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Cover Page, Introduction, Contents, Call for Papers, All in one file

CONTENTS

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Evaluation of the Antimutagenic Effect of Vitamin C against DNA Damage and Cytotoxicity Induced By Trimethyltin in Mice

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Abstract: The objective of this study is to investigate the utility of comet assay and chromosome aberrations analysis for detecting the possible antimutagenic activity of vitamin C to reduce the genotoxic effect of trimethyltin (TMT). TMT is one of the organotin compounds which is widely used as polyvinyl chloride heat stabilizers and marine biocides. In this study, male Swiss mice were treated interapretoneally (i.p.) with three tested doses 0.25, 0.50 and 1.0mg TMT/kg b.wt. for 1, 2 and 3 days. Alkaline comet assay in nucleated bone-marrow cells and chromosome analysis in spermatocytes were performed 24h after the last treatment. The amount of DNA damage in cells was estimated from comet tail length as the extent of migration of the genetic material. A significant increase in comet tail length indicating DNA damage was observed at all concentrations compared with control ($p<0.05$). The mean comet tail length showed a concentration- related and time-dependent increase. Also, the percentage of chromosome aberrations in spermatocytes was statistically significant ($p<0.05$) and showed dose and time dependent manner. Concurrent administration of vitamin C (VC) orally at 20mg/kg b.wt. with the highest dose of TMT for 1, 2 and 3 days reduced DNA damage in somatic and germ cells to a significant extent. In conclusion, our results indicated that vitamin C ameliorated DNA damage and genotoxicity induced by trimethyltin in mice somatic and germ cells *in vivo*. [Nature and Science 2009; 7(12):1-7]. (ISSN: 1545-0740).

Key words: trimethyltin, vitamin C, bone marrow, spermatocytes, comet assay, chromosome aberrations, mice.

1. Introduction

Among the various chemical agents that can induce damage in animals and humans, pesticides are a prime offender, chief among these are organotin compounds, which are used not only in agricultural pesticides, but also in many industrial processes, as material preservatives, and as components of antifouling paints **(Fent, 1996 and 2003)**. Organotins are widely dispersed throughout the ecosphere where they can be accumulated into the food chain **(Hodge *et al*, 1979)** via the tin geocycle **(Craig, 1988)**. Trimethyltin (TMT) is a potent toxicant that selectively kills cells in the central nervous system, immune system, spleen, lung, and kidney **(Brown *et al*, 1979; Snoeij *et al*, 1985; Philbert *et al*, 2000)**. TMT intoxication resulted in phenotypic changes indicative of apoptosis cell death, which includes chromatin condensation, nuclear fragmentation, mitochondrial dysfunction, reactive oxygen species production, membrane blebbing and caspase activation **(Stine *et al*, 1988; LeBel *et al*, 1990; Geloso *et al*, 2002; Jenkins and Barone, 2004)**. Administration of TMT at 2.8 mg/kg, i.p. induced neural damage seen in the dentate granule cells of mice **(Ogita *et al*, 2004)**. The induction of cytogenetic damage to mammalian cells by TMT has been indicated in a number of published reports *in vivo* and *in vitro* **(Ghosh *et al*, 1989; Ganguly *et al*, 1992; Ganguly, 1994; Dopp *et al*, 2007)**.

There is considerable evidence that the effects of mutagenic and carcinogenic agents can be altered by many dietary constituents. Vitamin C (VC) is an essential dietary nutrient required as a co-factor for many enzymes and a very efficient antioxidant, scavenging reactive oxygen and nitrogen species and protecting cells against free radical- mediated damage **(Sanchez-Moreno *et al*, 2003)**. Besides exerting antioxidant influence directly, VC can promote the removal of oxidative DNA damage from the DNA and/or nucleotide pool, through the upregulation of repair enzymes **(Cooke *et al*, 1998)**. The inhibitory effect of VC towards a number of mutagens/carcinogens was shown by many authors in humans and animals **(Mooney *et al*, 2005; Hassan *et al*, 2006; Fahmy *et al*, 2008)**. The goal of the present study is to evaluate the *in vivo* protective effect of VC against the DNA damage may be induced by TMT in mouse somatic and germ cells.

2. Materials and Methods:

2.1. Animals

Laboratory-bred strain Swiss albino male mice of 8-10 weeks old with an average weight of 27.5±2.5 g obtained from the National Research Center, Cairo, Egypt, were used. Animals were housed in groups (5 animals/ group) and maintained under standard conditions of temperature, humidity and light. The animals were given standard food and water *ad libitum*.

2.2. Chemicals

TMT was purchased from BDH Chemicals Poole, England. VC was purchased from Sigma, USA. All other chemicals used were of analytical grade.

2.3. Doses and experimental design

The experimental design involved: 1- Mice were treated i.p. with the doses 0.25, 0.5 and 1.0mg TMT/kg b.wt. for 1, 2 and 3 days. 2- Concurrent administration of VC at 20mg/kg b.wt. and 1mg TMT/kg b.wt. for 1,2 and 3 days. The samples were taken 24h after the last treatment from the bone-marrow cells for DNA damage detection by comet assay and spermatocyte cells for chromosome aberrations detection. In all experiments, the animals groups were treated with vehicle as a negative control, 20mg VC/kg b.wt. and 1mg mitomycin C/kg b.wt. as a positive control. All samples were collected after 24h.

2.4. Cell viability

To measure cytotoxicity, 15µl of each original cell suspension was mixed with15µl of 0.4% solution of trypan blue vital dye. Cells were analyzed with a light microscope and the percentage of viable cells was determined from 200 cells for each experimental group **(Zamorano-Ponce *et al,* 2004)**.

2.5. Comet assay

The comet assay was performed as described by **Tice *et al* (2000)**. Mice femurs were dissected out and bone marrow was aspirated from each femur into media solution. The cell suspension (25 µl) was mixed 1:10 with 250 µl molten low melting point (LMP) agarose, and samples of 75 µl of the mixture were rapidly spread on CometSlides. After gelling for 20 min at 4ºC in the dark, slides were put in a tank filled with lysis solution (2.5M NaCl, 0.1M EDTA, 10mM Tris base, 1% sodium lauryl sarcosinate and 1% Triton X-100) for 1h at 4ºC in the dark. Slides were then washed three times with neutralization buffer (0.4M Tris, pH 7.5) for 5 min and incubated in fresh alkaline buffer (0.3M NaOH and 1mM EDTA, pH>13) for 30min at room temperature to allow unwinding of DNA. Electrophoresis was then carried out at room temperature in fresh ice-cold alkaline electrophoresis buffer for 30 min (1V/cm; 300mA). After electrophoresis, slides were gently washed three times for 5 min in fresh neutralization buffer and exposed to 70% ethanol for 5 min. After drying at room temperature, slides were stained with 25 µl of ethidium bromide solution (20µg/ml) and covered with cover slip. Comets were examined at 200X magnification using a fluorescence microscope.

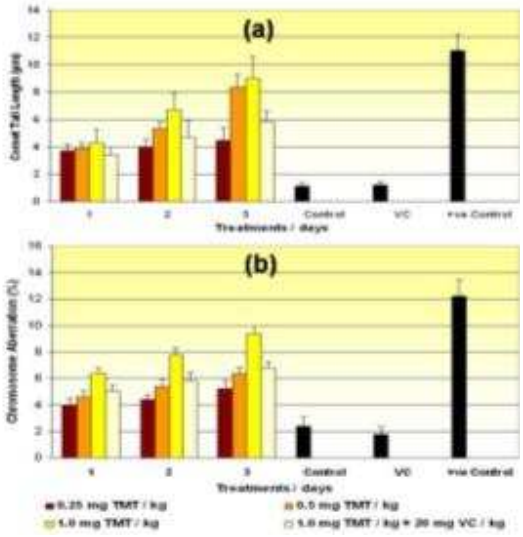

Figure 1: Diagram represents (a) Comet tail length (µm ± SE) (b) Chromosome aberration (% ± SE) induced by different doses of TMT for three times intervals compared with MMC as a positive control.

Table 1: The percentage of cell viability and comet tail length (µm) in mice nucleated bone marrow cells after treatment with TMT alone and in combination with vitamin C.

Treatments	Time of Treatment (Day)	(%) of Viable Cells	Comet tail length Mean ±SE
I. Control (vehicle)	-	93	1.14±0.23
II.VC 20mg/kg b.wt.	-	94	1.17±0.28
III. MMC (1mg/kg b. wt.) positive control	-	72	11.03±1.12[a]
IV. TMT 0.25mg/kg b. wt.	1	91	3.72±0.46[a]
	2	87	3.92±0.58[a]
	3	82	4.48±0.91[a]
0.5mg/kg b. wt.	1	85	3.90±0.48[a]
	2	80	5.31±0.52[a]
	3	78	8.37±0.97[a]
1.0mg/kg b. wt.	1	82	4.31±0.97[a]
	2	77	6.70±1.20[a]
	3	70	9.01±1.61[a]
V.TMT+V.C. 1.0mg/kg b. wt. + 20mg/kg b.wt.	1	84	3.42±0.55[a]
	2	81	4.71±1.19[ab]
	3	76	5.86±0.76[ab]

a) Significant compared to vehicle control ($p<0.05$)
b) Significant compared to TMT treatment at 1mg/kg b.wt. ($p<0.05$) (t-test)

Table 2: Number and mean percentage of diakinase metaphase I cells with chromosome aberrations in mice spermatocytes after treatment with TMT alone and in combination with vitamin C.

Treatments	Time of Treatment (Day)	XY un.	Auto. un.	XY+ Auto. un.	Chain (IV)	Total Aberrations	
						No.	Mean (%)±SE
I. Control (vehicle).	-	7	5	-	-	12	2.4±0.65
II.VC (20mg/kg b. wt.)	-	5	4	-	-	9	1.8±0.52
III. MMC (1mg/kg b. wt.) positive control	-	35	20	4	2	61	12.2±1.2[a]
IV. TMT							
0.25 mg/kg b. wt.	1	14	6	-	-	20	4.0±0.54
	2	15	7	-	-	22	4.4±0.40
	3	18	8	-	-	26	5.2±0.60 [a]
0.5 mg/kg b. wt.	1	16	7	-	-	23	4.6±0.42
	2	15	12	-	-	27	5.4±0.54 [a]
	3	18	13	1	-	32	6.4±0.48 [a]
1.0 mg/kg b. wt.	1	19	13	-	-	32	6.4±0.42 [a]
	2	22	15	1	1	39	7.8±0.56 [a]
	3	28	17	-	2	47	9.4±0.58 [a]
V. TMT+V.C.	1	20	5	-	-	25	5.0±0.50[a]
1.0mg/kg b. wt.	2	18	11	-	-	29	5.8±0.64[a]
+ 20mg/kg b.wt.	3	21	12	-	1	34	6.8±0.46[ab]

The total number of scored cells is 500 (5 animals/ group), XY un.: XY univalent; Auto. un.: Autosomal univalent
a) Significant compared to vehicle control ($p<0.05$)
b) Significant compared to TMT treatment at 1mg/kg b.wt. ($p<0.05$) (t-test)

Evaluation of DNA damage

Five good-quality slides were selected for each variable from the different treatment groups including controls. All slides were independently coded and scored. A total of 50 comets were scored for each variable. The comet tail length was measured with a calibrated ocular micrometer disk. The quantification of the DNA damage was made by calculating as comet tail length (µm) = total length of comet –head diameter. The significance of the effect of each treatment dose versus the solvent control and between treatments with protection versus treatment alone was evaluated by t-test.

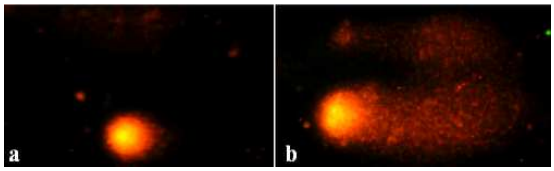

Figure 2: Photomicrographs of damaged DNA observed after the treatment of mice bone-marrow cells with TMT showing increase in tail moment. Comets were classified for qualitative evaluation into (a) Undamaged cell, (b) Highly damaged cell.

2.6. Chromosome aberrations

For spermatocytes preparation, animals from each group were injected i.p. with colchicine, 2-3h before sacrifice. Animals were sacrificed by diethyl ether at end of treatments. Chromosome preparations were made from testes according to the technique of **Evans *et al* (1964)**. 100 well spread diakinase-metaphase I cells were analyzed per animal (5animals/ group) for chromosome aberrations. Metaphases with univalents and chromosome translocations were recorded. The significance of treatment from the control data and treatment plus protective versus treatment alone was calculated using t-test.

3. Results:

3.1. Cell viability:

The cytotoxic effect of TMT was determined using three different doses for 1, 2 and 3 consecutive days. The percentage of viable cells reduced in a dose and time dependent. Oral administration of VC with the highest dose of TMT increased the percentage of cell viability for all the tested doses. The percentage of viable cells was always ≥70% after treatment for all experiments as recommended for comet assay by the International Workshop on Genotoxicity test Procedure (Tice *et al.*, 2000; Table 1).

3.2. Comet assay:

The amount of DNA damage in the cell was estimated from tail length as the extent of the migration of the genetic material in the direction of the anode. During electrophoresis, cell DNA was seen to more rapidly migrate towards the anode at the highest concentration than at the lowest concentration. Even the comet tail tended to increase when exposure was prolonged from 1 to 3 days. Mean tail length (µm) of comets obtained by TMT treatment is given in table (1). The trend of increase in comet tail length with increase in concentration and duration is depicted in figure (2). All the concentrations and their respective duration evoked significant DNA damage ($p<0.05$) compared with controls. The maximum DNA damage was recorded at the highest dose of TMT. This damage was reached to 9.01µm after treatment for 3 days with TMT in comparing to 11.03µm for positive control (MMC) and 1.14µm for negative control. The highest DNA damage reduced to 5.86µm after concurrent treatment of 1mgTMT/kg and VC (20mg/kg) for 3 consecutive days (Table 1, Figure 1a).

3.3. Chromosome aberrations:

The genotoxic effect of TMT on the germ cells was illustrated by examining the chromosome aberrations in mouse spermatocytes. Table (2) and figure (1b) represents the chromosome aberrations in mouse spermatocytes after treatment with different doses of TMT for the three time intervals.

It was demonstrated that the highest percentage of chromosome aberrations reached 9.4±0.58 after treatment with 1mgTMT/kgb.wt. for 3 days compared to 12.2±1.2 for positive control and 2.40±0.65 for negative control. This percentage was decreased to 6.8±0.46 after concurrent treatment of TMT (1mg/kg) and VC for the same period of time. The main types of chromosome aberrations observed were XY univalents and autosomal univalents (Figure 3).

4. Discussion:

The aim of the present study was to evaluate the protective effect of VC towards DNA damage induced by TMT in mouse nucleated bone-marrow cells and chromosome aberrations in mouse spermatocytes. DNA damage was carried out using comet assay, a molecular technique that has been increasingly employed to evaluate *in vivo* and/or *in vitro* DNA damage in individual cells **(Manas *et al,* 2009)**. Spermatocytes represent the only system in which the transmissible genetic damage from one generation to another takes place **(William and Hsu, 1980)**.

Our data showed that TMT induced statistically significant increase in DNA damage which represented by tail moment in bone-marrow cells and chromosome

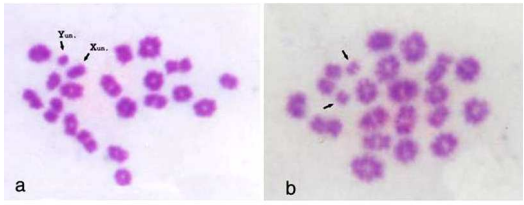

Figure 3: Metaphases spread from mice spermatocytes showing (a) XY univalents, (b) Autosomal univalents after treatment with 1mg TMT/ kg b.wt.

aberrations in spermatocyte cells in a dose and time response. The same observations showed by **Ghosh *et al* (1989)** and **Ganguly *et al* (1992)**. They demonstrated that TMT induced elevation in the frequency of micronuclei and sister chromatid exchanges in cultured human lymphocytes. Also, TMT had the ability to induce chromosome aberrations in mice bone-marrow cells *in vivo* 6h after treatment **(Ganguly, 1994)**. **Dopp *et al* (2007)** observed that di- and trimethyltin significantly induced micronuclei, chromosome aberrations, sister chromatid exchanges and DNA damage at non-cytotoxic concentrations in CHO-9 cells. It is worth to mention that other organotin compounds such as butyl- and phenyltin induced hyperdiploid cells (aneuploidy) and chromosome supercontraction even at low concentrations in cultured human lymphocytes **(Jensen *et al,* 1991)**.

With respect to the chromosome aberrations induced in mouse spermatocytes after 1, 2 and 3 days of treatments with TMT, it was observed that dissociated univalents dominated autosomal and XY-univalents. However, XY-univalents were more frequent. This type of abnormality has been discussed as an indicator of a mechanism of male sterility from radiations and mutagenic chemicals **(Cattanach *et al,* 1968; Rapp *et al,* 1977)**. Translocations in the form of chain IV were observed at low frequency. According to **William and Hsu (1980)**, chromosome translocations in germ cells have been observed in animals after treatment with ionizing radiation, but rarely in those treated with chemicals.

The DNA breaks that are detected in the comet assay and cytogenetic analysis may be resulted from: 1- The oxidative stress and the enhancement of the intracellular generation of reactive oxygen species (ROS) formed by TMT **(Sergent *et al,* 1999)**. These ROS can damage DNA and division of cells with unrepaired or misrepaired damage leading to mutations. Also, if these changes appear in critical genes, such as oncogens or tumor suppressor genes, initiation or

progression may result **(Loft and Poulsen, 1996; Pryor, 1997)**. 2- This DNA damage could be also originated from apoptotic cells. Several laboratories have reported that the onset of apoptosis can give comet images with cell aspect and tail parameter values of the same orders as those of cells with moderate DNA damages **(Florent *et al,* 1999; Choucroun *et al,* 2001)**. **Jenkins and Barone (2004)** reported that TMT had the ability to induce apoptosis in PC12 cells by initiating apoptotic pathway requiring oxidative stress, caspase activation and P38 protein kinase activity leading to cell death. Also, **Kawada *et al* (2008)** observed that i.p. injection of TMT at the dose 2.8mg/kg b.wt. led to a dramatic increase in the number of degenerating cells in the granule cell layer of the OB and AON of the mouse brain cells.

Interest in the chemopreventive functions of antioxidants has grown considerably in recent years. Evidence accumulated over the years shows that people with high dietary intakes of fruits and vegetables are less likely to develop cancer than people who have low dietary intake of these foods. While many chemopreventives in fruits and vegetables may have anticancer properties, much interest has focused on vitamin C **(Mayne, 2003)**. This study represents one of the premiere studies carried out to diminish the toxicity and the genotoxicity of the oxidative compound TMT by using the natural antioxidant compound VC. Vitamin C is a highly effective antioxidant. It acts as a reducing agent that can terminate free radical driven oxidation by being converted to a resonance-stabilized free radical. In this respect VC can protect indispensable molecules in the body, such as protein, lipids, carbohydrates and nucleic acids (DNA and RNA). VC also regenerates other antioxidants such as vitamin E **(Schneider *et al,* 2001**). Our results showed that concurrent administration of VC inhibited the DNA damage and chromosome aberrations induced by TMT in all tested doses. This ameliorative effect induced by VC may be resulted from enhancement of detoxification pathways that convert this reactive compound to less toxic and more easily excreted products **(Vijayalaxmi and Venu, 1999)** and/or through its action as the free radical scavenging efficiency (**Chaudiere and Ferrari-Iliou, 1999)**. In addition, numerous *in vitro* and *in vivo* studies have evaluated the protective effects of VC against several radical generating chemicals **(Blasiak and Kawalik, 2001; Blasiak *et al,* 2004; Robichova *et al,* 2004; Arranz *et al,* 2007)**.

