent image. Thus, African – American authors naturally considered language as a phenomenon that goes beyond a mere means of communication or a tool for exchanging thoughts and feelings. In fact it became a national obsession and it was employed to uncover the unspoken aspects of black community.

J. L. Dillard (1924-2009) studied linguistically the works published before the twentieth century such as works by Charles Waddell Chesnutt, Joel Chandler Harris and William Wells Brown who employed African American English. However later generation were more obsessed by black language. Zora Neale Hurston and Langston Hughes are most famous authors or poets of the Harlem Renaissance whose main themes were the black language. Greene believes that Hurston presented emphatically in her stories various aspects of African American language including phonetic differences, structural variety, different concepts and vocabularies. He admits that this generation especially Hurston paid more attention to the form of the language such as differences in phonics and articulation comparing to black writers of the previous generations. (Greene, 2002: 178) Hurston's characters have stronger ties with African culture with the use of colloquial and popular language. Greene also says that Ralph Ellison in his "Invisible Man" (1914-1994) reflected the language of African - Americans professionally by Using cultural elements of folklore and linguistic tools and labeled it as one of the most important literary works of African - Americans in the early second half of the twentieth century. He used the linguistic parameters, literary techniques with particular emphasis on language and syntax rules attempting to express the culture and history of African – Americans. The main character in "Invisible Man", Peter, is a trickster plays with rhetoric, rhyme, irony and ambiguity and tries to express more hidden meanings and to convey the concepts that cannot be transferred otherwise. Not surprisingly for most of other writers of the time such as Alice Walker, Toni Cade Bambara, Toni Morrison and Ishmael Reed language is a considerable theme. In this part the writer tries to explore Morrison's "*Beloved*" through Henry Louis Gates' 'Signifying Monkey'

1.4. The Signifying (g) Monkey

Henry Louis Gates is one of the most prominent literary critics of African – Americans. In a review article called "The Blackness of Blackness: A Critique of the Sign and the Signifying Monkey" he in order to clarify his theory describes a manner of speaking within African - American community. Gates borrows the term 'signifying' from Saussure, but with a distinctly different meaning.

He uses Bakhtin's ideas about language to define 'signifying'. Mary klages says the term 'signifying' in academic context has the same meaning of Saussure's term in his theory. But in African - American context 'signifying' is the name of a type of language that Gates connects it to jabbering, shouting, and signifying (Klages, 2008, 150). Gates pronounced and writes signifying as recorded in the African - Americans English language; signifying (g). According to Gates the origin of signifying roots in the myths and beliefs of African – American people. "Signifying" is defined by Gates as a verbal game which is based on insulting and is used as a defense mechanism. It is used in situations where other forms of power are not available. Gates relates this action to symbolic reactions of monkeys to their facing stronger animals of the forest. Monkeys are in a less powerful situation but they use their verbal playful skills. Gates indicates that African American people empties the sign from the normal signified and fill it with new ideas and concepts and in this manner, the relationship between signifying and signified of the standard

language is destroyed. At the same time new concepts and meaning are created.

All differences of African – American language with Standard English language arises from this action which all African Americans practice it consciously and sometimes unconsciously. And these playful uses of language create a discourse that stands side by side to Standard English. According to the linguistic theories of Derrida and Bakhtin, the speakers who are closer to the center of Language (standard language) experience more linguistic power. However ironically those speakers who are in the margin of language (speakers of dialects for instance) have a superior status because in the margin, Language is more flexible and plural. As a result of this instability of meaning the speakers are more able to use the language. (Klages, 2008, 151)

African – American vernacular has been marginalized as a non-standard language; it is plural, ambiguous and instable. Whereas standard English as an accepted 'proper' form is fixed, stable and monolithic. According to poststructuralist and postcolonial theories stability and inflexibility of language associate with monolithic systems of governments and dictatorship while multiplicity and plurality of language can be linked to multinational organization and democratic systems. With such mentality authors of African - American use language as a tool to counter the dominant discourse in literature. Morrison's narratives are often challenging Standard English through African-American vernacular employed in her novels.

1.5. 'Signifying (g) Monkey' in '*Beloved*'

In her work, Morrison indicates the condition of African Americans generally and black female specifically in a racist country full of injustice. She uses Components such as myth and imagination in addition to factors such as Racism, sexism and class differences in his novels. In one of her interviews Toni Morrison admits that the worst experience for any human being might be the loss of one's language. When asked what most prominent characteristic of her work is, she said in answer "her language". She says the most important thing for black people is language. African Americans love to play with words and enjoy it. "There are certain things that I cannot say without recourse to my language.

It is terrible to think that a child with five different present tenses comes to school to be faced with those books that are less than his language. And then to be told things about his language, which is him, that are sometimes permanently damaging." (Tylor-Guthrie, 1994, 124)

She added that In African - American literature, there are some layers of meaning which only for black readers or listeners are understandable.

One of the main themes of Morrison's narrative is the role of African - American language which is either in the form of linguistic elements or as thematic concepts. However these two elements are more frequently fused. Sethe the main character in "*Beloved*" sometimes tries hard to remember unsuccessfully anything of the language in which her parents and grandparents had spoken. But the only thing that she remembers is that when she was a little child she used to speak in another language; the language which has entirely disappeared. Nor can she find anything about her parents when she explores her past.

The vanished language is a part of her identity and like any other slaves she feels the gaps and emptiness which is one of consequences of losing one's language. (Morey, Ann-Janine (1997) After Mr. Garner' death, Schoolteacher has assumed responsibility for the farm. He conducted a pseudo-scientific study of the slaves, treating them in his study the way a biologist treats lab animals; writing and studying on "these creatures". When Cixous, another Character in "*Beloved*", was arrested on charges of stealing food, he tells Schoolteacher that if slaves eat enough with further working, master will benefit. But Schoolteacher says that in response "definitions are by definer not by defined" (Morrison, 1987: 190).

Sethe even once heard that Schoolteacher asked his nephew to look at Sethe and contrast her with an animal to classify her animal and human characteristics. Schoolteacher with his symbolic pseudo-scientific methods stands for the long-term established colonial discourse in which blacks are considered as uncivilized community and need to be civilized. Morrison criticizes the western historiography by which non-western nations are defined and formed. It is the reason that Seth prefers to kill her daughter to let people like Schoolteacher write her destiny. Beloved, another character in the novel, which is nineteen years old and appears suddenly in 124, is one of the most unconventional characters in Morrison's all narratives. She represents past and present history of African American community. She does not remember almost anything of her past.

She is sick, cannot breathe easily, her head is loose, her movements are like an immature child's, her speaking is less than a mature human's. Her real name is never known and her identity is complex. She is the embodiment not only of the baby's ghost but also the legacy of slavery. Among many other things she represents the history, language and culture of black community.

William L. Andrew associates the character of Beloved to Gates' signifying monkey (trickster). He

believes Beloved is the image of the non-rationality who cannot be defied easily. She simultaneously has several roles; Sister, daughter, lover and perhaps mother. Her relationship with other characters is both metaphorical and real. Her thought and words, are confusing. Even her physical figure is shifting and unstable. She belongs to the future and the past. She cannot be defined in words and in short she is pregnant with all possibilities (Andrews, 1999: 117). Paul D. is another character who joins a chain band and only his group members understand his unique language and words. He selects his words and expressions in such a way that almost every syllable has many different meanings. Sethe is another signifyin(g) monkey. On her way to escape Sethe is accompanied by Ella. Ella very soon recognizes that in Sethe's speech concepts and meanings go far beyond meaning of words. Therefore she attempts to understand the 'gaps', 'hole', 'silence' and unspoken words convey by her. However it is not an easy job to understand such language. Even for those who are from the same community filling the 'gaps' with meanings will be difficult. For example, Sethe and daughters' dialogue is not understandable for Paul D. This discourse that relies more on verbal techniques such as storytelling, rhetoric, diversity and ambiguity stands against monolithic and racist discourse which is based on writing and is represented by Schoolteacher.

The recent discourse created by African American narratives, which has all characteristics of Gates' "signifying (g)", evokes Bakhtin's world of dialogue. Mikhail Bakhtin, one of the well-known literary and language theorists, believes that the language has an ideological nature in terms of structure and content. He argues that any language is under a permanent changing process it should always examine how people use languages. Bakhtin's theory focuses primarily on the concept of dialogue.

His theory is rooted in uncertainty. Language is always "dialogic" both in speech and writing. Dialogue synthesizes multiple voices. When someone speaks or writes, she/he is not the only source of her/his speech. Rather her/his discourse like her/his identity is a mixture of multiple voices from Past and present. Speech and writing arose from pluralistic dialogue. Dialogue from Bakhtin's view is in contrast with monologue. Monologues include the utterance of one person that does not have the plurality of dialog. klages says Bakhtin believes that there are two forces at work in the use of language "centripetal " and " centrifugal." centripetal power leads the language to the integration and monologues and tries to eliminate the differences.

It makes the standard language and standard norms. However, centrifugal force propels the meanings to the margin of language which leads to plurality, diversity and democracy (Bakhtin, 189:1981).

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An Economic Study of the Most Important Oilseed Crops in Egypt

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Abstract: Egypt imports of vegetable oils about 676 thousand tons per year as an average during the period (1995-2010), which cost the state about 1.9 billion pounds annually. It is expected to increase the burden on the state budget in the coming years as a result of the increase in population and decreasing of oil crops production.Turns out the results of the study fluctuation of area and production oilseed crops under study (sunflower, soybean, peanut, sesame, cotton) between increases and decreases.The production of crops per feddan under study, had taken an increasing general trend statistically significant, except for the sesame crop productivity that increased at a non-statistically significant; as for the contribution of vegetable oils, both domestic or imported product in domestic consumption has increased from about 824 thousand tons in 1995 to about 1783 thousand tons in 2010; the average annual rate of about 1201 tons and the average per capita per year of vegetable oils had reached a minimum of about 4.2 kgs / year in 1999 and a maximum of about 18 kgs / year in 2010 and an average annual rate of about 11.3 kgs / year during the study period. The self-sufficiency rate ranged from a low of 19% in 2005 and a maximum of 54% in 2002, representing vegetable oils imported a significant proportion of the total Egyptian imports ranged from a minimum of 1.3% during the years 2001،2003and 2007; a maximum of around 4 % in 1995 and ranged from the cost of imports about 1.6 billion pounds, and in 2010 was the increase of 1.4% at a cost estimated at 3.9 billion pounds. The study examined the problems of production and marketing of oilseed crops, which lies in the lack of adoption of technological packages due to the presence of some obstacles, such as the high cost of implementation and the lack of improved seeds and lack of marketing information and offers crop for many diseases and pests. As for the means of the development of oil crops depend on the expansion of the cultivation of certain oil crops in the new lands until it exceeded from the circle of the competition of individual crops in addition to the organizing of the local marketing.

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Introduction

Oil crops are considered the main source of food for vegetable oils used in human food and in some food industries, it is also representing the secondary output (gain) including diet importance for animals and poultry, due to the importance that the vegetable oil is a good source of energy. Plant oils which are used in some industries supplying materials for manufacturing of margarine, paints, cosmetics and some medical industries.Vegetable oils contain the essential amino acids necessary for the human body and that the body can not configure inside and also considered the most important vegetable oils from the food industry in Egypt. Despite the importance of oil crops, the domestic production is not enough to meet the increasing demand for consumption, which led to the increasing gap in the production of vegetable oils; and moving toward increasing year after year due to increased demand, which is due to the steady increase in population with a lack of production, including the direction of consumers towards increased consumption of oils because they contain a small percentage of cholesterol due to increased health awareness.

Problem of the study:

The problem with vegetable oils in the imbalance between demand and supply them, Egypt achieved rate of self-sufficiency from vegetable oils amounts to about 54% in 2002, and took this rate to decline to about approximately 20% in 2010, and this increasing deficit in the ability of local production of vegetable oils to meet the food requirements of the consumer is one of the important things that occupy policymakers productivity related to food security.

Objective of the study:

The study aimed to the current situation of the most important oilseed crops in Egypt, (sunflower - soybean - peanut - sesame - cotton) during the period (1995-2010) through the study of the evolution of indicators for production of those crops (acreage, productivity and total production) in addition to the study the extent of stability in these indicators for production, as well as the study of consumption and imports, marketing and identify the problems of production and marketing of oilseed crops and the possibility of the development of its production in Egypt.

The method research and sources of data collection:

Descriptive and quantitative analyses were used to estimate trend in overall time, and calculate the coefficient of instability of the productivity indices, were calculated coefficient of instability through the following equation:

Instability coefficient = ∑ N (Yh -^Yh) / ^Yh x 100

Where Yh = actual value of the variable in the year h

^Yh = estimated value of the variable in the year h

h = 1-2 years of..... 15 and was achieved the optimum situation of the stability of indicators for production if the value of stability coefficient equal to zero.Increasing the value of this parameter from zero, it means there is a stability in the value of this indicator.

In this study was relied on data from published and unpublished, Ministry of Agriculture and Land Reclamation Ground and the Central Agency for Public Mobilization and Statistics and Statistical Yearbook and bulletins of consumption of goods.

Research plan:

To achieve the objectives of the study has addressed the current status of the most important crops of oilseed in Egypt and the domestic consumption of oilseeds and vegetable oils, and then study the evolution of the quantity of imports of oilseeds and vegetable oils during the study period (1995 - 2010) and then dealt with the problems of production and marketing of oilseed crops and the possibility of development in Egypt.

Research results:

First: the evolution of space, the productivity, and the production of oilseed crops under study: -

Table (1) shows the study of evolution in the area and production of the most important oilseed crops under study during the period (1995-2010), the cotton seeds, sunflower, soybean, peanut and sesame, as shown in Table (2) the trend overall time for area and productivity. The production of these crops is evident from the following tables: -

Table (1): The evolution of area and productivity of oilseed crops during the period (1995-2010)

Cotton seeds			Sesame			Peanut			Soybean			Sunflower			year
Production	Productivity	Area	Production	Productivity	Area	Production	Productivity	Area	Production	Productivity	reaa	Production	Productivity	Area	
383	0.54	710	**32**	**0.45**	**72**	**130**	**1.23**	106	**63**	**1.02**	62	67	0.9	74	1995
552	0.6	920	**37**	**0.49**	**75**	**126**	**1.21**	104	**40**	1.1	36	50	.092	54	1996
558	0.65	859	**34**	**0.5**	**67**	**125**	**1.23**	103	**35**	1.1	32	25	0.96	26	1997
387	0.49	**789**	**26**	**0.5**	**52**	**133**	**1.28**	104	**47**	1.11	43	32	0.97	33	1998
426	0.66	**645**	**33**	**0.5**	**65**	**180**	**1.28**	141	**19**	1.14	17	44	0.99	44	1999
477	0.92	**518**	**37**	**0.51**	**72.4**	**187**	**1.3**	144	**10**	1.17	9.2	27	0.98	28	2000
408	1.1	**731**	**35**	**0.51**	**67.9**	**205**	**1.36**	151	**15**	1.25	12.7	44	0.96	46	2001
650	0.92	**716**	**37**	**0.51**	**72.8**	**190**	**1.35**	141	**18**	1.45	14.1	35	0.95	37	2002
589	1.1	**535**	**36**	**0.51**	**71.5**	**196**	**1.33**	147	**29**	1.27	19.7	31	0.98	32	2003
701	0.98	**715**	**37**	**0.53**	**69.6**	**192**	**1.33**	144	**43**	1.29	34.220.1	45	0.97	46	2004
723	1.1	**657**	**37**	**0.55**	**66.9**	**200**	**1.35**	148	**26**	1.29	17.8	31	0.97	32	2005
600	1.12	**536**	**41**	**0.55**	**73.4**	**184**	**1.39**	132	**23**	1.27	19	36	1	46	2006
621	1.08	**575**	**42**	**0.56**	**75**	**218**	**1.41**	155	**26**	1.38	21	29	1.1	28	2007
362	1.16	**313**	**37**	**0.56**	**66**	**209**	**1.43**	146	**29**	1.39	25	33.6	1.12	30	2008
408	1.2	**340**	**41**	**0.55**	**75**	**170**	**1.4**	153	**39.1**	1.41	36	35.2	1.1	32	2009
377	1.02	**369**	**46**	**0.53**	**87**	**202**	**1.2**	158	**43.2**	1.19		36.8	1.04	35	2010
446.7	0.91	620	34.6	0.52	70.5	177.9	1.3	136	31.5	1.2	26.17	37.5	0.99	35.1	Mean

Area: - feddan. productivity: - tons / feddan. production: - thousand tons

Source: - Central Agency for Public Mobilization and Statistics, Statistical Yearbook - the number of sporadiction.

Table (2): The equations of the general trend of the area, productivity and total production of oilseed crops during the study period (1995 - 2010).

Growth rate%	Average period	T. calculated	F	R^2	Equations general trend	Statement	Yield
3.7	39.7	(1.85)	3.4	0.22	^Yh= 51.792 - 1.35 Xh	Area (feddan)	Sunflower
1	0.98	4.38**	19.14	0.62	^Yh = 1.03 + 1.67 Xh	Productivity (ton/fedd)	
3.3	37.8	(1.81)	3.3	0.214	Y= 47.5 - 1.34 Xh^	Production (ton)	
7.6	25.6	2.4*	5.75	0.23	^Yh = 40.36 - 1.9.72 Xh	Area (feddan)	Soybean
2.2	1.22	6.65**	44.2	0.79	Yh = 1.22 + 0.83 Xh^	Productivity (ton/fedd)	
4.9	30.2	(1.66)	2.76	0.19	^Y= 42.25 - 1.49 Xh	Production (ton)	
2.8	133.2	4.2 **	17.88	0.6	^Yh = 105.64 + 3.68 Xh	Area (feddan)	Peanut
1.51	1.32	16.6**	112.8	0.9	^Yh = 1.204 + 0.03 Xh	Productivity (ton/fedd)	
3.9	176.8	5.84**	34.08	0.74	^Yh = 129.52+ 6.84 Xh	Production (ton)	
0.35	69	(0.6)	0.354	0.03	^Y= 57.89 + 0.24 Xh	Area (feddan)	Sesame
2.8	0.25	9.6**	92.3	0.89	^Yh = 0.789 + 0.19 Xh	Productivity (ton/fedd)	
1.7	35.9	3.04**	9.3	0.44	^Yh = 0.487+ 42.95 Xh	Production (ton)	
4.1	658	3.73**	13.89	0.54	^Yh = 753.36 - 35.25 Xh	Area (feddan)	Cotton seeds
5.6	0.89	6.86**	47.1	0.8	^Yh = 0.78 0 + 0.06 Xh	Productivity (ton/fedd)	
1.9	559.5	(1.17)	1.36	0.102	^Yh = 352.5+956 Xh	Production (ton)	

yh ^ = estimated value of space and productivity and total production in the year
Xh = time element in the year () is not significant
* Significant at the level of 5% ** significant at the level of 1%
Source: calculated and collected in Table (1)

Table (3): The coefficients for each of the instability of the area, productivity and total production of the most important oilseed crops during the study period (1995 - 2010).