In conclusion, our results demonstrated that VC could be a suitable agent for preventing TMT- induced DNA and chromosome damage in an *in vivo* mammalian system.

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Crop Protection Problems in Production of Maize and Guinea Corn in Northern Guinea Savanna of Nigeria and Control Measures

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Abstract: The cultivation of Maize and Guinea corn in the northern Guinea Savanna of Nigeria is faced with lots of Crop protection problems which hinder full scale production of these crops in that ecological zone. The problems range from biotic factors like vertebrate and invertebrate pests, disease pathogens, nematode and weeds, to abiotic factors such as nutrient deficiencies, environmental conditions (climatic, edaphic), and agronomic, logistic or social problems. Addressing the various problems militating against the production of maize and Guinea corn in this zone will further help strengthen the national food reserve base and alleviate the devastating effects of the global food crisis particularly in Nigeria. [Nature and Science. 2009;7(12):8-14]. (ISSN: 1545-0740) .

Key words: Maize, Guinea-corn, biotic, abiotic, problems, control.

Introduction

Maize (*Zea mays* L.) and Guinea corn (*Sorghum bicolor* (L) Moench) are important food crops in Nigeria, widely grown in the savanna regions of the country. These crops form the staple foods for most of the population especially in areas adaptable for their production. Green maize (fresh grains) is eaten roasted or boiled on the cob. The ripe grains (of maize or sorghum) are cooked in combination with pulses or milled and boiled as porridge (Yoruba = Eko, Hausa = Kamu, Ibo = Akamu). Sorghum (Guinea Corn) uses vary from drinks to 'tuwo'. The stems are used for fuel and building of fences and local huts. Maize and guinea corn are used as basal ingredients of livestock feeds. They are rich in Carbohydrates. In spite of the importance of these cereals as sources of food for human consumption, their production is concentrated in the hands of peasant farmers whose average hectarage is very small, approximately 0.5 – 1.0 hectare per farmer. The technologies are basically traditional farming methods and systems.

An estimated one million hectares of land was planted to maize in the country in 1989/1990 and over 40% of this was cultivated in the northern states (NAERLS, 1982). This figure has been increasing steadily ever since, with the help of irrigation especially in the drier parts of the north (Sahel and Sudan). Average yield per hectare in the northern savannas on peasant farms is about 0.6 metric tonnes, while commercial farms average is about 2.0 metric tonnes/ha. Guinea corn, on the other hand, is grown in an estimated 300,000 hectares of land north of the Niger and Benue rivers, especially in areas generally too dry for consistent and reliable maize production. Average yield in both peasant farms and commercial setup is 0.40 metric tonnes/ha and 1.0 metric tonnes/ha respectively. Varieties of these cereals (maize and sorghum) planted in these areas are both local, improved local and hybrids. Plant breeders in I.A.R. (Institute for Agricultural Research, Ahmadu Bello University, Zaria) have produced suitable varieties adapted to different ecological zones of the savanna where the crops are grown. Suitable yields of the improved crops have also been packaged. However, a number of constraints (crop protection problems) militate against the production of those crops. These are discussed below and solutions proffered on identified problems.

Crop Protection Problems

Crop protection problems refer to all the biotic and abiotic factors which impede our quest to achieve self sufficiency in food production. These problems are common to both maize and sorghum in the savanna areas even though their importance may vary greatly. Some problems are confined to a single zone, others are generalized.

1. Biotic Factors

About 6 percent of the total food currently produced in this country is lost to pests and diseases. Maize and Sorghum are susceptible to various pests (vertebrate and invertebrate) and diseases (bacterial, fungal, viral, nematode infections) in different ecological zones of the northern savanna.

Pests

Vertebrate Pests

Samaru **(**Lat. 11^0 11^{11}N, Long. $07^0 38^{11}$E) is in the Savanna region which consists of derived Savanna (referred to as northern and southern Guinea zones), Sudan and Sahel ecological zones. These zones are hosts to various species of monkeys, birds, rodents

and other wild animals which cause extensive damage to maize and sorghum fields.

Monkeys moving in groups can cause up to 70% loss in peasant's maize fields and about 30% loss in commercial farms if their activities are not checked (Amadi, 1988 personal communication). Damage is done early in the morning between 6.00 am and 9.00 am; and between 4.30 pm and 6.30 pm. Succulent maize cobs are removed from the stalks and eaten up while excess harvest are littered on the fields and along bush paths. Monkeys menace on the farms can be checked by trapping and employment of hunters to track down the animals. Widening of farm paths to allow for regular traffic and placement of scare-crows in strategic areas in the farm may also be helpful.

Birds especially *Quelea quelea* and Doves cause great damage to Sorghum heads. Adult Quelea birds may not be as destructive as the young ones newly weaned. The adults feed on wild seeds of grasses until exhausted before raiding cereal fields. Damage done by the newly independent young Quelea (from 3 weeks of age) arises from extreme hunger, since their parents no longer feed them. The young Queleas are extremely persistent in their attack and may continue feeding even when the Sorghum stem they are on is shaken by hand. They may also pay a deaf ear to shouts or sounds produced by farmers to scare them away. Quelea birds can be controlled by using flame-throwers, explosives or aerial spraying of organo-phosphorus pesticides. The use of resistant varieties of Sorghum (those with hard kernels and more tannin content e.g. red types) may prove helpful in some localities.

Rodents particularly rats and grass cutters cause extensive damage to both maize and sorghum in the northern and southern Guinea Savannas. Rats and bush fowls attack newly sown seeds and young germinating seedlings causing wide gaps in crop rows. These gaps when extensive cause severe yield reduction and necessitate supplying to fill the gaps at extra costs. Seed dressing chemicals such as Apron Star, Apron plus etc, should be used to treat seeds before sowing to control these pests. Rats and grass cutters may cause damage on maize grains on the field. Rats climb up the stalk, reaching the cobs and feed on the grains while grass cutters cut the stem a few centimeters from the ground, subjecting the stalk to lodging. They later feed on the falling immature cobs. Rats also feed on stored grains of maize and sorghum thereby reducing its quality and quantity. Control of rats is by using bait poisons (both in the field and store) and by fumigation with phostoxin tablets during storage.

Invertebrate Pests

These include all arthropod insects, molluscs, etc. which attack maize and sorghum plants inflicting heavy losses to the farmers. Different stages of the plant growth (e.g. seedling, vegetative, flowering and heading) are susceptible and various parts (e.g. roots, stems, leaves, flowers and grains) are attacked resulting in colossal losses. Termites and mole crickets destroy seeds in the soil causing wide gaps within the crop rows and poor crop establishment. The roots of seedlings and mature plants may be attacked by termites resulting in extensive damage to the cereals. Control is by seed dressing chemicals and use of Dieldren sprayers on the habitat of termites.

The major insect pest problems on cereals in the field are the stem borers, (*Busseola fusca* and *Sesemia calamistis*); shoot flies (*Atherigona* spp); grasshoppers (*Zonocerus variegatus*) and army worms (*Spodoptera exempta* and *Helicoverpa armigera*). The stem borer attack is usually more serious in late maize than the early ones. These borers feed inside the plant stems and are well protected from both their natural parasites and insecticides. They cause two types of damage to the plants. First, is mechanical damage due to consistent feeding in the stem, weakening it, and thus rendering the stems susceptible to lodging (stem breaking or falling down) and withering (dead heart). Secondly, stem borers may cause characteristic perforations or windows on leaves called 'fenestrations' seen when the sheath opens exposing the perforations (NAERLS, 1982). This type of damage reduces the photosynthetic area of the leaves resulting in poor cereal yield, especially during high infestation. Stem borers can be controlled economically by cultural methods. This involves removal and destruction of infested plants and plant residues. Pesticides with contact and systemic action are very effective at the initial stage of infestation to get rid of the larvae before burrowing into the stems.

Similarly, *Sessemia calamistis* a polyphagous insect most associated with young seedlings can cause extensive tunneling of adult plants stems resulting in 'dead' heart and chaffy heads in sorghum. Control is similar to *B. fusca. Zonocerus variegatus* when occurring gregariously causes extensive defoliation of cereals. Spraying with Fernithrothion 50 EC, Endosulfan (granules) or Trichlorphon (granules) can effectively check its menace on the field. Other grasshoppers attacking maize and sorghum albeit sporadically include *Locusta migratoria* L., *Schistocerca gregaria* L. (desert locust) and *Oedaleus* spp. All these are gregarious pests which can stripe the plants of their vegetation leaving the stalk bare. Control is similar to that of *Zonocerus* spp.

Army worms, *Spodoptera* spp and *Helicoverpa armigera* occur sporadically but may destroy the crops completely. The larvae are gregarious during outbreak and they feed for about three weeks. Outbreak is associated with alternating wet and dry spells (Misari, 1993 personal communication). These worms cause severe yield reduction on cereals by feeding on developing grains cutting them into smaller bits. Deep ploughing immediately after the season's harvest exposes the pupae to direct sun-rays resulting in desiccation of the pupae. Chemical control using Uppercott ® (Cypermethrin + Dimethoate) gives a good control.

Sorghum shoot fly, *Atherigona socata* Rondani attacks young seedlings as soon as the plants emerge from the soil and can last for about six weeks. The larvae feed on the central bud of young shoot, causing the death of the growing points ('dead heart' effect). Fenithrothion 50 EC at the rate of two litres per hectare can be applied for control. Sorghum midge, *Contarina sorghicola* lays its eggs on flowering heads and on hatching, the larvae feed on developing ovaries. Control can be achieved by prompt spraying of the sorghum heads as soon as the pests are detected with a good insecticide.

Beside field pests, maize and sorghum are seriously attacked by storage pests. The most important storage pests include grain weevils (*Sitophilus zeamays*) and *Rhizopertha dominica* for maize crops; *Tribolium casteanum* or *T. confucium*, *Trogoderma* spp, *Sitotroga cerealella* and *Sitophilus* spp for sorghum. In some cases, infestation takes place on the field and continues in the store. Some others are confined to the store while infestation may be by insects already present where the cereal grains had previously been stored or by crops infestation between granaries during storage.

For control of storage pests, strict adherence to hygiene in the store as well as provision of air-tight cover is essential. Mixing or storing old grains with new ones during storage should be discouraged. Cereals stored for seed or consumption beyond one month should be fumigated with phostoxin or treated with Actellic e.c.

Diseases

Diseases play an important role in the reduction of the potential yield of cereal crops. Agents causing diseases include bacteria, fungi, viruses, nematodes, weeds and nutrient deficiencies. The geographical distribution of cereal diseases in the savanna ecological zones is influenced by temperatures (high/low), moisture (humidity), cultural practices and the type and diversity of germplasm used.

i) Pathogen Problems

In a survey for incidence and severity of diseases in both the northern and southern guinea savanna of Nigeria, Adeoti (1992) reported the occurrence of the common foliar diseases such as the rust, *Turcicum* blight, *Curvularia* leaf spot and *Maydis* blight induced by *Puccinia* spp; *Helminthosporium turcicum*; *Curvularia* spp and *H. maydis* in the order of severity. The 'Pokkha boeng' disease induced by *Fusarium moniliforme* was also found to be severe in many areas where it occurs (Adeoti, 1992) and the percentage yield loss ranges between 5 and 30%. Other important maize diseases occurring in the savanna ecological zones include smut (*Ustilago maydis*), Downy mildews, Maize leaf fleck and Maize streak. Similarly, some rusts, smuts and blight diseases have been recorded on Sorghum plants. These include common rust (*Puccinia graminis*) f.sp. *Sorghii*, loose smut (*Sphacelotheca cruenta*), Cover smut (*Sphacelotheca sorghii)* and Head smut (*Sporisorium reiliana*) (Adeoti, personal communication).

Control of most fungal, viral and bacterial diseases of maize and sorghum can be by the use of resistant varieties, seed dressing with Furadan or Apron plus; elimination of alternate host (for rusts); crop rotation, removal and burning of infected plants and spraying with systemic fungicides such as a mixture of Benomyl and Dithane M45, Delsene, Rovrus (for 'Pokkha Boeng' disease) and so on.

ii) Nematode Problems

Several species of nematode have been reportedly associated with both soil and root of sorghum and maize in the savanna ecological zones. These species include *Pratylenchus* spp, *Aphelenchoides* spp, *Tylenchus* spp, *Helicotylenchus* spp, *Ditylenchus* spp and *Scutellonema* spp (Chindo, 1991 personal communication). Infected plants fall down from the root level and on examination, the plant roots are shortened, tiller profusely with round stubs at the tips. Control of nematodes is achieved by the use of Furadan 3G and other fumigant nematicides e.g. Ethylene Di-bromide (EDB), Dichloropropenes (Telone) and Dichromochloropropane (Nemagon). Manufacturer's recommendations should be adhered to for effectiveness.

iii) Weeds

Weeds constitute a special class of pests which seriously limit the production of the major crops on any scale. They compete with the crops for nutrients, air, light and moisture. The most noxious of these weeds are the parasitic ones particularly striga

spp (known as witch weed and "wuta wuta" or "kuduji" in Hausa). There is still no "universally applicable" and most effective control for striga despite several years of research in Nigeria. However, some inexpensive control measures including crop rotation, the use of tolerant varieties, generous fertilizer application and hand pulling before flowering can be applied to ensure satisfactory crop yield.

Other 'stubborn' weeds which also reduce cereals yield in the savanna include *Rottboellia* spp, *Pennisetum purpureum*, *Cyperus* spp, *Dactylon* spp and some broad leaved plants (compositae). Stomp, Round up, Fusilade and 2, 4-D respectively offer good control of these weeds. Hand weeding is effective but must be timely and repeated thrice before the crops mature to ensure economic yield. Since most cereals are shallow-rooted, it is essential to ensure that no mechanical damage is done to the crops roots during hand weeding. In erosion prone sites, earthing up or remolding of ridges may be required to prevent excessive exposure of the roots to the sun.

iv Nutrient Deficiencies

Maize and sorghum are high nutrients demanding crops than other cereals (rice, millet and wheat). These crops require both the major nutrients (N, P and K) and the secondary nutrients (S, Mg, Ca, B, Fe, Cl, Cu etc.) in adequate amount to ensure good root establishment, vigorous and healthy growth and increased yields. Healthy seedlings and plants are less susceptible to pests and disease attack. Deficiencies of vital nutrients cause yield reductions through poor plant development and growth, thereby, predisposing the plants to pests and disease attack.

Plant nutrients are supplied as fertilizer formulations. The demand for fertilizers in Nigeria has increased in recent years forcing the Federal Government to remove fertilizer subsidies to the Nigerian farmers. The result is escalating prices of the product making it difficult for peasant farmers to purchase enough for their crop needs. Consequently, most field crops especially maize and sorghum planted all over the savanna ecological zones exhibit symptoms of nutrient deficiencies such as chlorosis, stunted growth, poor root development, early leaf fall, delayed flower opening, hasty maturity, improper setting of grains, poor resistance to disease agents and low yield. Since the prices of different brands are prohibitive, the Federal government may reconsider its stand on fertilizer subsidy in order to encourage farmers to produce more food crops. Availability of these fertilizers at the right time is also essential.

2. Abiotic Factors

Crop protection problems in the savannas can be precipitated by various abiotic factors including climatic, edaphic, agronomic, logistic and social contributors.

Climatic Problems

The areas north of Niger and Benue rivers can be classified as mainly savannas. The savanna consists of the southern and northern Guinea zones, Sudan and Sahel zones. The main characteristics of these ecological zones include poor rainfall (distribution and quantity), high temperatures, humidity, drought, high wind velocity and harmattan, etc. In the last ten years, the onset, distribution and even total amount of rainfall in the savanna zones have been erratic, resulting in crops failure (NAERLS, 1982). Maize crop is more water demanding than sorghum and the uncertainty in rainfall pattern and distribution affected the crop severely. If drought occurs at the time of silking, the result is poor pollination and serious loss of grain, even when the plants look well grown and healthy.

The Sahel zone (Katsina, Sokoto, Maiduguri, Kano, Potiskum, Nguru etc.) are particularly vulnerable to this problem where average maize yield on rainfed crop is below 400kg/ha as compared to national average ranging between 1000kg/ha and 2500kg/ha. Similarly, sorghum crop though tolerant to drought may be susceptible to drought during the reproductive growth stage. Late season drought causes sorghum midge and head bug outbreak.

Rainfall shortages and drought can be solved by constructing more dams for irrigation. Breeding of drought tolerant /resistant varieties of maize and sorghum as well as closer rows may reduce soil moisture loss at the end of the season. High humidity, day length and high wind velocity affect maize and sorghum yields. High humidity encourages pests and diseases attack; short day-length affects the photoperiod requirement of maize (usually about 12 hours) for high yield; while heavy wind causes lodging especially on tall local varieties as well as facilitating pests and diseases movement.

These problems can be ameliorated by planting resistant varieties and adopting relevant agronomic practices. Wind breaks/shelter belts may be established in strategic locations to check wind movement.

Edaphic Factors

Soils in the savanna parts of the country consist of sandy loam, clayey-loam and loess (wind deposited sand). Organic matter contents are generally low (< 0.5%) and plant nutrients are

critically low. In some places, soil water availability is very critical and in some others water logging is common-place (e.g. Fadama). Soils of the savanna are generally alkaline in nature but in some cases, soils with low pH values have been reported (UAC Agro, 1989 unpublished). Erosion due to wind and running water also create problems in some localities. These edaphic factors constitute an impediment to crop production in the savannas and can be remedied by various soil amelioration processes. These include application of cow dung, poultry droppings, farm yard manure, and leaf dropping of shelter trees (to improve the organic matter content and improve the physical properties of soil). Wind erosion can be checked by establishment of shelter belts in wind prone areas (Kano, Sokoto, Daura, etc.). Erosion due to running water (flood) can be checked through construction of water channels (gutters); embankments and levees; encouraging vegetation cover in susceptible areas. Soils with low pH can be reclaimed through liming to improve its nutrient availability to the crops.

Agronomic Factors

Various agronomic or cultural practices may predispose crops to attack by pests and diseases, in the following ways:

i) Sowing dates influence grain yield through number, head weight and length of total growth cycle. Ogunlela (1985) reported a marked reduction in grain yield when sowing of photosensitive variety of sorghum (L.187) was delayed beyond June at Samaru than at Mokwa. A major cause for sorghum failure under delayed sowing was shoot fly (*A. soccata*) attack. Adapted sorghum varieties should not be sown later than late June in the northern Guinea savanna to ensure good yield; and a little later in the southern Guinea savanna (Ogunlela, 1985). However, early sowing for early maturing grain varieties causes crop to mature during the rains leading to the problem of grain mould. Similarly, late sowing for late maturing varieties runs the risk of drought or early cessation of rains (Ogunlela, 1985).

ii) Planting depth also affects incidence of pests and diseases. Deep planting causes the seed to rot while shallow planting subjects the seed to predation by birds, rodents, termites; and may weaken the roots of seedlings. Solution to this problem is to plant at the recommended depth, usually between 2.5cm and 4.5cm on ridges or flat.

iii)

iii). Crops grown by hand labour and using wider row spacing encourage pests and diseases attack. High nitrogen predisposes crop to disease and lodging. Continuous cropping (monoculture) of these cereals throughout the year permits the maintenance of a high inoculum potential. Similarly, the practice of leaving maize in the field long after maturity tends to increase losses from ear rots, stalk rots and even pilfering.

iv). Seed Bed Preparation - Poorly prepared seed bed encourages shallow rooting, poor seed establishment, lodging and wilt due to soil water unavailability to plants. Good seed bed preparation is therefore essential to ensure good crop establishment and high yield. Deep plowing, harrowing and ridging facilitate water penetration, exposes eggs and diapausing pupae of pests to desiccation by the sun and ensures weed and erosion control in the field.

v). Removal of crop residue - Maize and sorghum stalks left over on the farm after harvest is a source of pest and disease attack next planting season. Their removal and burning will ensure protection of crops from this source of infestation. Guinea corn stalk used for fencing or building should be properly dried in the sun before use.

vi). Crop density and close spacing-This may be used to reduce pest infestation on the field by denying insect pests the opportunity to make soil contact during their life cycles due to extensive canopy cover.

vii). Farming systems - In some areas, farmers plant sorghum, groundnut and okra; maize, cowpea and pepper; or maize-soyabean in the same parcel of land in a single growing season. Each crop combination requires different agronomic practices, nutrients, sowing and harvesting periods. For a successful handling of a combination of crops, understanding of the farming systems is very essential. Invariably, this becomes a source of worry to the farmer due to poor planning and execution of work plans for each crop in the combination to ensure good yield. Crops with similar pests and diseases (e.g. sorghum and maize) should not be planted in a crop combination in order to avoid the perpetuation of their common pests.

viii). Timeliness is very important in crop protection programmes; a little delay can jeopardize efforts and render completely unprofitable all that have been incorporated into the farming enterprise. For instance, stem borer is an insect pest of economic importance on cereal crops. Larvae of these insects are found in the whorls feeding on the young unexpanded leaves and later bore into the stem. Control programme for these insect pests should be

directed at the larvae while feeding in the whorls. If spraying is delayed and the larvae have bored into the stem, the use of contact insecticides to control the insects at this stage is no longer feasible (Amatobi *et al*., 1988).