Yield	Area (%)	Productivity (%)	Production (%)
Sunflower	31	21	1
Soybean	62	3	1.3
Peanut	7.4	28.4	0.3
Sesame	0.2	1.8	0.3
Cotton seeds	0.02	14	0.05

Source: calculated and collected from Table (1), (2).

Sunflower crop: -

The area decreased from 74 thousand feddans in 1995 to 35 thousand feddans in 2010 had taken this decline in volatility during the study period, reaching below 1996, including an estimated 54 thousand feddans As for productivity per feddan, had tended to increase during the same period was kept to a minimum about 0.9 ton / fedd. in 1995 and capped at about 1.12 ton / fedd. in 2009, an increase of about 24.4% from what it was in 1995.Table (1) showed that a marked decrease in the total production of sunflower during the study period, dropping from 67 thousand tons in 1995 to about 36.8 thousand tons in 2010 by a decrease of about 50%. By studying the trend overall time for each of the area, productivity and production of sunflower crop was observed a direction of each of the area and the total production to decline at an annual rate decreased by an annual rate of about 3.7 %, 3.3 %, respectively, but that this decline is uncertain of destination statistical. This means that estimates of area and total production of sunflower revolves around the arithmetic average which indicates that all of area and production of the crop was not affected nearly by factors, which reflects the impact of the time element, but it was noted that productivity per feddan for this crop took a general trend increased during the same period, an increase of about 1 % and that this increase is certain of the destination at the statistical level of significance 0.01.

Soybean crop:

The area decreased of 62 thousand feddans in 1995 to 36 thousand feddans in 2010 which had taken this decline in volatility during the study period, reaching below the year 2000, including an estimated 9.2 thousand feddans and no later than 1998, including an estimated 43 thousand feddans. As for productivity per feddan has tended to increase during the same period amounted to about 1.02 ton / fedd in 1995 to

about 1.41 ton / fedd in 2009, an increase of about 35.3% from what it was in 1995. Table (1) shows the significant decrease in the total production of soybean during the study period, dropping from 63 thousand ton in 1995 to about 43 thousand tons in 2010.

Table (2) shows the area and production of soybean decreasing annually at an annual rate significant statistically amounted to about 7.6% and 4.9%, respectively, at 0.05 and that of the average area, which is about 26.17 thousand feddans and an average production of about 31 thousand tons while production had tended to increase at a rate of statistically significant annual growth estimated at 2.2% per annum.

Peanut crop:

The area of peanut has fluctuated, reaching in 1995 an estimated 106 thousand feddans and reached a maximum in 2010 by about 202 thousand feddans, an increase of about 52.4% and reached its lowest value of 170 thousand feddans. Studying the trend overall time for each of the area, productivity and production of peanut crop during the period (1995 -2010),it was observed the direction of area to grow annually at a rate of annual growth of significant statistically amounted to about 2.8% of the average area of 136 thousand feddans, 1.5 ton / fedd. of average productivity per feddan, which amounted to about 1.3 ton/fedd., 3.9% ton / fedd.,with an average production of about 177.9 thousand tons.

Sesame crop:

Table (1and 2) show that the area of sesame had wiggled between the increase and decrease as it became clear that the values of the area revolves around the arithmetic average of a 70.5 thousand feddans during the study period where the area was 72 thousand feddans in 1995 and reached a maximum in 2010, including an estimated 87 thousand feddans. This has led to the volatility that the equation of the trend overall time for the area of sesame has taken an increasing trend, but not statistically significant because most of the values revolve around the mean. As for productivity per feddan of sesame crop has increased from 0.45 ton / fedd. in 1995 to 0.53 tons in 2010 by an increase of around 11.7% were taken productivity per feddan general trend increasing and significant statistically significant at the level of significance 0.01 and the annual growth rate of approximately 2.8% of the average productivity per feddan during the study period, which is 0.52 ton / fedd., and for the total production of crop has taken a general trend increasing and significant statistically at the level of significance 0.01 and an annual growth rate estimated at 1.7% of the average total production during the study period and amounting to about 34.6 thousand tons.

Cotton crop:

Area was fluctuated where cotton was low 1995 and estimated at about 710 thousand feddans and reached the maximum in 1996 by about 920 thousand feddans and reached its lowest value of 313 thousand feddans have overall average for the area during the study period, 620 thousand feddans, and by studying the time trend for each year of the area, productivity and production of cotton crop area observed trend was decreasing annually at a rate of diminishing annual rate of about 4.1% at the 0.05 level of significance of the average area during the study period. Increased productivity per feddan where amounted to about 0.54 tons/fedd. in 1995 and reached a maximum of 1.2 ton / fedd. in 2009 and by studying the trend overall time for the production of cotton crop, it has taken a general trend growing and uncertain statistically significant at the level of significance 0.01 and the annual growth rate of about 5.6% of the average productivity per feddan during the study period, amounting to about 0.91 ton/fed. The fluctuation of the total production of cotton crop during the study period between increase and decrease, but it took a general trend increasing and that this increase is not significant statistically.

2 – coefficient instability of the productivity indicators for oilseed crops under study: -

Table (3) shows that the instability indicators for each of the area, the productivity and production of crops, sunflower, soybean, peanut, sesame, cotton, where it was found that the area of each of the cotton and peanut more stable than the area of sunflower and soybean where the coefficients of stability were (0.02%, 0.2%, 7.4% 0.31% 0.62%), respectively. For productivity per feddan has been observed the instability of productivity per feddan but the sesame more stable in productivity per feddan for the rest of the crops under study where the coefficient of stability was 1.8, followed by all of the soybean and cotton where the coefficients of stability were (3% 0.14%), respectively, compared to sunflower and peanut crops where the coefficient of stability was (21%, 28.4%) respectively. For the production is considered the cotton crop is more stable than the rest of the oil crops where the coefficient of stability for the production was 0:05 followed by sesame and peanut where the coefficient of stability was 0.3% for each, compared to the crops of sunflower and soybean where the coefficient of stability for each was 1%, 1.2%, respectively, and it is clear that sesame is ranked the first in terms of the stability indicators for production, followed by cotton crop, then peanut, sunflower and finally soybean.

Second: - The domestic consumption of oilseeds and plant oils: -

1 - domestic consumption of oilseeds: -

The consumption of oilseeds is directly or by seeds or used oils after drawn in human food also are consumed in animal feed, this can be seen from Table (4), the fluctuation of the total human consumption of oilseeds during the study period (1995-2010) was from a minimum 119 thousand tons in 1998 and a maximum of 2010, including an estimated 291 thousand tons, has reached the annual average about 212.6 thousand tons during the study period, and for the average consumption of capita per year has ranged from a minimum 3 kg / year in 2006 and a maximum of around 3.9 in 2010, while the average annual approximately 3.7 kg / year during the study period.

The data also indicate the quantity consumed of oilseeds locally produced or imported in the industry during the study period stood alone near about 477 thousand tons in 1995 and have been increasing until it reached its limit, including an estimated 1599 tons in 2010, while the average annual about 915.1 thousand tons during the study period, while the amount of seeds used has been shown a fluctuating quantity between increases and decreases.

Table (4) The end-uses of oilseeds in Egypt in thousand tonDuring the period (1995 - 2010).

Total consumption (1+2+3+4)	Wastage (4)	Seeds (3)	Industry (2)	Human consumption		Year
				Per capita / year kg	Total(1)	
747	10	29	477	3.9	231	1995
804	12	38	551	3.4	203	1996
939	12	37	686	3.4	204	1997
803	13	41	630	3.2	119	1998
856	14	39	676	3.5	127	1999
881	15	36	692	3.6	138	2000
920	16	48	682	3.7	174	2001
95	14	44	711	3.7	181	2002
955	17	28	650	3.8	260	2003
1040	19	27	751	3.5	243	2004
1292	26	25	1007	3.3	234	2005
1274	26	25	1007	3	216	2006
1780	40	25	1468	3.4	247	2007
1825	42	23	1500	3.4	260	2008
1901	45	25	1556	3.5	275	2009
1964	48	26	1559	3.9	291	2010
1080.08	23.06	32.2	915.1	3.7	212.06	Mean

Source: 1 - Ministry of Agriculture - Economic Affairs Sector, General Administration of economic resources – Bulletin of food balance of Arab Republic of Egypt, different numbers.

2 - Central Agency for Public Mobilization and Statistics, Statistical Yearbook - different numbers.

Table (5) The evolution of the amount of consumption of liquid vegetable oils and margarine and self-sufficiency rate in thousand tons during the period (1995-2010).

Self-sufficiency rate (4 /) 3X 100	Total domestic prodution (4(	Total consumption (1 +2(3)	Industry (2(	Per capita per year / kg	Total (1)	Other Oils	Margarine	Palm oil	Corn oil	Cotton seed and sunflower	soybean	year
40	364	900	76	10.4	824	74	102	203	19	329	97	1995
39	382	982	76	11.1	906	80	113	245	23	304	141	1996
35	388	1101	74	8.2	1027	43	190	248	22	466	58	1997
43	397	913	92	5.6	821	9	210	208	23	256	115	1998
39	420	1079	96	4.2	983	65	275	218	19	308	98	1999
39	450	1135	95	7.1	1040	54	307	220	27	372	60	2000
46	480	1045	98	7.1	947	43	304	223	25	289	63	2001
54	535	991	102	6.9	889	61	258	241	31	284	64	2002
47	520	1114	111	10.2	1003	56	243	410	40	234	20	2003
29	501	1735	98	14.0	1637	49	421	772	43	232	20	2004
19	323	1700	114	16.6	1586	78	310	989	40	154	15	2005
23	378	1644	116	15.6	1528	70	331	907	41	159	20	2006
22	390	1770	120	15	1650	75	380	950	42	180	23	2007
21.7	396	1819	125	16	1694	78	395	967	45	185	24	2008
21.3	395	1846	132	17	1714	82	406	984	47	190	25	2009
20.2	398	1922	139	18	1783	87	427	995	50	197	27	2010
33.6	419.2	1356	104	11.36	1201.3	62.7	292	554.3	33.5	264.9	23.1	Mean

Source: 1 - Ministry of Agriculture - Economic Affairs Sector, General Administration of economic resources - Bulletin balance of food to Arab Republic of Egypt, the number of sporadic.

2 - Central Agency for Public Mobilization and Statistics, Bulletin of annual consumption of goods - different numbers.

Table (6) The evolution of the quantity and value of imported oilseeds during the period (1995 - 2010). Quantity / thousand tons, Value / million pounds

Percentage Of the value of imports	Total value of imports	Total		Other oil seeds		Cotton seeds		Sunflower seeds		Peanut		Flax seeds		Sesame		Soybean		Year
		Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	
0.60	39884	224	110	0.27	0.73	-	-	34	14	-	-	6	5	126	25	48	55	1995
0.7	44219	310	191	1.38	0.33	-	-	21	21	0.703	0.526	7	6	152	39	129	124	1996
0.8	44884	375	215	0.12	0.36	-	-	6	4	0.546	0.438	72	24	152	46	145	140	1997
0.4	56025	247	177	0.46	0.24	-	-	2	2	3	2	20	18	112	38	108	115	1998
0.4	54399	225	187	1.81	0.71	-	-	5	3	0.195	34	18	19	125	45	75	85	1999
0.8	48645	371	357	0.49	0.08	-	-	-	-	-	-	42	53	167	92	162	212	2000
0.01	50660	564	441	0.71	0.13	-	-	9	11	0.871	0.168	11	11	227	69	316	350	2001
1	56480	549	399	3	0.32	-	-	0.39	0.001	0.245	0.03	37	25	186	50	322	322	2002
0.6	65082	403	192	2	0.35	-	-	-	-	0.364	0.32	45	21	145	39	211	132	2003
0.72	76718	577	256	4	0.59	-	-	20	10	0.179	0.035	69	3	147	27	400	215	2004
1.1	114687	1274	628	56	30	-	-	0.24	0.074	0.544	0.133	36	13	59	11	1122	574	2005
0.9	118373	1067	617	23	13	0.04	0.06	0.19	0.002	0.47	0.187	37	18	71	13	926	572	2006
1.7	152587	2547	1178	80	31	0.03	0.03	0.53	0.099	0.876	0.123	7	3	49	9	2409	1126	2007
0.84	287767	2429	991	95	18	0.04	0.04	0.62	0.12	1.5	0.19	17.5	2.5	65	10.5	2250	960	2008
1.3	249964	3284	680	123	15	0.06	0.04	0.95	0.18	2.2	1.9	18.5	1.7	99.5	11.8	3040	650	2009
1.4	300344	4069	512	142	13	0.07	0.05	2.4	0.24	3.9	4.5	34	1.5	114	13.7	3873	480	2010
0.83	110044	1157	445	33.3	7.7	0.053	0.045	6.3	4.1	0.97	2.8	29.8	14.1	124.8	33.6	962.8	569.5	Mean

Source: - Central Agency for Public Mobilization and Statistics, Statistical Yearbook, Bulletin of Foreign Trade, different numbers.

Table (7) The evolution of the quantity and value of imports of oilseeds group oils and margarine during the period (1995 -2011). Quantity / thousand tons, Value / million pounds

Percentage Of the value of imports	Total		Other oils		Margarine		Flax seeds oil		Olive oil		Cotton seeds oil		Corn oil		Soybean oil		Sunflower oil		Oil palm		Year
	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	
4	1611	739	195	111	25	11	10	5.5	2.5	0.35	211	96	26	14	178	81	408	172	557	289	1995
3.8	1666	797	295	142	29	13	13	6	4	0.27	45	21	25	10	223	106	475	225	558	272	1996
3.4	1522	762	162	71	20	8	9	5	4	0.66	0.80		27	11	63	34	637	324	608	309	1997
2.9	1629	711	270	97	41	20	19	9	3	0.40	23	10	36	15	231	97	360	163	646	300	1998
2.4	1289	640	140	61	21	15	16	8	5	0.62	7	4	22	7	290	149	364	180	415	215	1999
2	970	655	83	45	17	6	6	4	5	0.13	15	8	22	9	336	239	129	81	358	262	2000
1.3	657	475	15	7	9	4	9	5	4	0.54	9	4	22	11	283	180	105	54	202	210	2001
1.4	777	249	14	7	2	0.7	7	2	3	0.22	2.3	1	32	11	350	156	80	26	286	143	2002
1.3	865	265	6	2	4.4	0.8	8	2.5	2	0.25	-	-	51	13	340	107	417	123	36	17	2003
2.8	2215	878	76	23	17	2	4	1	8	2	-	-	63	14	245	92	449	115	1252	618	2004
2.3	2598	1070	150	54	58	11	9	1.8	3.7	0.23	4	2.6	82	43	289	88	453	116	1548	754	2005
2.3	2740	1205	103	43	34	7	-	-	6	0.39	21	6	50	15	340	45	451	127	1912	957	2006
1.3	1992	556	82	21	42	7	5	1	8	0.54	8.4	2	82	16	394	96	630	151	742	261	2007
0.8	2357	597	95	23	43	7.5	6	1.5	9	0.57	9.1	4	95	17	430	107	720	160	950	277	2008
1.1	2559	625	102	25	45	8	7	1.8	11	0.60	10.2	5	110	18	465	112	789	172	1020	283	2009
0.9	2721	648	107	28	48	9	8	1.9	13	0.65	12.8	7	130	20	492	125	945	182	966	275	2010
1.4	3908	646	165	16	163	13	15	1.7	56	0.33	14	8	126	14	718	239	1039	201	1612	153	2011
4.1	1886.6	676	112	45.6	36.3	8.8	8.9	3.4	8.6	0.56	23.1	10.5	58.9	15.2	301	145	497.1	144	715	273	Mewn

Source: - Central Agency for Public Mobilization and Statistics, Statistical Yearbook, Bulletin of Foreign Trade, different numbers.

2 - domestic consumption of vegetable oils: -

Table (5) shows that the contribution of vegetable oils, both domestic or imported product in terms of domestic consumption increased from about 824 thousand tons in 1995 to about 1783 thousand tons in 2010,by an increase of about 46.2%, while the average per capita in the year amounted to about 10.4 kg / year in 1995.It has been taken in terms of the decline was the smallest since the year 1999 is estimated at 4.2 kg / year and reached its maximum about 18 kg / year with an average of about 11.36 kg / year during the study period. The amount consumed from vegetable oils used in the industrial products has increased from about 76 thousand tons in 1995 to about 129 thousand ton in 2006, an increase of about 55% and an average estimated at about 104 thousand tons during the study period, the data show the table that the domestic production of vegetable oils has failed to meet the local needs of vegetable oils during the study period (1995 - 2010), where the self-sufficiency rate ranged between a maximum of 54% in 2002 and a minimum of 19% in 2005 with an average of about 33.6 during the study period.

Thirdly - the evolution of imports of oilseeds, vegetable oils and costs by Egyptian Pound: -

1- The evolution of the quantity and value of imports of oilseeds: -

Table (5) shows that the inability of domestic production of oilseeds and vegetable oils to cover the demand, and thus increasing the need to import them, leading to the increasing burden on the balance of payments, which can be seen from Table (6) that the soybean crop came as the first for the quantities imported from oilseeds, followed by sesame and linseed has ranged total imported quantities of oilseeds during the study period (1995 -2010) from a minimum of around 110 thousand ton in 1995 and a maximum of around 1178 thousand ton in 2007, and for the cost of imported amounted kept to a minimum in 1995 about 224 million pounds alone in 2010, including an estimated 4069 million pounds and an average of about 1157 million pounds during the study period. The proportion of imported oilseeds estimated 0.83% of the total value of Egyptian imports, which amounted to 110.04 billion pounds annual average during the study period.