Similarly, some insect pests that attack produce in storage usually commence infestation while still in the field. These fields to storage pests (e.g. *Sitophilus* spp on maize and sorghum) may cause extensive damage to stored produce if harvesting is delayed on the field. Timely harvesting of these crops is recommended to avoid further yield losses during storage.

ix). Pesticides are chemical formulations for the prevention and control of crop pests and diseases. Pesticides include herbicides, insecticides, fungicides, bactericides, nematicides, various protectants and growth regulators. A clear understanding of the mode of action is essential to ensure effective use. However, most Nigerian farmers seldom understand what pesticides to apply or look for to solve a specific field problem. Invariably, these farmers fall easy prey to Agro-chemical hawkers who are more interested in making money than solving the farmers' problems. Even when the correct pesticide is purchased by the farmer, the pesticide may not be effective due to staleness, late application after significant damage has been done to the crop; poor follow up of the recommended application schedule, incorrect method of application, sole reliance on pesticides in situations where other methods are more effective and fear of toxic effects on crops and man (Srivastava, 1974). Another constraint to pesticide use in the northern savanna is the lack of large amount of water required for conventional application. Even when water is available, cost of labour in carrying and applying large quantities of water can be quite high. The above problems can be solved by training and posting more extension workers to the villages to assist farmers solve their crop protection problems. Water shortages can be checked by digging wells or sinking bore-holes in the farms and by formulation of more ultra low volume (ULV) pesticides with hand sprayers.

x). Use of Resistant varieties - Farmers in the northern savanna seldom plant improved resistant varieties of maize and sorghum due to its high demand for fertilizers and good management practices before high yield is guaranteed. Again, local preferences (for example use of tall sorghum variety stems for fencing/building; or preference for red coloured to white sorghum) may restrict the adoption of resistant varieties in spite of its attendant benefits. Solution to this problem lies in effective extension services and mass literacy campaign to change farmer's orientation. Cost of purchase of resistant crop varieties must be farmer pocket- friendly

Logistic Problems in Crop Protection

Farmers in northern Nigeria seldom plan for their crop protection needs. Their pre-occupation is to clear and till the land, plant the seeds, apply fertilizers, remove unwanted vegetation and harvest the crop. This sequence has exposed a major problem inherent in our farm management system, at least, at the peasant farmer's level. It is only when crop protection problems surface (albeit when much harm have been done to the crop) that a 'fire brigade approach' to control pest is initiated. Crop protection problems such as pests, diseases and early cessation of rains should be anticipated and planned for, to ensure success.

Logistic problems have been recognized as a major hindrance to a virile and effective crop protection programme. For instance, farmers may recognize a problem situation in the field but may not understand its causes. This calls for training of more extension workers and sending them to the rural suburbs to assist farmers. Difficult field problems should be communicated to the research scientists for solution. Unfortunately, the number of trained extension workers is inadequate and ill-equipped. Most extension workers prefer living in the urban areas than staying in the rural areas where pipe-borne water and electricity are lacking. This situation can be reversed by training two extension workers from each village in northern Nigeria who will live among the rural people to render extension services to them.

Another logistic problem in crop protection is lack of monitoring group and ineffectiveness of plant quarantine and sanitation programmes in the country. To this end, it is suggested that the state governments in the northern savanna should as a matter of necessity establish pests and disease monitoring groups as well as effective plant quarantine laboratory in each state capital. At the national level, a system of quick response (within 48 hours) to a reported case of outbreak of pests and diseases should be adopted to salvage crops and prevent total failure and great loss to farmers.

Social Problem

Plant protection problems have its social aspect. For instance, an individual farmer cannot take effective measures against pests which ravage over a large territory; in which case a joint action with his neighbours is necessary. An example is illustrated with grass hopper (*Z. variegatus*) infestation. Preventive measures should be taken in the locations where the eggs are laid, such as burning old tree stumps, heaps of uprooted weeds and avoiding damp

places in general. Eggs are most effectively destroyed by raking them out so that they dry in the sun. Nest sites are comparatively rare, usually one or two per hectare (Amatobi, 1984). For effective control, the destruction of all nest sites over a large area by all farmers is necessary. One farmer acting alone has little effect, whereas joint action can reduce the succeeding population of grasshoppers by 70 – 80% (Amatobi, 1984).

Another social problem in crop protection is inadequate price incentives to farmers particularly when inclement weather strikes. Price incentive is necessary to guarantee farmers good reward for their efforts. Also, excess produce should be purchased by the state governments and stored as strategic grain reserves. All these would encourage farmers to produce more to feed the teeming population in the country.

CONCLUSION

Plant protection is currently considered as being synonymous with the use of pesticides whose utilization is the only barometer for ascertaining achievement in this respect. Other control methods which are relatively easy to adopt should be explored and exploited. A number of pests can effectively be checked by manipulation of cultural practices, for example, depth of planting, soil and water management, soil amendments (for nematodes), and dry season deep plowing for killing insects and pathogens in the soil. Growing maize and sorghum in areas where the environment is unsuitable for pests and diseases attack and where the crops have relative advantage for high yield potential is essential for good economic returns from the farm.

Besides, since the savanna ecological zones have diverse weather conditions and varied farming systems, pest and disease problems, the severity of their occurrence and control strategies differ according to conditions. By studying the occurrence and spread of pests and diseases in these agro-ecological zones for more than 3 years, a fairly good idea of the pest and disease situation can be worked out for that particular area.

Establishment of plant clinics at district or local government level, staffed with competent pathologists and an entomologists will ensure sound surveillance service with the aim of supplying technical assistance to the farmers and village extension workers in terms of diagnosis, control, correct use of pesticides and use of disease free planting materials.

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Genotoxicity of Dioxin and its Effect on the Immune Response of Goats Vaccinated with Brucella Melitensis Vaccine

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Abstract: The few cytogenetic studies performed in both humans and animals exposed in vivo or in vitro to dioxin reported conflicting data with or without chromosome damage. Therefore the aim of this work was to fulfill the genotoxicity of dioxin and its effect on immune response of sheep vaccinated with Brucella melitensis Rev.1 vaccine . A total number of twenty female goats were divided into four groups, each group consisted of 5 animals. The first group kept as control till the end of experimental period after 3 weeks post-treated. The second group was vaccinated with the Brucella Rev 1 vaccine. The third group was given an oral dose of 4 ml of stock standard solution of dioxin for 3 successive days. The fourth group was vaccinated with the Brucella Rev 1 vaccine and then given after that the dose of dioxin for each animal for 3 successive days. Blood samples were collected for detection of micronucleus, chromosome aberrations and Brucella antibodies titer. Both cytogenetic tests gave clear indications of high levels of chromosome damage in the dioxin treated group and dioxin vaccinated group compared with the control. Serological tests revealed decreased level of antibodies titer by both Tube agglutination test (TAT) and Mercaptoethanol test (MET) in vaccinated animals plus dioxin. In conclusion, dioxin may induce chromosome damage and lower the immune response of goats vaccinated with Rev.1 vaccine. The percentage of micronuclei and chromosomal aberrations decreased after vaccination with Rev.1 vaccine plus dioxin compared with dioxin alone. [Nature and Science. 2009;7(12):15-21]. (ISSN: 1545-0740).

Key words: Dioxin, Micronucleus, Chromosome aberrations, Rev.1 vaccine.

1. Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), commonly known as "dioxin" is formed as a byproduct in the manufacture or combustion of materials made of chlorinated phenols. It is considered to be one of the most potent man-made toxicants and is the prototype for a large class of halogenated aromatic hydrocarbons. Because of the demonstrated toxicity of TCDD and its environmental persistence, dioxin is considered to be a potential hazard to human health (Hays and Aylward, 2003). The toxicity of TCDD has been well characterized: exposure causes generalized wasting syndrome, involution of the thymus, hepatic toxicity, gastric lesions, sperm toxicity, tumor promotion, teratogenicity and embryocytotoxicity (Mimura and Fujii-Kuriyama, 2003, Fisher et al., 2005 and Fouzy et al., 2007).

Cytogenetic studies of persons living within dioxin-contaminated territories occupationally exposed to dioxin resulted in contradictory data (ATSDR, 1998, Zhurkov et al., 1987, Revazova et al., 2001, Iannuzzi et al., 2004 and Baccarelli et al., 2006). The micronucleus (MN) assay and chromosome aberration have been commonly used as a predictor of genotoxicity (Moore et al., 1995). The micronucleus test is widely employed in different areas in biological monitoring. It has become a tool to evaluate the mutagenic effect of drugs before they are commercialized (Masjedi et al., 2000 and Othman and Ahmed, 2004). Moreover, micronuclei have been shown to be a sensitive measure of chromosome damaging effect of environmental pollution (Amer et al., 1997).

Brucellosis is an endemic zoonotic disease in many parts of the world, notably in Mediterranean countries and the Middle East. The Brucella vaccine is considered the only practical method for controlling and eradicating of Brucella infection in small ruminants (Stournara et al., 2007). Since first developed in the mid-1950s, the Brucella melitensis vaccine strain Rev.1 has been used worldwide and its significant value in protecting sheep and goats in endemic areas was recognized (Banai, 2002). Suppression of primary humoral immune responses is one of the most sensitive sequela associated with exposure to TCDD, a ubiquitous environmental contaminant. This suppression

is characterized by a striking reduction in plasma cell formation and immunoglobulin M (IgM) secretion, and is mediated through a direct effect by TCDD on B cells (Holsapple et al., 1986; Sulentic et al., 1998). Previous studies in mice and B cell lines that differ in AHR (aryl hydrocarbon receptor) expression demonstrated the involvement of AHR in the suppression of humoral immune responses (Vecchi et al., 1983; Kerkvliet et al., 1990; Sulentic et al., 1998; Sulentic et al., 2000).

The impairment of the functional outcome of B cell differentiation, (*i.e.,* IgM secretion) by TCDD was previously shown to occur at TCDD concentrations that only modestly suppressed B cell proliferation, giving rise to the notion that TCDD impairs terminal B cell differentiation (Holsapple et al., 1986; Luster et al., 1988). However, little is known about the mechanism by which TCDD-mediated suppression of B cell differentiation occurs, and what other aspects of B cell differentiation, besides the IgM response, are impacted by TCDD treatment. In previous studies TCDD treatment of LPS-activated CH12.LX cells was shown to markedly reduce the mRNA levels of IgH, Igκ and IgJ as well as protein levels of XBP-1 **(**Yoo et al., 2004).

Although 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to influence immune responses, the effects of low-dose TCDD on the development of autoimmunity are unclear **(**Ishimaru et al., 2009). Therefore the aim of this work was to fulfill the genotoxicity of dioxin and its effect on immune response of sheep vaccinated with Brucella melitensis Rev.1 vaccine.

2. Materials and methods

2.1. Animals

Twenty mature female baladi goats (over 3 years old and about 30 kg live body weight) were used. Animals were kept under the routine mangemental system and fed on commercial concentrate mixture with rice straw and barseem *ad libitum*.

2.2. Dioxin Standard

The stock standard solution contained Pg WHO- TEQ (PCDD/PCDFS) of 17 congeners labelled with C13 and 17 native congeners at equal preparation. Total is 57.7826 Pg WHO. It was obtained from Freiburg, Germany (Rainer, 2002).

2.3. Brucella melitensis Rev.1 vaccine

Live attenuated Brucella melitensis strain. The recommended dose was 1x 10^9 colony forming units (cfu). It was obtained from Meral, Lyon, France.

2.4. Experimental Design

Goats were divided into four groups, each group consisted of 5 animals.

- The first group was kept as a control group till the end of experimental period lasted for 3 weeks post-treatment.
- The second group was vaccinated with the Brucella Rev 1 vaccine (2 ml for each animal).
- The third group was given an oral dose of 4 ml of stock standard solution of dioxin diluted with 5 ml distilled water , The amounts of stock standard solution of dioxin given to the goats were 6.9 µg which represent 0.23 µg /body weight and equal (1/3 of LD50) for guinea-pig (0.6 µg/kg body weight), Kociba et al. (1978) for 3 successive days .
- The fourth group was vaccinated with the Brucella Rev 1 vaccine and then given after that the dose of dioxin for each animal (4 ml of stock standard solution of dioxin diluted with 5 ml distilled water) for 3 successive days.

2..5. The in vitro micronuclei (MN) test

Blood samples were collected in vials containing heparin as anticoagulant. The in vitro micronuclei test with goat peripheral blood lymphocytes was carried out according to Fenech and Morley (1985). Whole blood cultures from the four groups were set up by adding 0.4 ml whole blood to 5 ml culture medium consisting of RPMI 1640 supplemented with 15 % fetal bovine serum, 2mM l-glutamine, antibiotics (100 units/ml penicillin and 100 µg streptomycin/ml) and 1.0 % phytohemagglutinin. Cytochalasin B was add to the cultures at 44 h post initiation at final concentration 5 µg/ml. 24 h later the cells were centrifugated, resuspended in hypotonic saline (75 mM KCL), centrifuged again and fixed twice in fixative (acetic acid and methanol 1:3) for 20 min. the cell suspension was dropped on wet slides and the air dried preparations were stained with 4 % Giemsa in Sorensen's buffer, pH 7.4. Scoring was done at 100 X magnification. 1000 binucleated cells/ experiment were counted for the presence of micronuclei. The data were statistically analyzed using Fisher exact test. Replicative index (RI), a measure of cell division kinetics was calculated by scoring 500 cell/sample , by counting the percent of cells containing 1,2,3 or more nuclei / individual.

RI= [(1x % mononuclear cells)+ (2x % bi) + (3 x% tri) + (4x % tetra)]/n.

2.6. Chromosomal aberrations

Blood samples were collected via sterile syringes from the four groups of goats. Lymphocyte cultures were prepared according

to Halnan (1977). Blood cells were cultured for 72 h at 38°C in 5 ml TCM-199, 1ml fetal calf serum and 0.1 ml phytohaemagglutinin (PHA). After incubation, cells were treated with colchicines (0.05%) for 2 h, then with a hypotonic (0.075M KCL) for 30 min. After fixation in acetic acid: ethanol (1: 3) solution, the cells suspension were dropped on wet slides then flammed to dry. The slides were stained with Giemsa stain and covered with DPX mounting media for chromosomal analysis. Chromosomal abnormalities were recorded in at least 100 metaphase spreads for each animal.

2.7. Serological examination for Brucella antibodies titer

Blood samples from vaccinated group and vaccinated plus dioxin group were collected and centrifuged at 3000 rpm /15 min. The obtained sera were kept at -20 ºC till used for detection of Brucella antibodies titer. Tube agglutination test (TAT) and Mercaptoethanol (MET) were made according to Alton et al. (1998) and Brucella antigens were supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt.

2.8. Statistical analysis

Data were subjected to statistical analysis according to Snedecor and Cochran (1982).

3. Results

The clinical symptoms of goat drenched dioxin (alone or with Brucella vaccine) were ranging from general depression, different degrees of inappetaness, poor body condition, pale mucous membranes, staggering gaits and respiratory manifestations.

A significant ($p<0.01$) increase in percentage of micronuclei in binucleated lymphocyte was observed in dioxin group than control (Table 1). In group of goats vaccinated and given dioxin, there was a significant ($p<0.05$) decrease in percentage of micronuclei than dioxin group.

Chromosomal aberrations in goat lymphocytes for all groups are presented in Table 2. The frequencies of chromosomal abnormalities increased significantly ($p<0.01$) in dioxin treated goat than control. The percentage reached 8.0 ± 0.51 in treated animals compared with 2.66 ± 0.58 for the control. The percentage of chromosomal aberration significantly ($p<0.05$) decreased in dioxin plus vaccinated group than dioxin group.

Serological examination of vaccinated goats with or without dioxin treatment using serological tests revealed decreased level of antibodies titer by both TAT and MET in vaccinated animals plus dioxin (Table 3). Results showed that the titer of antibodies by TAT significantly decreased in goats vaccinated and drenched dioxin (28±10.95) than in vaccinated (72±16). Meanwhile the titer of antibodies by MET showed non significant decreased in animals vaccinated and drenched dioxin (24±8.94) than in vaccinated (36±8.94).

Table (1): Percentage of micronuclei (MN) in binucleated goat blood lymphocytes vaccinated with Rev.1 vaccine and treated with dioxin.

Treatment	Number of Exp	No of binucleated cells	No of MN in binucleated cells	% of MN in binucleated cells ± S.E
Control	6	6000	32	0.53 ± 0.43
Vaccine	18	18000	109	0.60 ± 0.55
Dioxin	6	6000	194	3.23 ± 0.45**
Dioxin + Vaccine	10	10000	188	1.88 ± 0.31●

** Highly significant P< 0.01 comparing to control. ● Significant P< 0.05 comparing to dioxin.

Table (2): The types and mean percentage of chromosome aberrations in cultured goat blood lymphocytes vaccinated with Rev.1 vaccine and treated with dioxin.

Treatment	Number of Exp	Number of metaphases	Number of abnormal metaphases	Chromosome aberrations (Mean % ± S.E) without gaps	Number of abnormal metaphases			
					Gaps	Fragment and / or break	Deletion	polyploidy
Control	6	300	14	2.66 ± 0.58	6	4	4	-
Vaccine	18	900	46	3.55 ± 0.54	14	26	6	-
Dioxin	6	300	32	8.0 ± 0.51**	8	14	2	8
Dioxin + Vaccine	10	500	39	5.80 ± 0.42●	10	22	3	4

** Highly significant P< 0.01 comparing to control (T-test). ● Significant P< 0.05 comparing to dioxin (T-test).

Table (3): The titer of antibodies in vaccinated and/or vaccinated plus dioxin groups by using the serological tests.

	Tube agglutination test (TAT)		Mercaptoethanol test (MET)	
	Vaccinated	Vaccinated and drenched dioxin	Vaccinated	Vaccinated and drenched dioxin
The titer of antibodies	72* ± 16	28 ± 10.95	36 ± 8.94	24 ± 8.94

***** Significant P< 0.05 comparing to vaccinated and drenched dioxin by the same test.

4. Discussion

In the present study, dioxin exposed goats show mild signs of adverse healthy conditioned. Fouzy et al. (2007) reported similar findings in goats. Such clinical signs could be due to appetite suppressive effect of TCDD which related to its feedback mechanism originating in the periphery and not to a direct effect on appetite-regulating areas of the brain (Stahl and Rozman, 1990).