2 - The evolution of the quantity and value of imports of vegetable oils and margarine: -

Table (7) shows that palm oil is ranked the first in terms of value and quantity, followed by sunflower, and soybean, cotton seeds, corn, and other oils in terms of the relative importance of imported vegetable oils, has reached a total value of oil imports, with an estimated 657 million pounds and its maximum in 2010, with an estimated 3908 million pounds, representing the portion of imported oils which is estimated at about 4.1% of the total value of Egyptian imports during the study period.

Fourth: - problems of production and marketing of oilseed crops: -

Low acreage oilseeds crops, is due to several reasons, including: -

1 - self-sufficiency in oil output of the cottonseeds oil crops with other crops, delusion for cotton cultivation for several years.
2 - competition between oil crops and the major crops of strategic importance such as rice, maize and vegetable crops, especially in the old lands.
3 - the lack of profitability of some oil crops as compared with some other crops.
4 - Non-availability of extracting oil from the seeds of some oil crops such as sunflower seeds that need private transactions in addition to the cancellation of the contract on the receipt of these crops with factories.
5 - Non-availability of good varieties of these crops.

The problems of production and marketing of oilseed crops: -

The high cost of the application of technological packages, which stand in the way before the application, and the inadequacy of new strains improved to meet the demand or lack of time availability and high price, making farmers resort to commercial varieties with high mixing ratio as well as the lack of agricultural extension interested in this area and the lack of information. In addition to the marketing exposure for many oil crops from pests and diseases affecting the productivity of the crop.

Recommendations of the study: -

- The need for the development of oilseed crops in terms of cultivating in the new land, which does not make them compete with traditional crops in the territory of Valley and Delta, such as the successful cultivation of peanut in sandy soil and the successful cultivation of sesame in sandy soil, as well as the success of cultivating sunflower in calcareous soils and clay, and as a result of favorable weather conditions of Egypt, help on the success of the cultivation of these crops with higher productivity.

- The attention of the Ministry of Agriculture to produce new varieties of oil crops and to supply farmers,the private sector can contribute in solving the problems of concentrated feed after the crush of seeds and thus compensate the large gap in the feed, which is considered the first cause of the problems of meat production in Egypt.

- Effective regulation of domestic marketing to ensure product delivery delay farmers the price at the right time, the output by contracting between farmers and cooperatives, and the agency responsible for marketing and extraction plants.

- To find places to store the product to control the quality attributes, as well as reduce the cost of transport to the extraction factories, linking local prices to international prices to compensate farmers for escaped price by establishing a fund to offset oilseed prices through a tax on imported oilseeds and vegetable oils.

Conclusion:

Vegetable oils represent main source of edible oils can be used in human food and in some industries as a gain output of them in a bush important food for animals and poultry. The problem of the study, the Republic of Egypt suffer a large deficit in the production of vegetable oils, the need for national consumption, as Egypt imports of vegetable oils about 676 thousand tons per year as an average during the period (1995-2010), which costs the state about 1.9 billion pounds a year is expected to increase the burden on the state budget in the coming years as a result of the increasing in population and the lack of production from oil crops. The fluctuation of area and production of oilseed crops under study (sunflower, soybean, peanut, sesame, cotton) between increases and decreases. The area of each of sunflower, soybean and cottonseed has taken a general trend decreasing and statistically significant, while it took the area of each of peanut and sesame a general trend for increasing the productivity of the crops per feddan, study has taken all general trend growing and statistically significant except for the sesame crop productivity increased at a rate of non-statistically significance, as for the contribution of vegetable oils, both from the domestic product or imported in domestic consumption has increased from about 824 thousand tons in 1995 to about 1783 thousand tons in 2010 and an average annual rate of about 1201 tons, and the average per capita per year of vegetable oils has reached a minimum of about 4.2 kgs / year in 1999 and a maximum of about 18 kgs / year in 2010 with an average annual rate of about 11.3 kgs / year during the study period, and that the self-sufficiency rate ranged from a low of 19% in 2005 and a maximum of 54% in 2002.The imported vegetable oils has a significant portion of the total Egyptian imports ranged from a minimum of 1.3% during the years 2001.2003, 2007, and a maximum of around 4% in 1995 and ranged from the cost of imports, about 1.6 billion pounds, and in 2010 was the increase 1.4% at a cost estimated about 3.9 billion pounds. The study examined the problems of production and marketing of oilseed crops, which lies in the lack of adoption of technological packages due to the presence of some obstacles, such as the high cost of implementation, lack of improved seeds and lack of marketing information, and offers crop for many diseases and pests, and as for the means of the development of oil crops depend on the expansion in the cultivation of some oil crops in the new lands until the graduation from the department of competition of traditional crops as well as local marketing organization.

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Exploration of *Erythrina Excelsa* Baker and *Aneilema Beniniense* (P. Beauv.) Kunth Aqueous Extracts For The Management of Flea Beetles (*Podagrica* Spp) On Okra (Abelmoschus Esculentus)

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Abstract: Control of insect pests involves the management of pest populations to an equilibrium whereby their effect on yield is reduced. The efficacy of aqueous extracts of *Aneilema beniniense* and *Erythrina excelsa* plants were compared for effectiveness in the control of flea beetles (*Podagrica uniforma* and *Nisotra dilecta*) on okra during the 2013 cropping seasons at the Teaching, Research and Commercial Farm, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. The extracts were sprayed every week for 5 weeks, starting from crop establishment till fruiting. The results show that the two plant extracts were able to exercise significant ($P<0.05$) insect controlling influence against *P. uniforma* and *N. dilecta* and caused impressive reductions of both insect pests population and protected the okra plant from serious damage and increased pod yield in comparison with the control. Fruit yields were significantly ($P<0.005$) higher in plots treated with *A. beniniense* extracts compared to other treatments. From the results, *A. beniniense* and *E. excelsa* was recommended for use on farms managed by limited resource farms in Nigeria, since the technology is cheap, safe, environmentally friendly and easy to adopt in tropical countries.
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Keywords: effectiveness, impressive reductions, insect pests population, pod yield

Introduction

Okra *Abelmoschus esculentus* L. (Moench), is a commercial vegetable crop with considerable area under cultivation in Africa and Asia. In 2009-2010, the total world area under cultivation was 0.43 million hectares and the production stood at 4.54 million tons; with India being largest producer (67.1%), followed by Nigeria (15.4%) and Sudan (9.3 %) (Varmudy**,** 2011).

Okra plays an important role in the human diet (Kahlon et al. 2007, Saifullah and Rabbani 2009). by supplying fats, proteins, carbohydrates, phosphorus, calcium, iron, sulphur, fibre, minerals and vitamins **(**Lamont 1999, Owolarafe and Shotonde 2004, Gopalan et al. 2007, Arapitsas 2008, Dilruba et al. 2009). Okra fruit is usually boiled in water resulting in slimy soups and sauces, which are relished. The fruits also serve as soup thickeners. Okra seed can be dried, and the dried seeds are a nutritious material that can be used to prepare vegetable curds, or roasted and ground to be used as coffee additive or substitute (Moekchantuk and Kumar 2004).

Industrially, okra mucilage is usually used for glace paper production and also has a confectionery use. Okra has found medical application as a plasma replacement or blood volume expander **(**Savello et al. 1980, Markose and Peter 1990, Lengsfeld et al. 2004, Adetuyi et al. 2008, Kumar et al. 2010) and it is said to be very useful against genito-urinary disorders, spermatorrhoea and chronic dysentery (Nadkarni, 1927). Its medicinal value has also been reported in curing ulcers and relief from hemorrhoids (Adams, 1975). Tests conducted in China suggest that an alcohol extract of okra leaves can eliminate oxygen free radicals, alleviate renal tubular-interstitial diseases, reduce proteinuria, and improve renal function (Liu et al. 2005, Kumar et al. 2009).

Insect pest infestation is one of the most limiting factors for accelerating yield potential of okra. The crop is prone to damage by various insects; various growth stages of the crops are susceptible to the different insect pests and diseases (Ek-amnuay 2007, Fasunwon and Banjo 2010). Insect pests like crickets can be a problem during germination/seedling stage of the crop while the thrips, whitefly and other phloem feeders are common during vegetative stage (Fajinmi and Fajinmi 2010). The most destructive insect pests are two flea beetle species, *Podagrica uniforma* (Jac.) and *P. Sjostedti* (Coleoptera: Chrysomelidae) which are responsible of heavy defoliation (Odebiyi, 1980). Important yield losses are reported in Nigeria and Ghana (Obeng-Ofori and Sackey, 2003; Ahmed et al., 2007). These insects also transmit the okra mosaic virus which causes significant yield losses (Van Lommel et al., 1996).