The results of this study showed a significant increase in percentage of micronuclei in binucleated lymphocyte in dioxin and dioxin with vaccine groups. Micronuclei represent whole chromosomes or chromosome fragments that have been lost from the cell nucleus during mitosis or meiosis (Kirsch-Volders et al., 1997 and Junk et al., 2002). Heddle et al. (1991) suggest that micronuclei may form by one of four basic mechanisms:1) mitotic or meiotic loss of an acentric fragment; 2) a variety of mechanical consequences of chromosomal breakage and exchange; 3) mitotic or meiotic loss of whole chromosomes; 4) as a result of apoptosis. In this respect, Patterson et al. (2003) found that induction of apoptosis was accompanied by dioxin exposure.

Our data demonstrate that, there is a significant increased in structural and numerical chromosomal aberrations in dioxin treated group and group vaccinated with dioxin. Similarly, Perucatti et al. (2006) cytologically examined two herd of sheep with high levels of dioxins in the milk (50.65 and 39.51 pg/g of fat, respectively). Increases of both chromosome abnormalities (gap, chromosome and chromatid breaks) (17 and 8 times higher in the two exposed herds, respectively). Also, Iannuzzi (2004) recorded a significant percentages of chromosomal aberrations in the same two herds exposed to lower levels of dioxins (5.27 pg/g). Bertazzi et al. (2001) reported cytogenetic abnormalities in human and found to be linked to TCDD exposure. Ingel et al. (2001) found high level of correlation between emotional stress and individual dioxins blood contents (up $P \leq 0.001$) as well as between emotional stress and individual chromosome aberration level (up $P \leq 0.05$). In contrast, Revazova et al. (2001) found no personal correlation related to dioxins exposure in human by chromosome aberrations and micronuclei.

The result of micronucleus assay coincide also with chromosome aberrations in inducing

DNA damage. TCDD-induced oxidative stress and DNA damage may, in part, contribute to TCDD-induced carcinogenesis (Lin et al., 2007). The group of vaccinated animal with dioxin has decreased rate of chromosomal abnormalities than group of dioxin alone. The primary explanation for these effect could be the increasing general immune response of the animals due to vaccine lead to decrease the chromosomal damage. Gupta et al. (2007) cited that increasing immune response of goats vaccinated with brucella melitensis vaccine. But TCDD induce oxidative stress (Jin et al., 2008) which contribute to DNA damage (Lin et al., 2007).

In this study, we used Brucella vaccine, which is live attenuated bacterial vaccine, as a model of bacterial infection and estimation of the immune response due to TCDD exposure. Our results demonstrate that the Dioxin markedly suppresses the humoral immune response in the form of decreased titer of antibodies in the serum of goat experimentally exposed to Dioxins. Previous studies have demonstrated that the suppression of humoral immune responses is one of the most sensitive sequela associated with TCDD exposure. They demonstrated that B cells are directly targeted by TCDD (Holsapple et al., 1986; Sulentic et al., 1998) and that the AHR is required for suppression of the IgM response (Vecchi et al., 1983; Kerkvliet et al., 1990; Sulentic et al., 1998). However, the molecular mechanism responsible for the suppression of humoral immune responses by TCDD remains undeciphered. Collectively, these studies demonstrate that the suppression of the IgM response by TCDD is due to the impairment of B cell differentiation by dysregulation of Pax5 resulting in high-level expression of the Pax5a isoform, a potent repressor of XBP-1, IgH, Igκ and the IgJ chain (Yoo et al., 2004). Pax5 is known to induce genes responsible for the mature B cell phenotype, while suppressing genes involved in their terminal differentiation into plasma cells. Consequently, suppression of Pax5 promotes the terminal B cell differentiation program (Nera et al., 2006).

With respect to the two tests used in our study, Several serological tests have been used for detecting specific serum antibodies of brucellosis. The tube agglutination test (TAT), Rose Bengal test, Mercaptoethanol (MET), complement fixation test, indirect Coombs test, enzyme immunoassay (ELISA) and, more recently, an immunocapture-agglutination test (Díaz and Moriyon, 1989, Orduña et al., 2000 and Rubio et al., 2001). However, the interpretation of differences in results among these tests is due to every test depend on specific type of immunity.

In conclusion, dioxin may induce chromosome damage and lower the immune response of goats vaccinated with Rev.1 vaccine. The adverse effect of dioxin on chromosomes decreased in vaccinated animals.

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PHYTOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL: THE EFFECT OF GROWTH OF *Glycine max* ON THE PHYSICO-CHEMISTRY AND CRUDE OIL CONTENTS OF SOIL

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Abstract
The remediation of oil contaminated soils has been a major problem in oil producing countries and recently use of plants to clean such soils has been on investigation. In order to identify plants that can enhance the remediation of crude oil contaminated soil, the effect of the growth of *G. max* on the physico-chemistry and crude oil content of soil contaminated with different concentrations of crude oil was investigated in this study. The results revealed that the pH, moisture and organic matter contents of soils contaminated with crude oil were significantly affected by the growth of *G. max* at differently levels of significance ($P<0.001$, $P<0.01$ and $P< 0.05$). Crude oil loss was enhanced in soil with 25g crude oil in the presence of *G. max*. Although the growth of the *G. max* did not significantly affect the crude oil level in the 50g and 75g treatments, the soils became more favourable for plant growth as weeds sprouted from the contaminated soil vegetated with *G. max*. The implication of the findings of this study is that within 110 days, growth of *G. max* can lead to cleanup of crude oil contaminated soil and the reduction in toxicity of crude oil in soil. The ability of *G. max* to reduce the level of crude oil in oil polluted soil can help to restore polluted soils back for agricultural use. The high acceptability of *G. max* due to its high nutritional value, high adaptability and ease of propagation will make it an easy tool for remediation of soil contaminated with crude oil. [Nature and Science. 2009;7(12):22-30]. (ISSN: 1545-0740)

Keywords: Contamination, Crude oil, *Glycine max*, Cleanup, pH, Moisture, Organic matter

Introduction

Since commercial exploration of petroleum started in Nigeria in 1958 (Okoh, 2003), petroleum has continuously grown to be mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and water ways. The agricultural lands have become less productive (Dabbs, 1996) and the creeks and the fishing waters have become more or less dead (Okpokwasili and Odokuma; 1990; Odokuma and Ibo, 2002). Several civil unrests due to environmental degradation due oil exploration have also been witnessed in the Niger Delta region of Nigeria (Inoni *et al*., 2006).

The physical, chemical and thermal processes are the common techniques that have been involved in the cleaning up of oil contaminated sites (Frick *et al*., 1999). These techniques however have some adverse effects on the environment and are also expensive (Frick *et al*., 1999; Lundstedt, 2003). Recently, biological techniques like phytoremediation are being evaluated for the remediation of sites contaminated with petroleum. Phytoremediation is the use of plants and/or associated microorganisms to remove, contain or render harmful material harmless (Cunningham *et al*., 1996; Schwab and Banks, 1999; Merkl, 2005). It has been shown to be effective for different kinds of pollutants (contaminants) like heavy metals, radionuclides and broad range of organic pollutants (Schroder *et al*., 2002; Schnoor, 2002) According to Pivetz (2001), plants for phytoremediation should be appropriate for the climatic and soil conditions of the contaminated sites. Such plants should also have the ability to tolerate conditions of stress (Siciliano and Germida, 1998a). Njoku, *et al.,* (2008a) demonstrated that *G. max* germinates and grows in crude oil polluted soil. Also Frick *et al.* (1999) included *G. max* in the list of plants that can grow and remediate petroleum hydrocarbon contaminated sites. However, no record has shown that *G. max* can remediate crude oil polluted soil. It is therefore important to study the ability of *G. max* to affect the physico-chemistry (pH, moisture and organic matter contents) and the crude oil content of soil polluted with crude oil. The overall goal of this investigation was to evaluate the suitability of *G. max* for use in remediation of crude oil polluted soil.

The study is significant for some reasons. Firstly, phytoremediation has mostly involved the use of weeds (Aprill and Sims, 1990; Lee and Banks, 1993; Schwab and Banks, 1994; Qui *et al*., 1997; Banks *et al*., 2000). The use of food crops will improve the economic value of the technique (Van de Lelie *et al*., 2001). Secondly, although the conditions in the tropics favour phytoremediation, few researches have been carried on this technique in the tropics (Gallegos Martinez *et al*., 2000; Merkl *et al*, 2005a). There is the need therefore to evaluate the potentials of phytoremdiation in the tropics especially in Nigeria where pollution due to oil activities is high. In addition, the high nutritional value of *G. max* makes it acceptable by many and Njoku *et al*., (2008b) reported that *G. max* has the potential of growing in sandy loam soil, a soil type found in Niger Delta region of Nigeria.

Materials and methods

This study was carried out in the Biological garden of the University of Lagos, Akoka Lagos, Nigeria. The crude oil (Wellhead medium) was obtained from the SPDC Port Harcourt while the *G. max* was obtained from the Gene Bank Section of IITA Ibadan, Nigeria. The soil used is sandy loam soil and the treatments included 25g, 50g, and 75g crude oil mixed with 4000g of the soil filled in plastic containers. For each treatment, the control had no *G. max* grown on it. Both the treatments and the control were replicated thrice. Seven seeds of *G. max* were sown into each of the containers at 2cm depth and the containers were moderately watered regularly to keep the soils moist.

Soil samples were collected at the surface and 15cm depth from each container every 21 days (3 weeks) for 105 days (15 weeks). The collected soil samples were used to investigate the effect of *G. max* on the pH, moisture and organic matter contents of crude oil polluted soil. The soils from the surface and 15cm depths were usually mixed together and the mixture used for the study of the above physico-chemical features.

Result and Discussion

The growth of *G. max* generally reduced the acidity of the crude oil polluted soil. However on days 21 and 42, the growth of *G. max* led to increase in the acidity of crude oil polluted soil (Table 1). On days 21 and 63, the pH of the control differed significantly from those of soil

The soil samples used in the study of the effect of G. *max* on the crude oil content of the soil were collected on the 110th day of sowing of the seeds of *G. max* in the soils.

The pH of the homogenized soils was determined following the protocols outlined by Eckerts and Sims (1995). The soils were air-dried and sieved to remove large particles and debris. 5g of the sieved soils were mixed with 5mls of distilled water and stirred very well after which mixture was allowed to stand for 30 minutes. The electrode of a pH meter was put into slurry of the soil-water mixture and the pH of the soil was read off. The moisture content of the soil samples was determined according to the method of Schneekloth *et al*. (2002). The procedure of Schulte (1995) was used to determine the organic matter content of the soil samples.

The amount of crude oil the soil samples was determined using air-dried soils that were sieved through 1mm mesh. The crude oil in the soil was first extracted with n-hexane by shaking with a mechanical shaker for 30 minutes as was described by Okolo, Amadi and Odu (2005). The soil-crude oil-n-hexane mixture was filtered into a beaker of known weight through a Whatmann No.1 filter paper. The crude oil content of the filtrate was determined after heating the beaker at 40^{o}C to a constant weight (Merkl, Schutze-Kraft and Infante, 2005b). The amount of crude oil lost from the soil was determined as the amount of crude oil added to the soil minus that in the soil at the time of analysis.

The effect of *G. max* on the pH, moisture, organic matter and crude oil contents of the soils was determined by comparing each parameter in soil with *G. max* with that in soil with *G. max.* Statistical analyses of the data obtained were done using Graphpad Prism 5.0 package using a 2 way ANOVA followed by Bonferroni posttests at 5%, 1% and 0.1% significance levels. Correlation analyses were also carried out.

with 50g crude oil and *G. max* ($t = 2.701$ for day 21 and $t = 3.696$ for day 63) and those of the soil with 75g crude oil and *G. max* ($t = 2.985$ for day 21 and $t = 3.838$ for day 63). Negative correlations exist between the pH of soils with *G. max* and soils without *G. max* for each concentration of crude oil ($p = 0.350$, 0.083 and 0.683 for 25g, 50g and 75g crude oil

concentrations respectively). A perfect positive correlation exists between the pH of the soil with 50g crude oil and the soil with 75g crude oil ($p = 0.017$) while no correlation exists the soils with 25g crude oil and *G. max* and 75g crude oil and *G. max.*

The positive correlation between the pH of the soils and the amount of crude oil added to the soil may be an implication that crude oil pollution leads to increase in soil pH. This is similar to the findings of Andrade *et al*. (2004) and Ayotamuno *et al.* (2004) who observed increase in the pH of soils polluted with crude oil. In the opinion of Dibble and Bartha (1979), the higher pH of soils with *G. max* than in soils without *G. max* means that higher degradation of crude oil took place in soil with *G. max* than in soils without G. max. The trend of the pH over the period of studies was against the expectation going by the reports of Ayotamuno *et al.* (2004) and Merkl *et al*. (2005c). These researchers reported that the pH of soils decreased as a result of degradation of crude oil. This decrease in the pH of soil with degradation of crude oil could be due to accumulation of organic acids produced during degradation in the soil (Merkl *et al*., 2005a) or the production of acid radicals through nitrification (Tisdale and Nelson, 1975). However, since soil bacteria thrive better in neutral than in acidic soils (Song *et al*., 1986; Phung, 1988), the increase of the soil pH towards neutral condition means more favourable conditions for soil bacteria. Many researchers have reported that bacteria play good role in the degradation of crude oil (Atlas and Bartha, 1977; Amund and Igiri, 1990; Frick *et al*., 1999; Van Hamme, Singh and Ward, 2003). This means that as observed in this study, growth of *G. max* can enhance the bacteria population in crude oil polluted soil and thereby lead to higher degradation of crude oil in the soil. The continual increase in the soil pH as the period of the study increased means that there was continual increase in favourable conditions soil bacteria and for biodegradation (Dibble and Bartha, 1979).

Table 1: The effect of *G. max* on the pH of crude oil polluted soil. Values are means ± standard error of three replicates

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	4.73±0.233	5.30± 0.115	5.37±0.067	5.87±0.186c	5.37±0.067a	5.50±0.153b	5.60±0.153a
42	5.03±0.176	5.30±0.173	5.23±0.067	5.57±0.145	5.37± 0.033	5.07±0.120	6.23±0.433□
63	5.07±0.088	5.17±0.067	5.33±0.333	5.37±0.233	5.77±0.176a	4.97±0.088	5.97±0.240a□
84	5.03±0.067	5.13±0.088	5.37± 0.067	5.37±0.203	5.80±0.231	4.97±0.133	5.97±0.186□
105	5.00±0.115	5.13±0.033	5.37±0.120	5.37±0.233	5.87±0.233	4.97±0.088	5.97± 0.203□

Note: a = significant difference between treatment and control at $p<0.05$ significant difference between treatment and control, b= significant difference between treatment and control at $p<0.01$, c = significant difference between treatment control at $p<0.001$, * = significant difference between soil with *G. max* and soil without *G. max* at $p<0.05$,+ = significant difference between soil *G. max* and soil without *G. max* at $p<0.01$, □= significant difference between soil *G. max* and soil without *G. max* at $p<0.001$

The growth of *G. max* in soils polluted with 25g crude oil led reduction of the moisture content of the soil. The reverse was the case for the soils with 75g crude oil. In the case of the soils with 50g crude oil, the growth of *G. max* led to reduction of the moisture in the first 42 days and afterwards the growth of *G. max* enhanced the moisture content of the soil (Table 2). The control has positive correlation with the treatments and there is a positive correlation among the treatments.

Crude oil pollution causes among other things low permeability and low infiltration of water into the soil (Hutchinson *et al*., 2001; Andrade *et al*., 2004). These conditions can lead accumulation of water on the soil surface and an artificial drought in the subsurface layer of soil. This can lead to difficulty for the roots to absorb water and nutrients which in the water as the roots usually grow deeper into the soil subsurface layers. The growth of plant root into soil help to create pores in the soil and thereby enhance water penetration and infiltration in soil polluted with crude oil. This increased water penetration and infiltration could be the cause of low moisture contents of soil contaminated with 25g crude oil and that had *G. max* grown on it as observed in this study. This can help to eliminate water logging of crude oil polluted soil and can lead to increased aeration of the soil. The increased aeration can lead to increase in the activities aerobic microbes in the soil and this can lead to increase in the degradation of oil.

Since the phytotoxic effect of crude oil increases with the concentration of the crude (Cullie and Blanchet, 1958), the higher moisture content of the soil with 75g crude oil and *G. max* than in the soil with 75g crude oil and no *G. max* could be due to inhibition of root growth by such amount of crude oil. The inhibition of root growth can lead to low penetration of water and higher accumulation of water on the soil surface. Reduction of transpiration is one of the phytotoxic effects of crude oil (Baker, 1970). The reduction in transpiration also affects the rate at which water absorption and uptake as these are controlled by transpiration pull (Taylor *et al*., 1997; Kent, 2000). Therefore higher moisture content in the soil with 75g crude oil and *G. max* than in soil with 75g crude oil and no *G. max* could be attributed to reduced loss of water due transpiration and subsequent reduction in the rate of water absorption in such soil. A possible cause of the difference between the trend of moisture content in the soil with 25g crude oil and soils with 50g and 75g crude oil is that because better growth of *G. max* in soil with 25g crude oil led to more absorption of water from the soil than from soils with 50g and 75g crude oil.

Table 2: The effect of *G. max* on the percentage moisture content of crude oil polluted soil. Values are means ± standard error of three replicates.

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	13.04±0.211	13.50±1.381	12.24± 0.701	9.97± 0.573	10.31± 0.693	5.20±1.743	12.59± 0.763□
42	11.06±0.647	13.59±0.935	12.42± 0.739	10.05±0.427	11.12±1.832	3.34±0.975	13.32± 0.978□
63	13.16±0.230	14.03±0.420	13.06± 0.502	12.41±0.290	15.41±0.188	8.19±1.236	15.85± 0.593□
84	13.43±0.578	14.05±0.677	13.12± 0.430	12.74±0.133	15.52±0.133	8.16±1.196	15.97±0.477□
105	15.06±0.920	14.07±0.580	14.40± 0.534	13.66±1.420	16.45±0.423	8.86±0.700	14.84±0.629□

Note: a = significant difference between treatment and control at $p<0.05$, b= significant difference between treatment and control at $p<0.01$, c = significant difference between treatment control at $p<0.001$, * = significant difference between soil with *G. max* and soil without *G. max* at $p<0.05$,+ = significant difference between soil *G. max* and soil without *G. max* at $p<0.01$, □= significant difference between soil *G. max* and soil without *G. max* at $p<0.001$

The organic matter content of the soil was reduced by the growth of *G. max* in the first 42 days (Table 3). This might be due to the use of growth of *G. max* in the first 42 days might be as a result of the use of the organic matter by the *G. max* as it grew. Since the plants were in their early growth stages, they could possibly be absorbing nutrients from the soil and returning little or none to the soil. Such could have caused lesser accumulation of organic matter in the vegetated soil than in non-vegetated soil. Ayotamuno *et al*. (2004) reported similar

observation of lower organic matter contents in vegetated soil.

From day 63, the growth of *G. max* enhanced the accumulation of organic matter in the soils. The observed higher organic matter accumulation in vegetated soil as from day 63 has some interpretations. Firstly, it is possible that *G. max* started shedding its leaves from after the first 42 days and the decomposition of such leaves increased the organic matter content of the vegetated soil more than that of the non-vegetated soil. The release of organic carbon to the soil due to degradation of crude oil possibly led to accumulation of more organic matter in the vegetated soil than in the non-vegetated soil. This is because organic carbon is a major component of organic matter (Okolo *et al*., 2005). Also the fixation activities in the root nodules of the plant also had a possible impact on the amount of organic matter accumulated in the vegetated soil.

The organic matter content of the soils has negative correlation (p = -0.237) with the days of sampling and positive correlation (p = 0.767) with the amounts of crude oil added to the soil. This means that while the organic matter contents of soil polluted with crude oil decreases with time, it increases with the quantity of crude oil added to soil. Apart from the 25g treatment, the soil organic matter was significantly affected by the addition of crude oil and growth of *G. max* at different levels of significance (P<0.001, P<0.01, P<0.05) for the different days of study. The growth of *G. max* however did not produce any significant effect on the organic matter content within each concentration of crude oil. There was negative correlation between the organic matter content of the control and the treatments. The 25g and 50g treatments have a perfect correlation (± 1) and same applies to the 25g and *G. max* and 50g and *G. max* treatments

Table 3: The effect of *G. max* on the percentage organic matter content of crude oil polluted soil. Values are means ± standard error of three replicates.