The control of field insect pests of okra remains a major production constraint of farmers. In Nigeria, use of chemical insecticides is in vogue for the control of insect pest. Although synthetic insecticides application is popular and effective means of pest control their use

in okra production is limited because the crop was regarded as low value cash crop. Exclusive reliance on insecticides as a control strategy against insect pest has resulted in several undesirable effects, like pesticide pollution, resurgence of secondary pests, insecticide resistance, elimination of beneficial fauna and different human health problems. There is a need to explore alternative approaches to reduce the sole dependence on insecticides. The use of plants derived insecticides are in recent time being investigated by researchers as possible replacement for synthetic insecticides because they are supposedly safer and may be more readily available and affordable (Dudu and Williams 1991). Therefore an attempt was made to study the efficacy of *Erythrina excelsa* Baker and *Aneilema beniniense* aqueous extracts for controlling *P. uniforma* (Jaq.) and *N. dilecta* (Jaq.) (Coleoptera: Chrysomelidae) infestation and boost yield of okra *Abelmoschus esculentus.*

Material and Methods

Experiment site and field layout

The experiment was conducted during rainy season 2013 in the horticultural farm of the Teaching and Research Farm Rufus Giwa Polytechnic Owo, Ondo state, Nigeria. The land was cleared of all vegetation cover and was then ploughed and harrowed with a disc plough and disc harrow respectively to render the soil loose. The trial was laid out in a Randomized Complete Block Design (RCBD) in a total land area of 456m^2 measuring 24m x 19m; with three treatments and each treatment was replicated thrice. It was then partitioned into three blocks and each block was further divided into nine plots, with each plot measuring 7m x 5.9m (41.3m^2). A distance of 1 m was left as walkway between the blocks and the plots. Planting was done in 27th May, 2013. The seeds of an early maturing okra variety "NH-47-H" obtained from Ondo State Agricultural Development Programme, Akure, Nigeria and were directly sown at two seeds per hole at a planting distance of 60cm x 60cm and a planting depth of not more than 0.5cm; this was later thinned to one seedling per stand. Supplying was carried out 2 weeks after sowing and weeding was done manually when necessary; but no fertilizer application was made.

Preparation and application of treatments

The treatments were 10% (w/v) crude extracts each of *Erythrina excelsa* and *Aneilema beniniense* leaves (Table 1) collected from Owo and Irun Akoko, Nigeria and control (synthetic insecticide) purchased from Agro-chemical store in Owo, Nigeria. Each of these treatments was prepared by weighing 1.0kg of plant material with an electronic balance (DH-V1000/d model), homogenized with pestle and mortar and then allowed to seep overnight in 4 litre of water. The extracts were then filtered through muslin cloth to obtain aqueous extracts. The treatments were applied to the plants on the field at 20 days after sowing (DAS) when the plants were about 30 cm tall, using Spray well 16 L Knapsack sprayer model. Subsequent application of the treatments was carried out at weekly intervals till the plants reached the fruiting stage.

Data collection and Analysis

Number of *P. uniforma* (Jaq.) and *N. dilecta* (Jaq.) was counted before each spray and also at 24, 72, 120 hrs and 7 days after treatment. Five plants were selected at random in each plot and number of brown and blue flea beetles was counted from 2 leaves on top, 2 leaves on bottom and 2 leaves at middle. The collected data were pooled and mean population was worked out separately for 20, 27, 34, 41 and 48 days after sowing. The efficacy of the plant extracts has been worked out by comparing it with the untreated control plot.

Data collected was transformed using the square root transformation method to ensure homogeneity of the variance and normal distribution of the data. The data was later subjected to analysis of variance (ANOVA) using Genstat Release version 12.1 (Payne et al 2009). Means were separated using least significant difference at a probability of 5%.

Results

Table 2 shows that population of *N. dilecta* and *P. uniforma* before spraying were not significantly different from each other in all the assigned treatment plots on the okra plants. However, flea beetle population ranges from 1.7 – 1.9 insects/plant.

At 27 days after planting (DAP), insect population was significantly ($P< 0.05$) reduced by the application of Cypermethrine (Table 3) compared to the spraying effect observed in plots treated with plant extracts at 24 and 72 hrs after spraying (HAS). However, *P. uniforma* population increased slightly at 120hrs and 7 days after spraying (DAS) in plots treated with *E. excelsa* extract and at 7 DAS in plots treated with *A. beniniense* for *N. dilecta* respectively (Table 3). The increase in the insect population mighty is due to light shower recorded on the eve prior to insect count.

The result presented in Table 4 shows that days after second spraying (DAS), plot sprayed with synthetic insecticides maintained significant ($P<0.05$) reduction in insect population for both *P. uniforma* and *N. dilecta* compared to plots treated with plant extracts. Non-significant ($P>0.05$) difference was observed for *P. uniforma* population at 24, 72 and 120 HAS in all the treatments application. The result also shows that there exist no significant ($P>0.05$) difference in the insect population at 24, 72, 120hrs and 7 DAS in plots treated with plant extracts, except

for *P. uniforma* population observed at 7 DAS in which there exist significant difference (P<0.05) between *E. excelsa* and other treatments. In addition, there was no significant difference (P>0.05) on *P. uniforma* population on plots treated with Cypermethrin (0.7 insect/plant) and *A. beniniense* (0.9 insect/plant) respectively.

Table 5 shows the result for third spraying at 41 DAP with aqueous plant extract indicates that there is significant different (P<0.05) among the treatments evaluated with the synthetic insecticide significantly (P<0.05) maintained reduction in insect population for both species days after spraying (DAS). Though, the aqueous plants extracts does not exhibit any significant difference (P>0.05) to one another post treatments application. However, at 72 HAS *N. dilecta* population suppression was not significantly different (P<0.05) between the plant extracts and Cypermethrine treated plots.

Results presented in Table 6 shows the population of *N. dilecta P. uniforma* after the fourth spraying at 48 DAP. The result shows that there was no significant different (P>0.05) among the plots treated with synthetic insecticides and plant extracts post application hours and days, except for 24 HAS in which there exist significant difference between plant extracts and Cypermethrin. However, there was no significant difference between *A. beniniense* and Cypermethrin on *N. dilecta* population at 24 HAS. Above all, the result clearly indicates significant reduction in the beetle population compared to result obtained in Table 5.

N. dilecta = Blue flea beetle, *P. uniforma* = Brown flea beetle

The mean number of insect population after fifth spraying with aqueous plants extracts was presented in Table 7. The result shows that there are no significant difference (P>0.05) between the plots sprayed with synthetic and the aqueous plants extracts days after spraying (DAS). The insects population ranges between 0.7 – 0.8 insect/plant in all the treated plots.

The effect of botanical insecticides on okra yield attributes is shown Table 8. The result of the yield shows that there was no significant differences (p>0.05) between plots treated with *E. excelsa* and control (Cypermethrin) in terms of fruits number and fruit length, but there was significant difference (p<0.05) in fruit weight across the different treatment. *A. beniniense* has the highest value in fruit number (20.3 fruits), fruit length (27.9cm) and fruit weight (0.25kg/pod), while Cypermethrine had the lowest fruit weight (0.12kg/pod).

Discussion

Growing awareness of health and environmental issues associated with the intensive use of chemical inputs has led to interest in alternate forms of agriculture in the world.

Plant extracts often consist of complex mixtures of bioactive constituents plant metabolites may produce toxic effects if ingested leading to rejection of the host plant (Russel and Lane, 1993). The active compounds may act as antifeedants, disturb insect growth, development and inhibit oviposition (Gerard and Ruf, 1991; Emimal Victoria, 2010).

P. uniforma and *N. dilecta* is a major okra defoliator and fruit feeder (Parh et al., 1997) reported to cause heavy defoliation of up to 80% of the okra leaves surface (Dabire-Binso et al 2009). Therefore, substantial reduction or controlling of the population of flea beetles on the okra plants by the plant extracts also resulted in a significant reduction in the pest damage on leaves of the okra plants treated with the plant extracts. This experiment suggests that the two plants used as insecticide effectively reduced the level of insect infestations which consequently lead to high yield. This work confirms the findings of other workers (Krishnareddy, et al 1995; Ogunjobi and Ofuya, 2007; Adesina and Idoko, 2013; Adesina and Afolabi, 2014) which showed that okra plants treated with the plant extracts recorded higher yield as compared to the yield of the untreated control okra plants. Okra plants protected with the plant extracts recorded significantly lower leaf damage than the control. This may be due to the ability of the phytopesticides to control the population of the flea beetles on the okra plants as compared to the control.

The findings from this study shows that the plant extracts insecticidal potential manifest greatly at 41 and 48 DAP i.e. 4^{th} and 5^{th} spraying. This indicates that the plant extract is a slow acting insecticide and support the findings of Okuku *et al* (2007) and Adesina and Afolabi, (2014) who both reported the slow action of plant extract(s) in the control of cocoa mirids and flea beetles on cocoa and okra respectively. Also the result concurs with earlier observation raised by (Alao and Adebayo, 2011) that the delayed effect is one of the major problems of botanical insecticides.

Even though the effectiveness of botanicals is not superior to chemical insecticides, they are slow in their efficacy due to their repellent and antifeedent properties. The present study revealed that all the treatments showing insecticidal activity against okra flea beetle and considering their ecofriendly and non-toxic nature, these botanicals may be recommended it was found effective in reducing the population of flea beetle and achieving high yield of okra, thus could serve as an eco-friendly approach in future pest management strategies of okra in developing countries especially in poverty ridden societies.