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	0.89±0.118	1.95± 0.529	1.55±0.041	2.47±0.176	2.14 ±0.284	3.16±0.180	2.54± 0.170□
42	1.29±0.651	1.53± 0.073	1.15±0.367	2.22±0.115	1.65± 0.103	2.70±0.306c	2.21± 0.111□
63	0.90±0.096	0.96± 0.060	1.14±0.042	1.67±0.111	1.63±0 .071	2.05±0.140c	1.97± 0.119□
84	0.91± 0.096	0.99± 0.018	1.29±0.168	1.70±0.124	1.66 ±0.100	1.87±0.204c	1.98± 0.127□
105	0.91± 0.142	1.35±0.066	1.33±0.123	1.88±0.140	1.78 ±0.061	1.91±0.228c	1.95± 0.030□

Note: a = significant difference between treatment and control at p<0.05, b= significant difference between treatment and control at p<0.01, c = significant difference between treatment control at p<0.001, * = significant difference between soil with *G. max* and soil without *G. max* at p<0.05 ,+ = significant difference between soil *G. max* and soil without *G. max* at p<0.01, □= significant difference between soil *G. max* and soil without *G. max* at p<0.001.

The effect of crude oil the pH, moisture and organic matter content of soil observed in this study conforms with the reports of Njoku *et al*., (2008c) that these change with addition of crude oil to soil. Soil pH, soil moisture and soil organic matter contents have influence on the soil properties. The organic matter content of soil improves the binding processes in the soil. Such binding reduces water drainage and improves water retention ability of soil. Therefore the low organic matter in soil with 25g crude oil could be a cause of the low water accumulation in that soil. Excess binding of the soil particles together reduces root penetration and inhibit the absorption of materials. This can lead to malnourishment of plants even in the presence of abundant nutrients.

The amount of crude oil lost from the soil contaminated with 25g crude oil was enhanced by the growth of *G. max*. However in soils with 50g and 75g crude oil, more crude oil was lost

from soils without *G. max* than in soils with *G. max* (figure 1). It is however worthy to note that in this study weeds were observed to have sprouted out from the contaminated soils with *G. max* and none of such was observed in the non-vegetated soil. This shows that even though the growth of *G. max* did not produce any significant effect on the percentage of crude oil lost from the soils the plant can reduce the quantity and toxicity of crude oil in soils. This is shown by the lesser amount of crude oil left in soil with 25g crude and *G. max* than in soil with same amount of crude oil and no *G. max* and the sprouting of weeds from the soils with 50g and 75g crude oil and *G. max*. The sprouting of the weeds indicates that the toxicity of crude oil in the vegetated soils reduced to the extent of allowing for the growth of weeds in such soils. This confirms the findings of Siciliano and Germida (1998a) that plants may not reduce the concentration of contaminants and yet can reduce the toxicity of such contaminants. For example, Siciliano and Gemida (1998a), observed a reduced toxicity of 2,3-dichlorobenzoic acid and 3-chlorobenzoic acid without reduction in the contaminant concentration in vegetated soil. The reduction is also a mechanism of phyoteremediation going by the definition of phytoremediation as a technique of rendering harmful materials harmless using plants and their associated microbes (Cunningham *et al*. 1996; Pivetz, 2001). Conversely, the absence of such weeds from soils without *G. max* indicates that the soil has not reach the level that will enable plants to grow.

The effect of *G. max* on the removal of crude oil from the soil polluted with 25g crude oil is similar to the findings of Aprill and Sims (1990), Lee and Bank (1993), Schwab and Banks, (1994) and Merkl *et al*. (2005b) who reported higher degradation of petroleum hydrocarbon in vegetated soils than in non-vegetated soil. The higher removal of crude oil observed in this study conforms with the reports of Frick *et al*. (1999) who listed *G. max* as one of the plants that can remediate petroleum hydrocarbon (anthracene) polluted soil. It also conforms with the suggestions of Njoku *et al*. (2008b) who suggested that *G. max* can be tried for its efficacy to remediate crude oil polluted soil. The removal of crude oil by *G. max* possibly occurred through one of the several mechanisms of phytoremediation. Such mechanisms include polymerization of the contaminants (Adler *et al*., 2004), interaction of the plant with fungi and bacteria (Siciliano and Germida, 1998) and production of root exudates and plant materials which serve as source of carbon, nitrogen and phosphorus for petroleum degrading microbes (Horvath, 1972; Rajaram and Sethunathan, 1975; Alexander, 1977; Smith, 1990; Burken and Schnoor, 1996). Nitrogen fixed in the soil by legumes reduces plant/microbes competition for nitrogen and thereby increase plant growth exudates production. This increases the ability of plants to increase the degradation of pollutants.

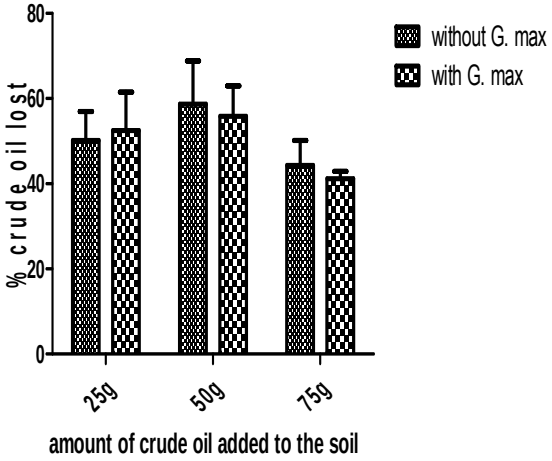

Figure 1: The effect of *G. max* on the removal of crude oil from polluted soil. Values are means ± standard error of three replicates.

Conclusion

The findings of this study indicate that growth of *G. max* in crude oil contaminated soils affects the physico-chemistry of the soil enhancing the degradation of crude oil. For instance, the significant effect that the growth of *G. max* produced on the pH and moisture content of the soil with 75g crude oil indicates that *G. max* affects the physico-chemistry of crude oil contaminated soil. It can also be inferred from the findings of this study that the growth of *G. max* in crude oil contaminated soils reduces the toxicity of crude oil in the soil. This is going by the sprouting of weeds in the soils with *G. max* and none of such soils without *G. max*. We suggest that to soil augments like cow dung should be added to crude oil contaminated soil to enhance the increase the efficacy of using *G. max* in remediating crude oil contaminated soils as Njoku *et al.* (2008a) have reported that

addition of cow dung to crude oil contaminated soils enhances the growth of *G. max* in such soil.

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An easy experiment for dark matter

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Abstract: Physicists have computed that this universe was born nearly 13.7 millions years ago. It is generally believed that singularity / big bang caused the origin of this universe. After big bang, the forces were formed and matter came to existence. There are three different forms of matter: i) solid, ii) liquid and iii) gas. According to Einstein's special relativity, matter and energy are equivalent. Whenever energy is transformed into mass, shape and volume it is called matter. Matter is made up of tiny particles like electrons, protons and neutrons. Matter, antimatter and dark matter are the three kinds of matter. Antimatter is also made up of particles such as electrons, protons and neutrons but having negative sign. Perseverance of antimatter is experimentally existent but very expensive. The nature and the properties of dark matter is still a burning issue in physics. There are many conjectures, hypotheses and so called theories for dark matter. But it is mathematically manifested. Several experiments have been performed to deduct dark matter. Unfortunately no experiment is able to establish the existence of dark matter. All those attempted tests are very difficult to carry out and grasp. On the other hand, in this brief work the authors propose an easy laboratory test for the detection of dark matter. [Nature and Science 2009;7(12):31-32]. (ISSN: 1545-0740).

Key words: Big bang, matter, antimatter, dark matter and experimental establishments.

PACS: 47.27.Ak, 95.36+x, 87.64 mf, 95.35+d, 98.80 Cq

Introduction

Physical theories demonstrated beyond any doubt about the properties of matter. Regarding antimatter, many concrete results have been found. The existence of dark matter is proved only by mathematical equations but it lacks experimental verification. In physics, experiment is the supreme judge. So far only proposals have been proposed. And they are not scientific but philosophical. Physicists generally only believe that dark matter is made up of dark particles and dark energy. Their blind belief is that dark matter came into existence along with matter. They have a calculation that from 30% to 95% of this universe is constituted by dark matter. This dark matter is generally believed that it does not emit light and never interacts with electro magnetic force. In the following lab test the authors attempt to appraise the dark matter.

Experiment

Choose a convenient dark room whose roof is made up of tiles. Make an artificial hole by slightly rearranging a tile. If this dark room is facing north, the convenient time for doing this experiment is between 8.00 am to 9.00 am or 4.00 pm to 5.00 pm. during sun light. This test mainly depends on climatic conditions. Particularly the sun should be visible and bright to the naked eye. Choose an ideal time and lock the doors and windows of the above mentioned room. While the sun's light rays moves from top to bottom in the dark room, along the light path countless number of very tiny particles can be easily seen. For this viewing, no sophisticated equipments/apparatus are required. What are the physical phenomena of this result?

Discussion

Several difficult and costliest experiments have been proposed in the past by top experimentalists, astro physicists, astronomers and organizations [1-35]. But these tests never produced positive results. These experiments did not directly detect or denote dark matter. Only they have hypothised and guessed. In our experiment, the pin pointed tiny particles might be **clusters of dark matter** [9 & 24]. If these particles are not clusters of

dark matter, it must be either superstrings or gravitons. Let us note that superstrings or gravitons dominate the whole of quantum gravity. And till this date, there is no experimental evidence in support of both superstrings or gravitons. The authors politely believe that the experimentally invented particle can not belong to a third category.

Conclusion

The visible of particle in the path traveled by the sun light in the dark room is very bright for the naked eyes. These particles may be dark matter or dust particles. Future studies will decide this.

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Matched Quick Switching Variable Sampling System with Quick Switching Attribute Sampling System

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Abstract: This paper presents a methodology for finding a Quick Switching Single Sampling variables inspection system matching a given Quick Switching attribute sampling inspection system. Here matching implies the same Acceptance Quality Level (AQL) $(p_{0.95}, 0.95)$ and Limiting Quality Level (LQL) $(p_{0.10,} 0.10)$ of the Operating Characteristic (OC) curves for both the systems. [Nature and Science 2009;7(12):33-39]. (ISSN: 1545-0740).

Key words: Operating Characteristic Curve, Acceptance Sampling, Acceptance Quality Level and Limiting Quality Level

1. Introduction

Hamaker (1979) has given a method of constructing single sampling variables inspection plans such that the OC curve of a given single sampling attributes inspection plan and the OC of the variables plan have the same indifference quality level (p_0) and the same relative slope (h_0) of the OC curve at p_0. Bender (1975) has given a table for single sampling variables inspection plans matched at the points $(p_{0.95}, 0.95)$ and $(p_{0.10,} 0.10)$ with attributes inspection plans given in Table II-A of MIL-STD-105D (1963). Bender (1975) achieves this matching by means of an iterative computer program involving non central t-distribution.

Romboski (1969) has studied a new system, comprising of normal and tightened plans, called Quick switching system (n; c2,c1) [(n,c_2) and (n,c_1) are normal and tightened single sampling plans respectively with $c_2 > c_1$] proposed by Dodge (1967). Taylor (1996) investigated how to evaluate and select Quick Switching Systems. Soundarajan.V and Palanivel.M (1997) and Soundarajan.V and Palanivel.M (2000) has investigated on Quick Switching Variables Simple Sampling (QSVSS) Systems.

2. Operating Procedure

Step 1: From a lot, take a random sample of size n and count the number of defectives, d

i) If $d \le c_2$ accept the lot and repeat the step 1 for the next lot.
ii) If $d > c_2$ reject the lot and go to step 2.

Step 2: From the next lot, take a random sample of size n and count the number of defectives, d

i) If $d \le c_1$ accept the lot and go to step 1repeat the step 1 for the next lot.
ii) If $d > c_1$ reject the lot and repeat the step 2.

Based on this procedure, a quick switching single sampling variables inspection system can be operated as follows:

Step 1: Draw a sample of size n_σ from the lot, inspect and record the measurement of the quality characteristic for each unit of the sample. Compute the sample mean $\bar{x}$

If $\bar{x} + k_{N\sigma}\sigma \le U$ (where U is the upper specification limit) accept the lot and repeat step 1 otherwise, reject the lot and follow step 2.

Step 2: Draw a sample of size n_σ from the next lot, inspect and record the measurement of the quality

characteristic for each unit of the sample. Compute the sample mean $\bar{x}$,

If $\bar{x} + k_{T\sigma}\sigma \le U$ accept the lot and go to step 1 otherwise, reject the lot and repeat step 2.

3. Preliminaries

According to Romboski (1969) the OC function of Quick Switching Single Sampling Attributes System (n;c2,c1) is given by

$$P_a(p) = \frac{P(d \le c_1)}{1 - P(d \le c_2) + P(d \le c_1)} \quad (1)$$

By assuming the Poisson model, one obtain

$$P(d \le c_1) = \sum_{i=0}^{c_1} \frac{\exp(-np)(np)^i}{i!}$$

$$P(d \le c_2) = \sum_{i=0}^{c_2} \frac{\exp(-np)(np)^i}{i!}$$

and $P_a(p_1) = 0.95$ & $P_a(p_2) = 0.10$ (2)

For a quick switching single sampling variables inspection system QSVSS $(n_\sigma; k_{N\sigma,} k_{T\sigma})$ [$(n_\sigma, k_{N\sigma})$ and $(n_\sigma, k_{T\sigma})$ are the normal and tightened single sampling plans respectively, with $k_{T\sigma} > k_{N\sigma}$] with known standard deviation (σ), the fraction non-conforming in a given lot will be P=F(-v)

with $v = \frac{(u - \mu)}{\sigma}$ (3)

and the OC function of the system has been given by

$$P_a(p) = \frac{P(Z_u \ge k_{T\sigma})}{1 - P(Z_u \ge k_{N\sigma}) + P(Z_U \ge k_{T\sigma})} \quad (4)$$

where

$$P(Z_u \ge k_{N\sigma}) = \phi(w_1), \quad P(Z_U \ge k_{T\sigma}) = \phi(w_2)$$

with

$$w_1 = (v - k_{N\sigma})\sqrt{n_\sigma} \text{ and } w_2 = (v - k_{T\sigma})\sqrt{n_\sigma}$$

and $L(p_1) = 0.95$ & $L(p_2) = 0.10$ (5)

4. Matched QSS Variable System with Attribute System

The procedure for obtaining a Quick switching Variable Sampling Inspection System $(n_\sigma; k_{N\sigma,} k_{T\sigma})$ matching a given attributes system QSS (n;c_2,c_1) of Soundarajan.V and Arumainayagam S.D. (1990) is as follows:

For the QSS (n; c_2,c_1) with p_1=0.014, p_2=0.05, α =0.05 and β =0.10, from Table 1 the following values are determined. $\frac{p_2}{p_1} = \frac{0.05}{0.014} = 3.571$

The nearest value of 3.571 in the table is 3.5815, which has associated with it value of $np_1 = 1.2104$ $c_1 = 1$ and $c_2 = 3$.

The sample size is then determined by

$$n = \frac{np_1}{p_1} = \frac{1.2104}{0.014} = 86.45 \cong 86$$

The attributes system is designated as QSS (86, 3,1)

From equation (1)

$$P_a(p_1) = \frac{p(d \le c_1 / p_1)}{1 - p(d \le c_2 / p_1) + p(d \le c_1 / p_1)}$$

For the above system

$$P(d \le c_1 / p_1) = \sum_{i=0}^{c_1} e^{-np_1}(np_1)^i / i! = 0.659$$

$$P(d \le c_2 / p_1) = \sum_{i=0}^{c_2} e^{-np_1}(np_1)^i / i! = 0.9653 \quad (6)$$

Therefore $P_a(p_1) = 0.95$

Similarly

$$P_a(p_2) = \frac{p(d \le c_1 / p_2)}{1 - p(d \le c_2 / p_2) + p(d \le c_1 / p_2)}$$

$$P(d \le c_1 / p_2) = \sum_{i=0}^{c_1} e^{-np_2} (np_2)^i / i! = 0.0699$$

$$P(d \le c_2 / p_2) = \sum_{i=0}^{c_2} e^{-np_2} (np_2)^i / i! = 0.3709 \quad (7)$$

Therefore $P_a(p_2) = 0.10$

Thus the OC curve of the QSS (86, 3, 1) passes through $(p_{0.95}, 0.95)$ and $(p_{0.10}, 0.10)$. To obtain Quick switching sampling variable system we consider the OC function

$$P_a(p_1) = \frac{\varphi(w_2)}{1 - \varphi(w_1) + \varphi(w_2)} = 0.95$$

where

$w_1 = (v_1 - k_{N\sigma})\sqrt{n_\sigma}$ and $w_2 = (v_1 - k_{T\sigma})\sqrt{n_\sigma}$

If $P_a(p_1) = 0.95$ then

$\varphi(w_2) = 0.6590$ and $\varphi(w_1) = 0.9653$

[From (6) & (7)]

$w_2 = (v_1 - k_{T\sigma})\sqrt{n_\sigma} = 0.41$ and

$w_1 = (v_1 - k_{N\sigma})\sqrt{n_\sigma} = 1.82 \quad (8)$

$$P_a(p_2) = \frac{\varphi(w_2)}{1 - \varphi(w_1) + \varphi(w_2)} = 0.10$$

$w_1 = (v_2 - k_{N\sigma})\sqrt{n_\sigma}$ and $w_2 = (v_2 - k_{T\sigma})\sqrt{n_\sigma}$

If $P_a(p_2) = \beta = 0.10$ then

$\phi(w_2) = 0.0699$ and $\phi(w_1) = 0.3709$

[From (8) & (9)]

$w_2 = (v_2 - k_{T\sigma})\sqrt{n_\sigma} = -1.48$ and

$w_1 = (v_2 - k_{N\sigma})\sqrt{n_\sigma} = 0.33 \quad (9)$

If $p_1 = 0.014 \Rightarrow v_1 = 2.2$

$p_2 = 0.05 \Rightarrow v_2 = 1.64$

Through (10) and (11) one can get

$n_\sigma = 13$, $k_{N\sigma} = 1.713$ and $k_{T\sigma} = 2.068$

From the given Quick switching sampling attribute inspection system (86;3,1) one can obtain the QSVSS as

1. $n_\sigma = 13.011 = 13$
2. $k_{N\sigma} = 1.713$
3. $k_{T\sigma} = 2.068$
4. $v_1 = 2.2$, $v_2 = 1.64$

Thus the quick switching variable sampling system (13: 1.7132, 2.068) is such that the OC curve passes through $(p_{0.95}, 0.95)$ and $(p_{0.10,}\, 0.10)$. The equivalence of the OC curves is illustrated in Table 3.

Though in practice the sample size n_σ would have to be rounded to 13, the OC curve for the Quick switching variable sampling system has been computed with the fractional value n in order to better demonstrate the closeness of the agreement of the OC curves. One can imagine the saving of the variable system with attributes system when the sample size 13 is compared with 86.

5. Selection of variable Systems Matching Quick Switching Attribute System Indexed by AQL and LQL

Table 2 provides matched variables system to that of attributes systems indexed by AQL and LQL. For example, for given $p_1 = 0.012,\ \alpha = 0.05\ \ p_2 = 0.05$ and $\beta = 0.10$, the "OR" value is 4.1666, the nearest value of 4.1666 in the Table 3.9 is 4.2069, which has associated with the attribute system n=91, $c_2 = 3$ and $c_1 = 2$ one can obtain the corresponding matched Quick switching sampling variable system from Table 2 as $n_\sigma = 16$, $k_{N\sigma} = 1.7623$, and $k_{T\sigma} = 1.9030$. Table 1 provides $\frac{p_2}{p_1}$ together with the value of np_1, c_2 *and* c_1 $(\alpha = 0.05, \beta = 0.10)$.