Table 1. Plant used as botanical insecticides

Botanical Name	Common Name	Family Name	Plant part used
Erythrina excelsa	Coralbean	Leguminosae	Leaves
Aneilema beniniense	Aneilema	Commenlinaceae	Leaves

Table 2. Mean number of flea beetles counted on okra plants before application treatments

Treatments	*N. dilecta*	*P. uniforma*
E. excelsa	1.7[a]	1.8 [a]
A. beniniense	1.8 [a]	1.7 [a]
Cypermethrine	1.7 [a]	1.9a

N. dilecta = Blue flea beetle, *P. uniforma* = Brown flea beetle

Table 3. Effect of plant extracts on the population of *N. dilecta P. uniforma* after the first spraying at 27 DAP.

24h	72h		120h		Day 7			
Treatments	Blue	Brown	Blue	Brown	Blue	Brown	Blue	Brown
E. excelsa	1.2 [a]	1.4 [a]	1.7 [a]	1.5 [a]	2.0 [a]	2.1 [a]	2.1 [a]	2.4 [a]
A. beniniense	1.3 [a]	1.2 [a]	1.6 [a]	1.4 [a]	1.8 [a]	1.3b	2.1 [a]	1.6[b]
Cypermethrine	0.8 [b]	0.8 [b]	0.7 [b]	0.7 [b]	0.7 [c]	0.7 [c]	0.7 [b]	0.7[c]

N. dilecta = Blue flea beetle, *P. uniforma* = Brown flea beetle

Table 4. Effect of plant extracts on the population of *N. dilecta P. uniforma* after the second spraying at 34 DAP

24h	72h		120h		Day 7			
Treatments	Blue	Brown	Blue	Brown	Blue	Brown	Blue	Brown
E. excelsa	1.3 [a]	0.9 [a]	1.4 [a]	0.8 [a]	2.0 [a]	1.3 [a]	1.2 [a]	1.0 [a]
A. beniniense	1.3 [a]	0.8 [a]	1.4 [a]	0.9 [a]	1.7 [a]	1.0 [a]	1.1 [a]	0.9 [b]
Cypermethrine	0. 7 [b]	0.7[a]	0.7 [b]	0.7 [a]	0.7 [b]	0.7 [a]	0.7 [b]	0.7 [b]

N. dilecta = Blue flea beetle, *P. uniforma* = Brown flea beetle

Table 5. Effect of plant extracts on the population of *N. dilecta P. uniforma* after the third spraying at 41 DAP.

24h	72h		120h		Day 7			
Treatments	Blue	Brown	Blue	Brown	Blue	Brown	Blue	Brown
E. excelsa	1.0 [a]	1.0 [a]	1.2 [a]	1.2 [b]	1.1 [b]	1.1 [b]	1.2 [b]	1.1 [b]
A. beniniense	1.0 [a]	1.0 [a]	1.1 [a]	1.1 [b]	1.2 [b]	1.0 [b]	1.1 [b]	1.0 [b]
Cypermethrine	0.7 [b]	0.7 [b]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]

N. dilecta = Blue flea beetle, *P. uniforma* = Brown flea beetle

Table 6. Effect of plant extracts on the population of *N. dilecta P. uniforma* after the fourth spraying at 48 DAP.

24h	72h		120h		Day 7			
Treatments	Blue	Brown	Blue	Brown	Blue	Brown	Blue	Brown
E. excelsa	1.0 [a]	0.9 [a]	1.0 [a]	0.6 [a]	1.1 [a]	0.8 [a]	0.9 [a]	0.9 [a]
A. beniniense	0.9 [b]	0.9 [a]	0.9 [a]	0.9 [a]	0.9 [a]	0.9 [a]	0.8 [a]	0.9 [a]
Cypermethrine	0.7 [b]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]

N. dilecta = Blue flea beetle, *P. uniforma* = Brown flea beetle

Table .7 Effect of plant extracts on the population of *N. dilecta P. uniforma* after the fifth spraying at 41 DAP.

24h	72h		120h		Day 7			
Treatments	Blue	Brown	Blue	Brown	Blue	Brown	Blue	Brown
E. excelsa	0.8 [a]	0.8 [a]	0.8 [a]	0.8 [a]	0.8 [a]	0.8 [a]	0.8 [a]	0.8 [a]
A. beniniense	0.8 [a]	0.7 [a]	0.8 [a]	0.7 [a]	0.8 [a]	0.8 [a]	0.8 [a]	0.8 [a]
Cypermethrine	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]

N. dilecta = Blue flea beetle, *P. uniforma* = Brown flea beetle

Table 8. Mean yield attributes of okra fruits on plots treated with aqueous plant extracts

Treatments	Fruit number	Fruit length	Fruit weight
E. excelsa	15.3[b]	20.8[b]	0.16[b]
A. beniniense	20.3[a]	27.9[a]	0.25[a]
Cypermethrine	13.3[b]	21.1[b]	0.12[c]

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Floristic Diversity Assessment on the Afforested Bank of Manasbal Lake, Kashmir

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Abstract: Floristic diversity study was carried out on the afforested bank of Manasbal Lake, Kashmir during the year 2009. The study was carried out in two vegetational strata's, the tree and the herbaceous layer. The various phytosociological parameters like species richness, dominance and evenness index exhibited variations in both the strata in all the sites.

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Key words: Floristic diversity; Manasbal Lake; Vegetational strata

1. Introduction

The state of Jammu and Kashmir is very famous all over the world for its lofty mountains, fascinating valleys, water bodies and lush green forests. Among various water bodies present in Kashmir valley, Manasbal lake is one of them. It is located about 30 km north of Srinagar city and is considered as the supreme gem of all Kashmir lakes with lotus *(Nelumbo nucifera*) nowhere more abundant or beautiful than on the margins of the lake during July and August. It is the deepest lake of Kashmir valley and perhaps the only one that develops stable summer stratification. On the south of the lake is a hillock called 'Ahtung' which is used for limestone extraction. The eastern part is mainly mountainous and towards the north is an elevated plateau known as 'Karewa' The north-western bank of the lake was barren and was prone to soil erosion, then Faculty of Forestry, SKUAST-K in the year 1992 under took reclamation of the area, under operational research project on Agroforestry funded by ministry of environment and forests and planted both coniferous and broad leaved tree species at the site. The afforestation programme was launched with the aim of preventing the soil erosion and heavy influx of nutrients into the lake which otherwise causes heavy growth of aquatic biomass in the lake causing trouble not only to the fish flora but also to navigation. Restoration of pristine glory of lake is of paramount importance in the tourist industry of Kashmir.

The floristic diversity which few years ago was considered unimportant by ecosystem ecologists has now been shown to be significantly important for many aspects of ecosystem functioning. The floristic diversity has been a source of amazement and scientific curiosity and increasingly a source of concern (Elourard et *al.,* 1997). Floristic diversity is becoming a significant component as it is used and exploited variously for food, fodder, timber, medicines, recreation etc. More than 70,000 plant species are used in traditional and modern medicine. Maintaining healthy floristic diversity can play a vital role in climate change mitigation and the worlds protected areas-national parks, marine reserves, wilderness areas and so on are essential in safeguarding this role. The sustainability of floristic diversity can be assessed only on the plant species there in (Gentry, 1992). Thus along with the understanding of floral diversity characteristics, the studies in relation to the other component i.e. in terms of their quantitative characters have become imperative for their proper management.

The present research therefore has been attempted on the afforested bank of Manasbal Lake to understand the floral diversity which is an important aspect of forest biology entailed the status and contribution of various species in structure and function of ecosystem.

2. Materials and Methods

The study site lies between 70º-40' East longitude and 34º-15' North latitude at an elevation of 1583 meters above sea level and is about 30 km north of Srinagar city. The maximum temperature of the study site touches as high as 33º C in the month of July where as minimum temperature drops as low as –4ºC in the month of January. The annual precipitation of the area is about 700mm and most of the precipitation is received in the form of snow during winter months. The site was taken up for afforestation by the Faculty of Forestry, SKUAST-K in the year 1992. During afforestation fourteen tree species were planted in the area viz. *Acer negundo,Aesculus indica, Ailanthus altissima, Albizia Julibrissin, Catalpa bignonoides, Cedrus deodara, Cupressus torulosa, Celtis australis, Morus alba, Populus deltoides,*

Prunus armeniaca, Robinia pseudoacacia, salix alba and Ulmus wallichiana (Anonymous, 1993). The area is about four kilometers in length where as its width ranges between fifty to hundred meters. The place is globally known for its beauty and is always figuring among the top tourist resorts of Kashmir valley. The research site was completely barren and was prone to soil erosion, then Faculty of Forestry took afforestation of the site and planted both coniferous and broad leaved species under a research project funded by ministry of environment and forests. The objectives of afforestation were to check the soil loss and heavy influx of nutrients into the lake which otherwise causes growth of aquatic weeds thus causing eutrophication.

The study was carried out during the year 2009. Four sites were selected for the present study. The size and number of quadrants were determined by species area curve (Ambasht and Ambasht, 1995).The quadrants were drawn in thorough consultation with statisticians and sampling was done in presence of the statistician. Total eight quadrants of size 10×10m for trees and eight quadrants of size 1×1m were randomly placed for the herbaceous layer. Frequency, diversity, dominance, IVI (importance value index), basal area and GBH (Girth at breast height) for both the layers were determined (Curtis, 1959). General diversity index (H), Species richness index (d_2) and Eveness index (e) were calculated after Shannon- Wiener (1963), Menhinick (1964), Pileou (1975) and Simpson (1949) respectively.