Table 1: Values of $\frac{p_2}{p_1}$ for QSS

c_2	c_1	$\frac{p_2}{p_1}$ **for** $(\alpha = 0.05, \beta = 0.10)$	np_1
1	0	8.2128	0.3078
2	1	5.3013	0.7695
2	0	4.3834	0.6437
3	2	4.1703	1.3178
3	1	3.5815	1.2104
3	0	3.1344	1.0046
6	5	2.9126	3.2363
5	3	2.8568	2.4721
7	6	2.7163	3.9318
6	4	2.6543	3.1486
4	0	2.5404	1.3754
6	3	2.4340	3.0089
5	1	2.3707	2.0915
9	7	2.2849	5.2963
6	2	2.2509	2.8056
5	0	2.1989	1.7504
9	6	2.1444	5.1774
6	1	2.1001	2.5239
8	4	2.0837	4.2658
9	5	2.0220	5.0129
8	3	1.9662	4.0358
7	1	1.9160	2.9508
8	2	1.8669	3.7427
7	0	1.8259	2.5051
8	1	1.7833	3.3725
9	2	1.7491	4.1974
9	1	1.6281	3.7899
9	0	1.6281	3.2613
11	2	1.5871	5.0852
11	1	1.5436	4.6135
12	0	1.4611	4.3941

Table 2 .Quick Switching Variable Sampling System Matched with Quick Switching Attribute Sampling System

$\frac{p_2}{p_1}$	Attribute QSS			Variable QSS		
	n	c_2	c_1	n_σ	$k_{N\sigma}$	$k_{T\sigma}$
8.3333	128	1	0	9	2.2330	2.5769
5.2857	110	2	1	11	1.9597	2.1714
4.4615	99	2	0	10	1.9064	2.4358
4.2069	91	3	2	16	1.7623	1.9030
3.5714	86	3	1	13	1.7164	2.0700
3.1333	67	3	0	10	1.5446	2.2538
2.9250	81	6	5	22	1.3985	1.4828
2.8438	77	5	3	18	1.4526	1.6736
2.7200	79	7	6	23	1.3060	1.3855
2.6522	69	6	4	20	1.3028	1.4945
2.5238	66	4	0	11	1.4100	2.2126
2.4390	73	6	3	19	1.3241	1.6401
2.3793	72	5	1	15	1.3794	1.9467
2.2857	76	9	7	24	1.1380	1.2789
2.2500	78	6	2	18	1.3542	1.8165
2.2000	70	5	0	13	1.3293	2.1944
2.1447	68	9	6	23	1.0638	1.2883
2.0952	60	6	1	15	1.1758	1.8582
2.0789	56	8	4	19	1.0100	1.3703
2.0263	66	9	5	22	1.3592	1.0450
1.9692	62	8	3	21	1.0651	1.5342
1.9286	70	7	1	17	1.1847	1.9097
1.8654	72	8	2	19	1.1419	1.7512
1.8095	119	7	0	18	1.4385	2.3461
1.7742	109	8	1	12	1.2344	2.2838
1.7600	168	9	2	28	1.5433	2.1039
1.7037	140	9	1	23	1.1431	2.1716
1.6316	172	9	0	25	1.5130	2.4070
1.6000	170	11	2	27	1.4242	2.1047
1.4762	209	12	0	28	1.4421	2.4526

Table 3. Values of [p, L (p)] for Quick Switching Attribute Sampling System and the equivalent variable system of the proposed method

p	L(p) of attributes QSS	L(p) of Variables QSS
0.01	0.9846	0.9855
0.02	0.8331	0.8107
0.03	0.5044	0.4518
0.04	0.2393	0.2224
0.05	0.1041	0.0956
0.06	0.0444	0.0434
0.07	0.0191	0.0203
0.08	0.0087	0.0099
0.09	0.0042	0.0048
0.10	0.0021	0.0024

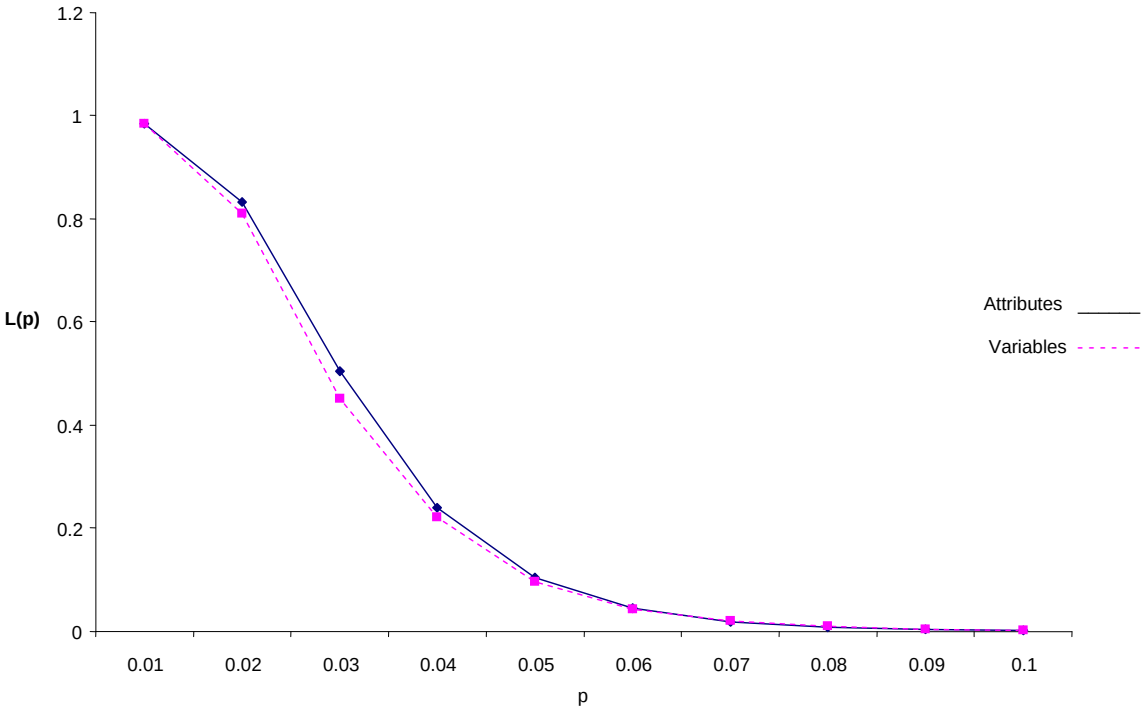

Figure. 1 OC curves - Attributes QSS vs. Variables QSS

6. Conclusions

The methodology used in this paper could be adopted for other developed sampling system to match with other sampling system. In the shop floor, we could apply both the given attribute quick switching systems as well as variable quick switch systems. From the Table 2 and Table 3, one can conclude that the variable quick switching sampling system is better one.

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10/12/2009

Accumulation of Some Heavy Metals in *Clarias anguillaris* and *Heterotis niloticus* from Lake Geriyo Yola Nigeria.

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ABSTRACT:

The accumulation of Cr, Cu, Mn, Ni, Zn, As and Pb in two fish species (*Clarias anguillaris* and *Heterotis niloticus)* from Lake Geriyo Yola Adamawa state Nigeria were investigated in April 2007 the hottest month, atmospheric temperature 42°c. The levels of heavy metals accumulated were assayed using energy dispersive x-ray fluorescence (EDXRF) spectrophotometer and the results were given as mg/kg dry weight. The concentration of heavy metals in the two fish species indicated that *C. anguillaris* accumulated higher levels of Cr, Mn, and Ni than the maximum permissible limits in fish by World Health Organization (WHO 1985), while the levels of Cu, Zn, and Pb are lower. *H. niloticus* accumulated higher levels of Mn and As than the maximum permissible limits in fish by WHO (1985), while Cu and Zn levels are lower. The order of heavy metals accumulation in *C. anguillaris* was Cr > Mn > Pb > Ni > Zn > Cu. Similarly, in *H. niloticus*, the order was Mn > Zn > As > Cu. In all of these heavy metals, the accumulation of Mn and Cr in *C. anguillaris and Mn in H.niloticus* was more pronounced than the other metals. The result indicates that Lake Geriyo is contaminated with Cr, Mn, Ni, and As with respect to the two fish species. This definitely affects the aquatic life of the freshwater fish. These levels of heavy metals accumulated by the two fish species might be due to the increase in agricultural influx waters and some anthropogenic activities within the catchment's area of the Lake. [Nature and Science 2009:7(12):40-43]. (ISSN:1545-0740)

Key words: Accumulation, Lake, Nigeria, heavy metal, *C. anguillaris*, *H. niloticus*

1. Introduction:

The contamination of freshwater with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threats to public water supplies but also their damage caused to the aquatic life (Canli. et al., 1998). The natural aquatic systems may extensively be contaminated with heavy metals released from domestic and industrial wastes, agricultural activities, physical and chemical weathering of rocks, soil erosions, as well as sewage disposal and atmospheric deposition (Alloway and Ayres, 1993).

Aquatic organisms such as fish are capable of accumulating heavy metals in their living cells to concentrations much higher than those present in water, sediment and micro flora in their environment (Forstner and Wittmann; 1981). The presence of heavy metals in river, Lake or any aquatic environment can change both aquatic species diversity and ecosystems due to their toxicity and accumulative behavour (Heath; 1987).

The increasing importance of fish as a source of protein and the interest in understanding the accumulation of heavy metals at the trophic levels of food chain, extend the focus towards fish (Deb and Santra; 1997).

Heavy metals in fish, increases with the increments of the metal levels in water, sediment and fish food organism (Arvind; 2002).

Heavy metals like Cu, Co, Zn, Fe and Mn at low concentrations are essential metals for enzymatic activity and many biological processes. Other metals such as Cd, Pb, and Hg have no known essential role in the body of living organisms, and are toxic even at low concentrations. The essential metals also become toxic at high concentration (Bryan, 1976; Alloway and Ayres; 1993).

This study was carried out to quantify the accumulated heavy metals in *C. anguillaris* and *H. niloticus* from Lake Geriyo Yola so as to ascertain their suitability for human consumption and aquaculture.

2. Materials and Methods:

2.1 Study Area:

Lake Geriyo is located at the outskirt of Jimeta metropolis on the North-west region (longitude 12° 25'E and between latitude 9° 8'N and 9° 17'E).

It experiences some influx of waters during rainy season and pollution load coming up stream from river Benue. The Lake has been subjected to intense irrigation, which might have attributed to the major factor for high levels of heavy metals in the lake and fish species.

Temperature ranges from 20°c, a cold and dusty winds in December to January and intense heat with temperatures of 30°c to 42°c in March to April (upper Benue River Basin Development Authority, (U.B.R.B.D.A. 1985).

2.2 Sampling

Two fish species (5 – 10 individual of each fish species) namely, *C. anguillaris* weighing 2543 ± 16.046g and *H. nilloticus* weighing 3032 ± 14.352g were randomly caught by the local fishmen using set gill nets of various sizes and traps set overnight prior to collection. The fish samples were immediately bought at the bank of the Lake during the month of April 2007. The fish sample was stored in a cooler with ice and was brought to the laboratory.

2.3 Fish Sample Treatment.

The modified version of transmission-emission (T-E) method (Kump 1996; Angeyo et al 1998 and Funtua 1999) was used. The fish samples were dissected with clean stainless steel instruments on the same day. The tissues from 5-10 individual of the same fish species were dried in an oven at 105°c until a constant weight was reached. The dried tissues of the same fish species were ground to powder, sieved to grain size of less than 125µm and were homogenized.

A quantity of (0.5g) of the powdered fish samples for each fish species was weighed separately and three drops of organic binder was added to each fish sample and were pressed with 10 tons hydraulic press to form pellets of 19mm diameters of each fish sample, three replicate of pellets of each fish sample was prepared.

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2.5 Determination of Heavy Metals in Fish Samples.

The modified version of transmission-emission (T-E) (Kump 1996; Angeyo et al 1998 and Funtua 1999) was used. The pellet of each fish sample was put into the X-ray fluorescence spectrophotometer sample holder and it was bombarded with high energy electrons of ^{109}Cd (22.1KeV).

Fluorescent X-ray was produced which passes to silicon-lithium detector, through Mo target as a source of monochromatic X-rays. The spectrum of energy generated on the detector was processed by a multi-channel Analyzer (MCA) to obtain analytical data.

The intensity of fluorescent X-rays on the detector is proportional to the concentration of individual element of interest in the sample.

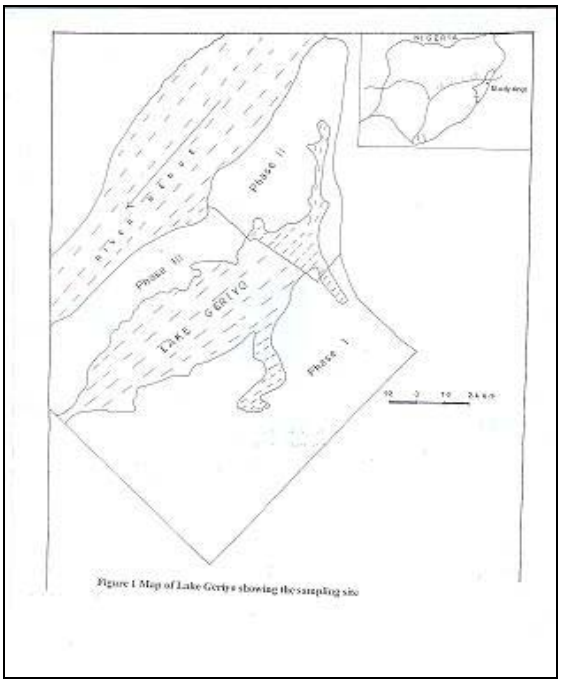

Figure 1. Map of Lake Geriyo showing sampling site

3. Results

The values of the heavy metals in the two fish species are presented in (Tables1). In this study, *C. anguillaris* a bottom feeder and *H. niloticus* a surface feeder were investigated for heavy metals. These two fish species are so common in Lake Geriyo.

Cr in *C. anguillaris* and Mn in *H. noliticus* showed a more pronounced high accumulation values (Table 1).

Cr value is 9.010 ± 1.453mg/kg dry wt in *C. anguillaris.* Cu values ranged from 0.643 ± 0.001mg/kg dry wt in *C. anguillaris* to 0.684 ± 0.001 dry wt in *H. niloticus.* For Mn, the values are a minimum of 1.245 ± 0.001mg/kg dry wt in *C.*

anguillaris, while the maximum value was 9.963 ± 1.250mg/kg dry wt in *H. niloticus*. The value of Ni is 0.953 ± 0.020mg/kg dry wt and it was present in *C. anguillaris* only.

The Zn values fluctuated between 0.752 ± 0.002mg/kg dry wt and 1.502 ± 0.001mg/kg dry wt for *C. anguillaris* and *H. niloticus*. As value was 1.042 ± 0.001mg/kg dry wt for *H. niloticus* only. Pb value was 1.012 ± 0.001mg/kg dry wt in *C. anguillaris only*

From the above results, *C. anguillaris* tended to accumulate more heavy metals (Cr, Mn, Cu, Ni, Zn and Pb) than *H. niloticus* (Cu, Mn, As, and Zn).

4. Discussion

Fish species living in contaminated waters tend to accumulate heavy metals in their organs and tissues. Various heavy metals are accumulated in fish body in different amount (Jezierska and Witesta; 2001)

Fish species mostly absorbed heavy metals from its feeding diets, sediments and surrounding waters resulting to their accumulation in reasonable amounts (McCarthy and Shugart; 1990).

The accumulation of heavy metals in fish species are found to be influenced by several factors like temperature P^H of water, conductivity, rainfall, hardness, salinity and also by biotic community interactions (Arvinda; 2002). Microhabitat utilization, feeding habits, age, sex and fish species also determine the accumulation pattern of heavy metals (Kotze; et al 1999).

C. anguillaris accumulated higher levels of Cr, Mn and Ni, while *H. niloticus* accumulated Mn and As to higher levels (Table 1), when compared to the maximum permissible limits by World Health Organization (WHO 1985) in fish.

The levels of Cu, Zn and Pb in *C.anguillaris*, Cu and Zn levels in *H. niloticus* were low when compared with the maximum permissible limits by WHO 1985 in fish.

The differences in the level of heavy metals accumulated by the two fish species respectively could be attributed to the differences in their metabolic rates, feeding habits, age, sex and fish species (Kotze et al; 1999). Body size and health which are closely related to growth and metabolism has been shown to attribute most of the variations in heavy metals content of fishes (Moriarty, et al; 1984).

These heavy metals Cr, Cu, Mn and Zn are concentrated in sediments, aerobic and anaerobic bacteria in Lakes, rivers and streams (Shahunthala 1989).

They are also found to be incorporated and accumulated in food chain which are passed to fishes (Forstner and Wittmann, 1981)

C. anguillaris being a bottom feeder may have accumulated Cr, Cu, Mn, and Zn from the sediment and bacteria in the Lake.

The accumulation of Ni and Pb by *C. anguillaris* could be attributed to the fact that Ni and Pb are naturally found on the surface waters due to weathering of materials and soil erosion (USEPA, 1997).

Cu and Zn essential metals, their levels are low in both fishes. It could be attributed to homeostatic regulation of intracellular metals due to undesirable intracellular interaction which could be restricted, through bonding of the heavy metals to protein to form metallothionein (MT) (Roesjadi, 1992). Pb level in *C. anguillaris* is low, it could be attributed to the fact that Pb do not induce MT formation in tissues of fishes (Arvind 2002).

The sources of heavy metals in Lake Geriyo could results from the exposure of high concentration of metals from the metal scrap market, due to the activities of the blacksmiths by the Lake. It could also be due to the dumping of the municipal wastes on the bank of the lake, others are agricultural activities within the catchments area of the lake, most especially the intense irrigation practices.

Table 1 Mean concentration of Heavy Metals in two fish species from Lake Geriyo Yola in (mg/kg, dry weight) and set standard. WHO (1985)

Metals / Fish Samples	Cr	Cu	Mn	Ni	Zn	As
C. anguillaris	9.010± 1.453	0.634.± 0.001	1.245± 0.001	0.953± 0.020	0.752± 0.001	BDL
H. nlioticus	BDL	0.684± 0.001	9.963± 1.250	BDL	1.502± 0.001	1.042± 0.001
WHO (1985)	0.15	3	0.5	0.6	10-75	0.02

5. Conclusion:

The levels of Cr, Mn, and Ni in *C. anguillaris*, Mn and As in *H. niloticus* were higher than the maximum permissible limits by WHO (1985) in fishes.

The results indicate that heavy metal contamination would affect the fishes, end user and consumer. Hence, a scientific method of detoxification is essential to improve the health of these fishes in any polluted environmental condition

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11/11/2009

Performance Of Pre-Winter Planted Rooted Cuttings Of Greater Yam (*Dioscorea alata* L.)

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Abstract : Nodal cuttings of *Dioscorea alata* from 45 day old plants were grown *in vivo* by treating them with different concentrations of IBA (100, 200, 500, 1,000 ppm), Rootex (a type of root promoting substance,the powdered contain 3gm IBA/Kg) and a control. For each treatment 2 node, 1 node and 0.5 node cuttings of 4 - 5 cm were used as explants. In the nursery there was a 100% survival of 1 node cuttings treated with 500 ppm of Indole 3-butyricacid (IBA) followed by Rootex 1 node, Rootex 0.5 node and control one node. From the results it was concluded that 2 node and 0.5 node cuttings were not suitable for *in vivo* rooting of yam with growth regulators. Single node cuttings with growth regulators are preferred for large scale production of propagules in an *in vivo* system for *D. alata*. [Nature and Science 2009; 7(12):44-47]. (ISSN: 1545-0740).

Key words: Rootex, IBA, Nodal cuttings Grater yam, Propagule

1. Introduction

Among tropical tuber crops the greater yam (*Dioscorea alata* L.) occupies an important position because of their food, nutritional, medicinal and industrial significance. *Dioscorea alata* is an old monocot from the family *Dioscoreaceae* (**Ayensu , 1972**). In India *D. alata* tubers are consumed mainly in the southern and northern states. They are also cultivated as a cash crop in some area where they are more important than potato. These crops have wide adaptability and are less stringent in input needs like fertilizer application, irrigation and plant protection. They are efficient converters of solar energy to dry matter.

In yams under ground tubers are seriously affected by pathogen accumulation (Gautt *et al*., **1969; Malauri *et al,* 1998**) which reduces the quality of planting material. Transportation of high volumes of planting material for field planting is difficult. About 2,500 to 3,000 kg of planting material is required to plant 1 ha. Thus the cost of planting material increases the cost of production. Under conventional propagation the rate of multiplication of tubers is very slow as a piece of tuber has only one or two sprouts. To counter the scarcity of planting material an attempt was made to standardize and develop an *in vivo* rapid multiplication method through the use of nodal cuttings treated with hormone. From the results it was clear that propagule production of yams through the use of nodal cuttings, for yam cultivation, is the best solution. It is cheaper, saves time and is resistant to pests and diseases.

2. MATERIALS AND METHODS

The experiment was conducted at the Botanical garden of the Post Graduate Botany Department, Utkal University, Vanivihar, Bhubaneswar. Nodal cuttings with different size node segments (2 node, 1 node and 0.5 nodes) were taken as explants from 45 days old vines of *D. alata* plants. Concentrations of IBA of 100, 200, 500 and 1,000 ppm and Rootex were used and an untreated control. In each chemical treatment a 2 node, 1 node and 0.5 node cuttings was used .The experiment had 18 treatments and 3 replicates in a randomized block design (**Panse and Sukhatme, 1978; Nanda *et al*, 1968**). Before starting the experiment the nursery bed was prepared (90cmX 90cm) by mixing with sun dried sand and cow dung and was sterilized with *Tricoderma viridae* followed by Steptocycline at 0.015%. Beds were watered twice daily depending on rainfall and the status of the environmental condition of the day. Nodal cuttings with different numbers of nodes were prepared from the vines and placed in different concentrations of IBA for 5 - 10 minutes. The nursery bed was wetted and the nodal segments were planted, one by one in the nursery bed at close spacings. For the Rootex treatment the nodal cut surface was dipped in to the solid mass. The cutting was then planted. Beds were watered every evening to maintain the humidity.

At 15 days data on nursery bed survival, root length, root number, microtuber weight and field survival was recorded and compared with the control (Table 1) using statistical analysis as proposed by **Panse and Sukhatme (1978)**.

3. RESULT AND DISCUSSION

From the results (Table 1) it was concluded that though greater yam is mainly propagated vegetatively through tubers it would be cost effective for farmers to use an alternative method of plantlet production from nodal cuttings. (**Bilderback, 1993**). There was a 100% survival of 1 node cuttings treated with 500 ppm of IBA, Rootex 1 node, Rootex 0.5 nodes and control 1 node. Two node and 0.5 node cuttings were not suitable for rooting. Half node cuttings treated with Rootex also had 100% survival in the nursery (**Waitt, 1960; Hartmann *et al*, 1997**)

In the field survival was highest in 1 node cuttings treated with Rootex (86.7%) followed by Rootex treated 0.5 node cuttings (81.7%). It was therefore decided to use Rootex 1 node cuttings for commercial production of propagules of *D. alata*. Root length was longest in 1 node cuttings treated with Rootex (14.0 cm). This was followed by 0.5 node cuttings treated with Rootex (13.3 cm). A similar result was obtained for root number (**Maharana and Singh, 1974; 1978**). The latter two treatments gave larger tubers than the other treatments. Tuber weights were 11.6 g in 1 node cuttings treated with Rootex followed by 0.5 node cuttings also treated with Rootex (9.7 gm). Tuber size was small, due to poor vegetative growth in all IBA and control treatments irrespective of 2 node, 1 node or 0.5 node cuttings pre-winter for off season

cultivation of this important crop (Lamotta and Whigham, 1971; Whittle, 1978).

4. CONCLUSION

From the results it can be concluded that vine cutting with different growth regulator treatments can be used as an alternative method for propagule production of greater yam to sustain cost effective yam cultivation even in the off season. As field survival was highest with 1 node and 0.5 node cuttings treated with Rootex it is recommended that 1 node cuttings, treated with Rootex, be use for commercial production of *D. alata* propagules.

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Table 1. Performance of rooted cuttings of pre-winter planted *D. alata* in nursery bed and field evaluation

Treatments	Survival (%)		Root length (cm)	Number of roots	Tuber size (gm)
	Nursery	Field			
IBA 100 ppm, 0.5 node	83.3	56.7	8.0	6.67	2.33
IBA 200 ppm, 0.5 node	75.0	60.0	7.7	6.33	2.33
IBA 200 ppm, 1 node	95.0	60.0	8.7	7.33	3.33
IBA 100 ppm, 1 node	91.7	55.0	9.0	7.67	2.33
IBA 200 ppm, 2 node	61.7	16.7	7.0	2.00	2.33
IBA 500 ppm, 0.5 node	63.3	60.0	7.3	3.00	3.33
IBA 500 ppm, 2 node	65.0	20.0	7.0	2.33	2.33
IBA 500 ppm, 1 node	100.0	61.7	7.7	3.00	5.33
IBA 1,000 ppm, 2 node	58.3	18.3	7.0	1.33	3.33
IBA 1,000 ppm, 1 node	93.3	66.7	4.0	1.67	7.00
IBA 1,000 ppm, 0.5 node	58.3	50.0	4.0	1.67	6.33
Rootex 2 node	71.7	30.0	10.3	7.00	6.67
Rootex 1 node	100.0	86.7	14.0	14.00	11.67
Rootex 0.5 node	100.0	81.7	13.3	11.33	9.67
Control 2 node	63.3	16.7	7.0	5.33	2.00
Control 1 node	100.0	60.0	5.7	6.67	5.00
Control 0.5 node	71.7	55.0	6.0	6.00	3.33
S.E.M. ±	2.88	2.58	0.94	0.76	0.45
C.D. (0.05)	7.99	7.15	2.63	2.13	1.26

10/22/2009

Response of Vine Cuttings to Rooting in Different Months in Three *Dioscorea* species

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Abstract: Availability period of vine cuttings of different Dioscoreas in the field reveals that *Dioscorea alata* L. can be maintained throughout the year for collection of vine cuttings. On the other hand vine cuttings of *D. oppositifolia* L., can be collected from June to December and *D. hispida* Dennst., can provide cuttings from June to October. Earlier or latter to this no cuttings will be available. Each vine cutting should have one or two nodes. One of the nodes should be in soil. One leaf must be there in each cutting. Since the leaf is responsible for photosynthesis and transpiration the area of the leaf may be kept in a comfortable size by trimming to maintain a balance between transpiration, photosynthesis and respiration. [Nature and Science 2009; 7(12):48-51]. (ISSN: 1545-0740).

Key words: Dioscorea, Vine cuttings, Rooting response

1. Introduction

Yams (*Dioscorea* spp.) constitute a staple food crop for over 100 million people in the humid and sub humid tropics. These are climbing plants with glaborous leaves and twining stems, which coil readily around a stake. They are perennial through root system but are grown as annual crops. The major yam growing region of the world are Asia, South America and West Africa (**Coursey,1967**; **Ayensu and Coursey,1972**).The tubers of *Dioscorea* have a dual agricultural function: first, as source of food for millions of people and secondly, as planting material (**Hahn, 1995**; **Craufurd *et al*, 2006**). The cultivated forms of this vegetative propagated crop have a large genetic diversity. Commercial scale of yam production meant for marketing or in domestic purpose, most farmers depend on different sources of seed tubers as planting material. Among these vine cuttings, mini setts and microtubers are preferred as seed tuber for yam planting (Abraham *et al*., 1986). In traditional practices the rural farmers generally use 100 to 600 gm or more tuber pieces as seed tuber which was 10 to 30% of the total yam yield. So the conventional farming by taking seed tuber is more costly than the vine cuttings and mini sett method and microtuber for yam planting. In yams under ground tubers are seriously affected by pathogen accumulation (**Malauri *et al*, 1995**) which reduces the quality of planting material. Transportation of high volumes of planting material for field planting is difficult. About 2,500 to 3,000 kg of planting material is required to plant 1 ha. Thus the cost of planting material increases the cost of production (**Onwueme, 1978**). The aim of this study is thus to standardize the response of vine cutting to rooting in different month of three Dioscorea species viz. *D. alata, D. hispida* and *D. oppositifolia* as chief method for production of disease free quality planting material for large scale propagation particularly in yam cultivation.

2. Materials and Methods

The experiment was conducted at the Botanical garden, Post Graduate Botany Department, Utkal University, Vanivihar, Bhubaneswar. Vine cuttings were collected in different months from field grown plants of *D. alata, D. hispida and D. oppositifolia* with one node and leaf was trimmed to half or remained as such with equal area. Immediately after collection the cuttings were dipped in water. The lowest node was treated with root promoting hormonal powder (ROOTEX-P, Bass Laboratories Ptv. Ltd.). Before starting of the experiment the nursery bed was prepared (90 cm X 90 cm) by mixing with sun dried sand and cow dung and was sterilized with Tricoderma viridae followed by Steptocycline at 0.015%. The nursery bed was wetted and the vine cuttings were planted, one by one in the nursery bed at close spacing by dipping with 0.02% bavistin to prevent further fungal incidence. Beds were watered twice daily using a rose can by considering the rainfall and status of the environmental condition of the days. Further in each month well rooted cuttings with leaf were planted in the field with standard agronomic practices. Various parameter viz. days taken for rooting, length of root (cm), No. of primary roots / cutting, No. of lateral roots /cutting, survival (%) in nursery bed and field, days taken for axillary bud development were recorded.

3. Results and Discussion

Vine cuttings can be established very quickly and an effective means of producing disease and nematode free planting material. Propagation of yam by vine cutting is not yet practiced in commercial plantings

because of intensive care required for rooting and low tuber yield from rooted cuttings, but it has a future. Standardizations of propagation of yam by vine cuttings will reduce the cost of cultivation and incidence of pest and diseases. In this trial, several aspects were investigated to standardize the methods of in vivo propagule production for sustainable means of yam cultivation in three *Dioscorea* species viz. *D. alata, D. hispida* and *D. oppositifolia*. The major problem militating against yam production in various tropical and subtropical regions is shortage of planting material which constituted about 33% of total cost outlay(**Orkwor and Asadu, 1997; Marfo *et al*, 1998**).The Yam nodal vine cuttings have offered valuable hope to break through in solving the problem of inadequate planting material. The study there fore was aimed at identifying the most effective, cheap, easy and readily available method for yam planting.

The rooting response of vine cuttings of all the three *Dioscorea* (*D. alata, D. hispida* and *D. oppositifolia*) species were observed month wise for development of propagules by planting in the nursery bed. Vine cuttings of *D. alata* rooted quickly i.e. in 7.87 days during August but longest time was taken during May (27.16days).Vine cuttings rooted quickly between July to October i.e. within a period of 7.87 to 11.05 days due to high humidity. During April-May (summer) and November, December and January (winter months) rooting was delayed due to temperature variations. Survival percentage was highest during rainy months of July to October (82.47%, 85.44%, 88.81%, 85. 12 %). The number of primary roots was highest during October (4.37) and lateral roots was highest during August (47.15) with maximum root length (21.26 cm) but reduced to a minimum of 5.56 cm in May. After transferring the material from nursery bed to the field survival percentage was highest in rainy season i.e. in the month of August (86.94 %). After the rainy seasons the axillary bud development was maximum (30.31) in the month of October. During summer and winter months, use of agro shed nets reduced the adverse effect of high temperature and low temperature (Table.1).

Similarly the vine cuttings of *D. hispida* were also capable to produce roots during June to October. The vines wither after October and completely dry by the end of December. D. hispida plants sprout quickly but leaf, development takes more time. Vine cuttings root quickly during September (15.49 days) with highest percentage of survival (82.91) in the nursery bed. Number of primary (4.20) and lateral (20.11) roots with maximum root length (3.50 cm) was found in the rainy season in the month of September. Survival % in field (46.69%) and time taken for axillary bud development (23.21 days) was quicker after rainy season i.e., in the month of October (Table 2). The vines of *D. oppositifolia* produced plenty of leaves from June onwards up to December. August and September is ideal for rooting of vine cuttings. During these months the number of primary roots (4.42), lateral root (20.98), and length of root (6.22cm), survival percentage in the field (63.90%) was highest in comparison from October to December. But minimum days (20.38 days) were taken for bud development in rainy season i.e. in the month of July. However, August to October was better as compared to other months. Rooted cuttings survived better (91.91%) during this period in the month of September in nursery bed. The growth and development of *D. oppositifolia* is quite different than *D. alata*. The vine cuttings withered during December with the production of microtuber (Table 3).

Amin *et al*. (1997) reported that in Shorea leprosula plantlet production through cutting in which rooting decreases when the leaf area increases. Hanses (1986) reported that cutting position and stem length also influence the rooting ability in leaf bud cuttings of *Schefflera arboricola*. Wang and Bogher (1988) reported that in Golden pothos nodal position and length of cuttings also determine the root retention for in vivo establishment. Vander and Escobar (1990) reported that expanded potato production in developing countries using cuttings as a source of good quality planting material is the simple low cost methods for root induction and establishment of the in vivo propagules for potato cultivation in warm tropical sites of Philippines. He also reported that there were no major differences in root and shoot development from cuttings differing in size and age. **Balogun *et al.* (2004)** reported that single node cuttings of two genotypes each of Dioscorea alata and *D. rotundata* from both plants grown in screen houses and in vitro plantlets were cultured in a tuberization medium. The screen house explants had significantly higher plantlet tuberization and primary nodal complex formation, and more tubers and primary nodal complexes per plantlet than in vitro explants, whereas in vitro explants performed better only in nodes per plantlet. It appears that in vitro tuberization is explant-, species- and genotype-dependent, the greatest variation being due to explant source. This is a first report of microtuber production from nodal explants of *D. rotundata* produced in a screen house. Production of microtubers from the vine cuttings of three Dioscorea species (*D.alata, D. hispida* and *D. oppositifolia*) revealed that the life span of the vine was highest in *D. alata* (63.81days) and least in D. oppositifolia (55.28 days). During this period, tuberisation was completed and the leafy cuttings withered.

Table 1. Rooting of vine cuttings of *D. alata* in different months

Sl. No.	Months	Days taken for rooting	Survival of cutting (%)	No. of primary roots / cutting	No. of lateral roots /cutting	Length of root (cm)	Survival (%) in (field)	Days taken for axillary bud development
1	January	19.60	41.96	2.94	20.64	12.73	13.20	24.20
2	February	16.60	46.26	2.72	23.30	13.79	18.07	21.28
3	March	14.57	61.93	3.09	28.06	13.15	33.59	26.07
4	April	23.83	73.65	1.73	18.38	11.48	37.04	26.23
5	May	27.16	64.25	1.90	13.81	5.56	43.05	25.86
6	June	17.42	75.66	2.04	18.30	11.20	74.20	21.07
7	July	10.85	82.47	3.70	40.92	17.23	85.23	18.28
8	August	7.87	85.44	2.87	47.15	21.26	86.94	19.27
9	September	9.45	88.81	3.11	40.73	17.40	78.93	28.40
10	October	11.05	85.12	4.37	35.57	15.74	64.89	30.31
11	November	20.74	66.25	3.88	28.24	11.60	38.24	18.48
12	December	24.94	51.92	2.84	20.73	11.29	36.39	27.42
S.E.M. ±		0.91	0.53	0.35	1.55	0.78	1.45	1.44
C.D. (0.05)		2.66	1.55	1.04	4.54	2.31	4.25	4.22

Table 2. Rooting in vine cuttings of *D. hispida* in different months

Sl. No.	Months	Days taken for rooting	Survival of cutting (%)	No. of primary roots / cutting	No. of lateral roots /cutting	Length of root (cm)	Survival (%) in (field)	Days taken for axillary bud development
1	June	25.13	56.09	1.25	7.30	1.09	21.97	24.24
2	July	21.25	66.32	2.28	12.63	1.22	43.93	24.84
3	August	17.31	77.93	3.28	20.13	2.80	46.69	25.91
4	September	15.49	82.91	4.20	20.11	3.50	45.90	23.53
5	October	25.76	57.59	1.89	8.02	2.78	22.32	23.21
S.E.M. ±		0.84	1.81	0.19	0.88	0.04	1.03	1.06
C.D. (0.05)		2.73	5.92	0.63	2.87	0.13	3.37	3.46

Table 3. Rooting in vine cuttings of *D. oppositifolia* in different months

Sl. No.	Months	Days taken for rooting	Survival of cutting (%)	No. of primary roots / cutting	No. of lateral roots /cutting	Length of root (cm)	Survival (%) in (field)	Days taken for axillary bud development
1	June	28.67	75.40	1.37	8.20	2.50	38.32	21.24
2	July	22.20	84.79	1.62	7.15	2.71	40.66	20.38
3	August	17.67	91.30	4.42	20.98	4.76	60.23	22.51
4	September	15.68	91.91	3.80	20.04	6.22	62.90	24.77
5	October	15.44	72.94	4.12	17.84	4.38	63.90	24.71
6	November	19.58	67.47	2.62	12.10	3.64	29.03	24.78
7	December	23.13	56.93	2.43	10.77	4.54	21.66	25.28
S.E.M. ±		0.62	1.09	0.09	0.58	0.43	0.77	0.64
C.D. (0.05)		1.92	3.36	0.28	1.80	1.35	2.37	1.97

4. Conclusion

Propagation of yams by vine cuttings is very useful for rapid multiplication of elite clones and as an alternative method of yam planting material production in commercial scale of yam cultivation. Though vine cutting is not yet practiced in commercial plantings but it has future. Vine cuttings of all the three Dioscorea responded well to rooting. D. alata can be propagated through out the year whereas *D. hispida* from June to October and *D.oppositifolia* from June to December.

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10/28/2009

A Comparative Study Between Endoscopic Clip Application versus Argon Plasma Coagulation (APC) In Management Of Bleeding Peptic Ulcer

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Abstract: Acute upper gastrointestinal bleeding (UGITB) remains an important emergency situation. In the last two decades, major developments took place influencing incidence, etiology and outcome of patients with acute UGIB.Peptic ulcer bleeding is the most significant complication of ulcer disease,being responsible for 50% of all cases mortality..Patients and methods:Thirty group i included 15 patients suffering from acute ugib were randomely divided into two groups : patients who were treated with endoscopic clip application.groupii included 15 patients who were subjected to endoscopic apc.all patients were classified according to forrest clssification and the clinical rockall score.results:there were insignificant diffirance between the two groups as regard rockall score ,site of the ulcer,and rebleeding between the two groups.also there were significant diffrence between the two groups regarding forrest classification.rebleeding was significant with higher rockall score in group i but it was insignificant in group. [Nature and Science 2009; 7(12):52-60]. (ISSN: 1545-0740).