3. Results

3.1 Floristic diversity

Total 24 species belonging to 17 families were recorded. All these families show very less variation in terms of number of species. Certain families such as Sapindaceae, Fabaceae, Salicaceae, Asteraceae and Poaceae were more dominant and the remaining were monospecific (Table 1).

Table 1. Floristic diversity on the afforested bank of Manasbal Lake, Kashmir

S.No.	Species	Family
1.	*Acer negundo*	Sapindaceae
2.	*Aesculus indica*	Sapindaceae
3.	*Ailanthus altissima*	Samaroubaceae
4.	*Albizia julibrissin*	Fabaceae
5.	*Catalpa bignonioides*	Bignoniaceae
6.	*Celtis australis*	Cannabaceae
7.	*Cedrus deodara*	Pinaceae
8.	*Cupressus torulosa*	cupressaceae
9.	*Morus alba*	Moraceae
10.	*Populus deltoides*	Salicaceae
11.	*Prunus armeniaca*	Rosaceae
12.	*Robinia pseudoacacia*	Fabaceae
13.	*Salix alba*	Salicaceae
14.	*Ulmus wallichiana*	Ulmaceae
15.	*Tulipa stellata*	Liliaceae
16.	*Cynodon dactylon*	Poaceae
17.	*Stellaria media*	Caryophyllaceae
18.	*Taraxacum officinale*	Asteraceae
19.	*Poa bulbosa*	Poaceae
20.	*Salvia moorcroftiana*	Limiaceae
21.	*Euphorbia helioscopia*	Euphorbiaceae
22.	*Chenopodium album*	Chenopodiaceae
23.	*Conyza Canadensis*	Asteraceae
24.	*Trifolium pratense*	Fabaceae

3.2 Vegetational composition of tree and herbaceous layer

3.2.1 Tree Layer

Diversity values of woody species found in the area are presented in (table 2).

Table 2. Plant diversity of the woody species

Details of Sites	General Diversity index (H)	Species Richness Index (d_2)	Index of Dominance	Evenness Index (e)
Site I	0.202	0.46	0.003	0.53
Site II	0.576	0.68	0.002	0.74
Site III	0.469	0.53	0.013	0.87

Site I

In this site, only four species were recorded of which the highest density was recorded for *Robinia pseudoacacia* followed by *Ailanthus altissima* (6.0/m^2), *ulmus wallichiana* (3.75/m^2) and *Populus deltoides* (3.12/m^2) and IVI values for *Robinia pseudoacacia* (72.06) was maximum followed by *Populus deltoides* (56.06), *Ailanthus altissima* (39.27) and *Ulmus wallichiana* (34.57).

Site II

This site had nine tree species *Cupressus torulosa* exhibited maximum density (2.0/m^2) followed by *Prunus armeniaca* (1.25/m^2), *Salix alba* (1.12/m^2), *Aesculus indica* (1.0/m^2), *Catalpa bignonioides* (0.87/m^2), *Robinia pseudoacacia*

(0.75/m^2), *Acer negundo* (0.62/m^2), *Celtis australis* (0.50/m^2) and *Morus alba* (0.25/m^2).

IVI values reflected that *Robinia pseudoacacia* (19.60) occupied first position followed by *Cupressus torulosa* (16.65), *Prunus armeniaca* (13.20), *Catalpa bignonioides* (8.89), *Aesculus indica* (8.36), *Salx alba* (6.80), *Celtis australis* (6.16), *Morus alba* (5.19) and *Acer negundo* (3.68).

Site III

On this site only five species were recorded. The highest density (4.75/m^2) was exhibited by *Albizia julibrissin* followed by *Morus alba* (3.57/m^2), *Populus deltoides* (3.25/m^2), *Ulmus wallichiana* (2.87/m^2) and *Robinia pseudoacacia* (2.25/m^2). The trend of IVI was *Robinia pseudoacacia* (7.40), *Albizia julibrissin* (7.25), *Morus alba* (6.21), *Populus deltoides* (4.08) and *Ulmus wallichiana* (3.97).

Site IV

This site had seven tree species. The highest density was recorded in *Robinia pseudoacacia* (12.25/m^2), followed by *Ulmus wallichiana* (10.75/m^2), *Aesculus indica* (9.12/m^2), *Ailanthus altissima* (8.87/m^2), *Cedrus deodara* (8.12/m^2), *Prunus armeniaca* (6.37/m^2) and *Celtis australis* (4.50/m^2). The IVI values were highest for *Robinia pseudoacacia* (20.29) followed by *Ailanthus altissima* (18.79), *Celtis australis* (13.02), *Prunus armeniaca* (10.22), *Ulmus wallichiana* (9.12), *Aesculus indica* (8.91) *and Cedrus deodara* (6.21).

3.2.2 Herbaceous layer

Diversity values of herbaceous species are presented in (Table 3).

Table 3. Plant diversity of the herbaceous layer

Details of Sites	General Diversity index (H)	Species Richness Index (d_2)	Index of Dominance	Evenness Index (e)
Site I	0.402	0.32	0.0014	0.82
Site II	0.479	0.41	0.0020	0.73
Site III	0.512	0.32	0.0006	0.86
Site IV	0.501	0.28	0.00103	0.81

Site I

Herbaceous layer of this site had a very poor diversity. Only three species were recorded. The density of these species ranged from 5.09- 2.50. Highest IVI was recorded for *Cynodon dactylon* (10.95) followed by *Stellaria media* (9.14) and *Teraxacum officinale* (10.10).

Site II

Low variation was observed at this site also. Only four species were recorded and their density ranged from 0.62 – 12.05/m^2. The highest IVI was recorded for *Tulipa stellata* (25.40) followed by *Poa bulbosa* (24.13), *Salvia moorcroftiana* (15.80), *Euphorbia helioscopia* (15.09) and *Chenopodium album* (6.55).

Site III

Similar to the site II, this site also showed the presence of only five species. The highest density was found for *Conyza canadensis* (20.57/m^2) followed by *Trifolium pratense* (16.25/m^2), *Tulipa stellata* (13.59/m^2), *Cynodon dactylon* (10.37/m^2) and *Salvia moorcroftiana* (5.78/m^2). *Tulipa stellata* (26.40) had highest IVI followed by *Conyza canadensis* (24.17), *Trifolium pratense* (20.69), *Cynodon dactylon* (15.41) and lowest was for *Salvia moorcroftiana* (13.02).

Site IV

Poor diversity with the presence of only three species was seen at this site and their density ranged from 2.6- 14.25/m^2. The grass *Tulipa stellata* was dominant with (27.04) IVI followed by *Salvia moorcroftiana* and *Cynodon dactylon* with IVI (14.03) and (10.72) respectively.

4. Discussions

Considering IVI as an indicator of dominance, *Robinia pseudoacacia* and *Tulipa stellata* dominated the woody species and herbaceous layer respectively in all the studied sites except for one site in herbaceous layer where the *Cynodon dactylon* exhibited a higher value. Generally the diversity index for the Indian forests ranged between 0.83 - 4.1 (Parsthasarathy *et al.,* 1992; Visalakshi, 1995). But in this area the species diversity values for the woody layer ranged between 0.202 – 0.576 (Table 2) and the species diversity value for the herbaceous layer ranged between 0.402-0.512 which are very low (Table 3). When compared to the Indian standard (Yadava and Supriya, 2006). This brings out to be an example of accelerating species extinction with reducing diversity affecting the forest ecosystem. The reason behind this decreased diversity may be human dominance as it has been proved by earlier workers that a high rate of anthropogenic activities result into reduction in diversity (Lindenmayer *et al.,* 2008; Goparaju *et al.*, 2005).

Though many a times there occurs an increase in undergrowth species diversity due to such activities because planting of trees had made the conditions congenial for the invasion of local herbaceous species which are mostly shade loving (Verma *et al.*, 2005)

have also reported in their study that planting of trees encouraged the invasion of local species and therefore, diversity of herbs was found maximum under plantations. (Ram *et al.,* 2004) but manmade disturbances usually leads to the forest degradation due to insufficient recovery time and also contribute to the disappearance of economically, ecologically or medicinally important plant species. As this area is situated on the bank of Manasbal lake and being an important tourist spot of Kashmir, it requires special protection and conservational measures which include complete fencing on the bank of the lake so as to protect the plantation against cattle and human interferences to create a more favorable environment for the establishment of the flora and facilitate to attract fauna. If conserved properly this afforested area will definitely act as a catalyst for successful natural forest succession. Conservation of such sites will also help in providing the basic needs of the locals and visitors of the area (Amin *et al.,* 2007).

5. Conclusion

The present study thus concludes that there were fourteen species of trees and ten herbaceous species. Tree species planted on the bank of the Manasbal lake had modified the microclimate and thus new, sciophytic herbaceous species have grown under the cover of plantations. Thus specific ameliorative steps in terms of a proper protection from human interferences and scientific management of this area are imperative for making this a biodiversity rich site in Kashmir valley.

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