Key words: Clip application ,APC ,peptic ulcer

Introduction

Since the late 1980s, endoscopic hemostatic therapy has been widely accepted as the first-line therapy for upper-gastrointestinal bleeding.. Most clinical trials demonstrated a reduction in both recurrent bleeding and the need for surgical intervention when endoscopic hemostasis was used (Alan Barkun et al., 2003). Endoscopic therapy can be broadly categorized into injection therapy, thermal coagulation, and mechanical hemostasis. When analyzed separately, injection therapy, thermal-contact devices, and mechanical treatment all decrease the frequency of recurrent bleeding and rate of surgical intervention. (Park et al., 2004).Argon plasma coagulation (APC) is a noncontact type of coagulation that is easier to target to bleeding sites. A high-frequency current is transmitted by the ionized, electrically conductive argon gas. The argon gas flows onto the target surface, even if approached tangentially. APC has been used successfully to obtain hemostasis during open surgery. The use of APC in digestive tract endoscopy was first described in 1994. It is being applied more and more widely in the treatment of different GI pathologic disorders, hemorrhagic lesions in particular. (Canard et al., 2001). The only mechanical therapies widely available are endoscopically placed clips and band ligation devices. Endoscopic clips usually are placed over a bleeding site (e.g. visible vessel) and left in place(Church et al., 2003).. This consisted of a stainless steel clip (of size approximately 6 mm long and 1. 2 mm wide at the prongs) with a metal deployment device (that could be used to insert the clip into the endoscopic camera, and deployed outside the camera) enclosed in a plastic sheath. These clips were initially reloadable (Devereaux, 1999).

Risk Assessment, Prognstic

Indices:Numerous prognostic scores have been devised to aid the gastroenterologist in the management of upper gastro- intestinal bleeding, stratifying individual patients by risk

of re-bleeding and death. These scores range from the simple, endoscopy-based analysis of ulcer appearance described by Forrest et al (Table 1), through pre-endoscopic clinical scores such as the ‘clinical’

Rockall scores, to combined clinical and endoscopic evaluation, best exemplified by the classical Rockall (Van Leerdam, 2008).Such a scheme should aid in making clinical decisions, as to both the need for urgent intervention and the prediction of continued or recurrent bleeding in the context of endoscopic therapy. (Sung, 2005).

Table 1: Rockall Risk Scoring System for Patients with Peptic Ulcer Disease

Features		Points
Age (years):		
< 60		0
60 to 79		1
> 79		2
Shock :		
No shock (SBP ≥ 100, pulse < 100 bpm)		0
Tachycardia (SBP ≥ 100, pulse ≥ 100 bpm)		1
Hypotension (SBP < 100)		2
Comorbid illness:		
No major comorbid illness CHF, ischemic heart disease, other major comorbidity.		0 2
Liver or renal failure, disseminated cancer		3
Diagnosis :		
Mallory-Weiss tear, no other lesion identified and no stigmata of recent h hemorrhage		0
All other pathology causing bleeding (except cancer)		1
Upper gastrointestinal tract cancer		2
Major stigmata of recent hemorrhage:		
None or dark spot only		0
Blood in upper gastrointestinal tract, adherent clot, visible or spurting vessel		2
Score	**Rebleeding**	**ortality**
< 3 points	6. 2 %	0. 2 %
3 or 4 points	13 %	6. 8 %
> 4 points	25 %	20 %

SBP = systolic blood pressure (mm Hg); bpm = beats per minute;
CHF = congestive heart failure
Quoted from(Best Practice & Research Clinical Gastroenterology, 2008). (Rockall,1995, debate abounds as to the benefits of using such scores, with fears that th).

Table 2: Forrest classification of stigmata of recent haemorrhage and associated re-bleeding rates.

Forrest class	Type of lesion	Risk of rebleeding if u untreated
IA	Arterial spurting bleeding	100%
IB	Arterial oozing bleeding	55%
IIA	Visible vessel	43%
IIB	Non bleeding ulcer with an adherent clot	22%
IIC	Hematin covered flat spot	10%
III	No stigmata of hemorrhage	5%

Quoted from (Best Practice & Research Clinical Gastroenterology, 2008). (Forrest ,1974).

PATIENTS AND METHODS:

This study was conducted on 30 patients presenting with hematemesis and upper GIT endoscopy was done after resuscitation of the patients and showed a bleeding peptic ulcer.The patients were selected according to Forrest classification between group IA (spurting bleeding) to IIB (non bleeding ulcer with an adherent clot).A score was calculated to them according to Rockall's score.All participants were subjected to: Resuscitation including IV fluids, packed RBC transfusion until become hemodynamically stable. Routine laboratory investigations: complete blood count, liver and kidney function tests, prothrombin time, partial thromboplastine time. Upper GIT endoscopy and the patients with selection criteria of bleeding ulcer randomely subjected to one of the two options of treatment : Group I: Consisting of 15 patients subjected to clip application using a metallic clips (Hemoclip), Group II:Consisting of 15 patients in which Argon Plasma Coagulation (APC) was done using an argon plasma coagulator unit.Clip application device: clip application was done using a metallic clips (Hemoclip; Olympus America , rotational clip fixing device HX`6UR`1 through flexible ndoscopes). The clip fixing device length is 23mm and maximum insertion portion diameter is 2. 8 mm with processing port. Clips are loaded onto the fixing device and drawn into a sheath. At the target lesion, the clip is advanced out of the sheath, oriented with the rotational handle, and then deployed. The mechanism of hemostasis is mechanical compression.

Technical Detailes:

Hemoclips have 2 components: metallic double pronged clips and a delivery/deployment catheter-handle assembly The prongs of the clip are applied with pressure onto the target tissue and pinched closed by manually squeezing the catheter handle assembly (Chuttani et al., 2006). The endoscopic clipping device was introduced by Olympus Corporation (Tokyo, Japan). The terms ''endoclip'' and ''hemoclip'' have been used for this device. The delivery/deployment catheter consists of a metal cable within a metal coil sheath, enclosed within a 2. 2 mm Teflon catheter. The tip of the metal cable has a hook onto which the clip is attached. A handle consisting of 2 sliding components controls loading and deployment. A rotation mechanism on the handle allows directed orientation of the clip. The clips are 1. 2 mm wide multiangled stainless steel ribbons with an opening angle of 90 degrees or 135 degrees. Clips open from 6 mm to 12 mm, depending on the specific clip.The clips are configured to be withdrawn into the outer Teflon sheath for delivery through the endoscope accessory channel (minimum 2.8 mm). The device may then be removed and additional clips loaded and the process repeated. Precision in clip loading and deployment are required for effective use.

Difficulties and Complications:

Clipping is easiest when the endoscope can be kept in a straight position, with the possibility of axial push into the tissue. Tangential access to the lesions sometimes results in poor anchoring of the clip in the gastric wall. The fundic region can also be a challenge, because the firing mechanism is often weakened when the scope tip is retroflexed. Clips also work poorly through the working channel of a duodenoscope, and the elevator must be minimally engaged to allow release of the clip .

APC in Group II:

Argon Plasma Coagulation (APC) was done using an argon plasma coagulator unit(TERNO ABC TOM 201, Germany). Spray mode was used with 2 power/gas settings (respectively, 40 and 70 W and 1.5 to 3 L/min). Probe of 2.3 mm was used with endoscopes with corresponding channel diameters (2.8- mm diameter accessory channels). The maximum coagulation depth achieved by APC is 3 to 4 mm, which minimizes the risk of perforation. Continuous suction was applied to remove smoke and prevent overinflation of the GI tract.The APC apparatus includes a high-frequency monopolar electrosurgical generator, source of argon gas, gas flow meter, flexible delivery catheters, grounding pad, and foot switch to activate both gas and energy. Probes are available that direct the plasma parallel or perpendicular to the axis of the catheter. APC systems (ERBE Elektromedizin, Tübingen, Germany; and Conmed, Utica, N.Y.) include an electrosurgical unit that generates a high frequency electrical current, an argon gas cylinder, and a gas flow meter. Disposable probes for endoscopic application consists of a flexible teflon tube with a tungsten monopolar electrode contained in a ceramic nozzle located close to its distal end. APC probes are available in a variety of diameters and lengths (2.3 mm

OD [220 cm, and 440 cm length], and 3.2 mm OD [220 cm length]). A foot switch synchronizes argon gas release with the delivery of electrical current. Generators deliver an output voltage of 5000-6500 V.

Follow up: After endoscopy, all patients were closely monitored clinically for one weak looking for symptoms and signs of bleeding . All patients received the same proton pump inhibitor, and Blood transfusion was given to maintain the hemoglobin level above 8g/dL. Clinical recurrent bleeding was defined as signs of bleeding: vomiting of fresh blood, passage of melena with pulse rate higher than 100beat/min,decrease in systolic blood pressure exceeding 30mmHg, after the early stabilization of pulse, blood pressure, and or decrease in hemoglobin concentration by at least 2 g/dL over a 24-hour period. In case of rebleeding endoscopy was repeated as an emergency procedure and the same primary endoscopic management was used. Indications for surgery ; where failed endoscopic treatment on second endoscopy, recurrence of bleeding after a second therapeutic endoscopy, or a total blood transfusion requirement of greater than 8 units to maintain a hemoglobin level of 10 g/dL.Results:Data Management: Data were collected, revised, verified then edited on P. C. Data were then analyzed statistically using SPSS statistical package version 15. The following tests were done .Student t-test = Unpaired Student T-test was used to compare between to two groups in quantitative data.-Chi–square test = the hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship.-Mann whitney = A nonparametric equivalent to the t test

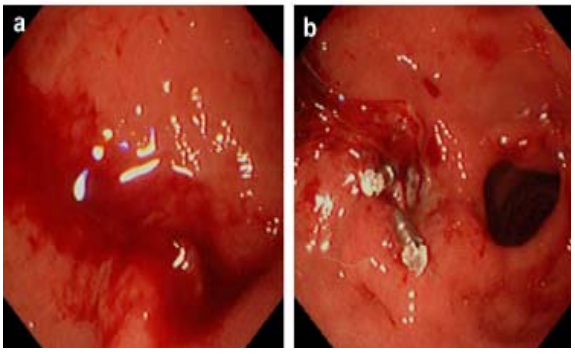

Figure 1. Showing control of bleeding of bleeding gastric ulcer after 2 hemoclip application in patient of clip application group .

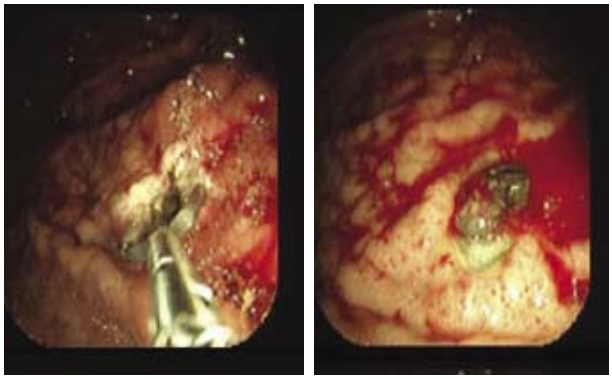

Figure 2. Showing control of bleeding gastric ulcer (blood clot) after hemoclip application in patient of clip application group)

Table (3) shows insignificant difference between the 2 groups regarding the presence of ulcer with visible vessel.(P-value was >0.05 which is non significant).

		Ulcer With Visible Vessel		
		Negative	Positive	Total
Group I	N	13	2	15
	%	86.67	13.33	100.00
Group II	N	12	3	15
	%	80.00	20.00	100.00
Chi-square	X2	0.240		
	P-value	> 0.05 (N.S)		

		Ulcer With Oozing Vessel		
		Negative	ositive	Total
Group I	N	8	7	15
	%	53.33	46.67	100.00
Group II	N	11	4	15
	%	73.33	26.67	100.00
Chi-square	X^2	1.292		
	P-value	> 0.05 (N.S)		

Table 4. Shows insignificant difference between the 2 groups regarding the presence of ulcer with oozing vessel. (P-value was >0.05 which is non significant).

		Forrest's Classification				
		IA	**IIA**	**IB**	**IIB**	**Total**
Group I	**N**	0	2	7	6	15
	%	0.00	13.33	46.67	40.00	100.00
Group II	**N**	5	3	4	3	15
	%	33.33	20.00	26.67	20.00	100.00
Chi-square	**X^2**	8.981				
	P-value	<0.05 (S)				

Table 5 Shows significant difference between the 2 groups regarding Forrest's classification.(P-value was <0.05 which is significant

Group	**Rockall's Score**				**T-test**	
	Range		**Mean**	**SD**		**P-value**
Group I	3.000	8.000	4.933	1.668	-0.585	> 0.05 (N.S)
Group II	3.000	9.000	5.333	2.059		

Table 6 Shows insignificant difference between the 2 groups regarding the Rockall'S Score. (P-value was > 0.05 which is non significant).

		Rebleeding		
		Negative	**Positive**	**Total**
Group I	**N**	12	3	15
	%	80.00	20.00	100.00
Group II	**N**	10	5	15
	%	66.67	33.33	100.00
Chi-square	**X^2**	0.682		
	P-value	> 0.05 (N.S)		

Table 8 Shows insignificant difference between the 2 groups regarding occurrence of rebleeding. (P-value was >0.05).

Group	Rebleeding		Forrest's Classification					Chi-square	
			I A	IIA	IB	IIB	Total	X2	P-value
Group I	Negative	N	0	0	6	6	12	9.643	< 0.01(H.S)
		%	0.00	0.00	40.00	40.00	80.00		
	Positive	N	0	2	1	0	3		
		%	0.00	13.33	6.67	0.00	20. 0		
Group II	Negative	N	3	3	2	2	10	2.100	> 0.05 (N.S)
		%	20.00	20.00	13.33	13.33	66.67		
	Positive	N	2	0	2	1	5		
		%	13.33	0.00	13.33	6.67	33.33		

Table 9. Shows highly significant difference in rebleeding incidence in different Forrest's classes in group I.(P-value was <0.01).while there was insignificant difference in rebleeding incidence in different Forrest's classes in group II (P-value was >0.05).

Rockall's Score	Rebleeding					
	Negative		Positive		T-test	
	Mean	SD	Mean	SD	t	P-value
Group I	4.417	1.240	7.000	1.732	-3.014	< 0.05 (S)
Group II	5.900	2.234	4.200	1.095	1.587	> 0.05 (N.S)

Table 10. Shows significant occurrence of rebleeding with higher Rockall's Score in group I . (P-value was <0.05). While there is insignificant occurrence of rebleeding with higher Rockall's Score in group II (P-value was >0.05).

Rockall's Score		Rebleeding		
		Negative	Positive	Total
<5	N	10	2	12
	%	33.33	6.67	40.00
≥5	N	12	6	18
	%	40.00	20. 00	60.00
Chi-square	X 2	1.02		
	P-value	> 0.05 (N.S)		

Table 11. Shows insignificant relation between <5 or ≥5 Rockall's score and the occurrence of rebleeding in the whole patient population. (the P-value was >0.05). There was also insignificant difference in the 2 groups regarding occurrence of rebleeding in relation to the site of ulcer GU or DU. (P-value was >0.05).

Forrest's Classification	No of Clips							
	Clips 2		Clips 3		Total		Chi-square	
	N	%	N	%	N	%	X2	P-value
IIA	1	6.67	1	6.67	2	13.33	0. 877	>0.05 (N.S)
IB	5	33.33	2	13.33	7	46.67		
IIB	5	33.33	1	6.67	6	40.00		

Table 12. Shows insignificant relation between the number of needed clips and forrest classification in group I. (P-value was > 0. 05).

	No of CLIPS					
	2		3		T-test	
	Mean	SD	Mean	SD	t	P-value
Rockall's score	4.727	1.679	5.500	1.732	-0.783	> 0.05 (N.S)

Table 13. Shows insignificant relation between the number of clips in relation to Rockall'S Score group I. (P-value was >0.05).

DISCUSSION

Peptic ulcer bleeding is the most common cause of upper gastrointestinal bleeding, responsible for about 50% of all cases Mortality is increasing with increasing age and is significantly higher in patients who are already admitted in hospital for co-morbidity. Risk factors for peptic ulcer bleeding are NSAIDs use and H. pylori infection (van Leerdam, 2008).In patients with ulcers presenting with ongoing bleeding or high risk features (Forrest I, IIa, IIb), surgery was frequently required in the past to solve the situation. However, endoscopic therapy has been well documented to treat these ulcers. (Aabakken, 2008).The timing of the initial endoscopy has been debated. In general, red hematemesis indicates emergency upper endoscopy, while black hematemesis and/or melena without haemodynamic instability can wait until normal working hours. However, from a logistic point of view, early endoscopy has been advocated to ensure optimal utilisation of resources .In this study there is no significant difference in both groups regarding age, shock, presence of comorbid illness or liver cell failure, ulcer size, rockall score and site of ulcer; factors known to affect prognosis in many previous studies.the study showed that the rate of rebleeding was slightly higher in APC group despite of being statistically insignificant.Also there was no significant relation between the rate of rebleeding and the size of the ulcer..Few reports have concerned the indication for and efficacy of each hemostatic therapy according to location,depth,and size of ulcer and bleeding activity of the exposed vessel. if the ulcer is large or deep,the possibility of complications including further ulceration,recurrence of bleeding,and perforation is high and great care is required in performing the procedure if the bleeding ulcer is located on the posterior wall or lesser curvature of the gastric body or on the posterior wall of the duodenal bulb, the hemostatic rate is lower than for other therapies because of the technical difficulty of approaching the lesion. Chung et al. (1999)In the present study although there was no statistical significance difference in rebleeding incidence in both groups there was highly significant difference in rebleeding incidence in relation to different Forrest's classes in group I.(P-value was <0.01 which is highly significant).while there was insignificant difference in rebleeding incidence in different Forrest's classes in group II.Also, the rate of surgical interference of both groups was 0% In recent years, the Rockall score has been used to select patients with a low risk of re-bleeding for early discharge. Almost all patients in this low risk group belong to patients without any stigmata of recent hemorrhage (SRH). However,

patients with a SRH are a high-risk group for further re-bleeding and also mortality. It is therefore important to determine whether the Rockall score could be useful in patients who have undergone endoscopic therapy for UGIB, to identify high-risk patients and thus improve their management and outcome (Bessa et al., 2006).In the present study we assessed correlation between high risk Rockall's score (>5) and occurrence of rebleeding, which rebleeding is 6.67% in low risk Rockall's score(<5). While rebleeding is 20.00% in high risk Rockall's score (≥5). However, this is statistically non significant. but incidence of rebleeding in relation to high risk Rockall's score is significant in group I. This did not go in agreement with *Saperas et al. (2008)*, who concluded that the Rockall scoring system accurately identifies patients at high risk of death, but not of rebleeding. Inspite that our study partially goes with *Church and Palmer(2001)* who observe good correlation between the Rockall score and both the probability of re bleeding and mortality in patients undergoing endoscopic therapy for peptic ulcer hemorrhage.In the present study the mortality rates between the two groups were the same which is 0% in the two groups despite of significantly higher need for surgery in group II .This goes with Sung et al. (2007) and Chung et al. (1999) who concluded that there was no difference in all-cause mortality irrespective of the modalities of endoscopic treatment. Sung et al. (2007)in a meta-analysis of 15 studies reported that regardless of improvements in sustaining hemostasis by clipping leading to less rebleeding and fewer interventions with surgery, mortality has not been reduced. and there is no indication of a reduction in the death rate. Nevertheless, it is a mystery that despite successful control of hemorrhage in many studies using various combinations of endoscopic and pharmacological therapies, the mortality rate remains unchanged..Conclusion: Endoscopic application of hemoclips have a less rebleeding rate than Argon plasma coagulation for treatment of bleeding peptic ulcer ,although this was statistically insignificant .Meanwhile APC is still less costy ,and easy for jonyor endoscopists in emergency units. Clinical and endoscopic assessment (through Rokal score and Forrest classification)could help in making best choice for endoscopic manageme

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Antimutagenesis of vitamin AD3E mixture to mutations induced by flouroquinolone drug ciprofloxacin on mice

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Abstract: Recently, considerable interest has developed regarding the presence of pharmaceutical in the environment. Human, livestock, birds, aquatic animals, plants and different organisms have been shown to be adversely affected by drugs persisting in soil and water. The genotoxic effect is one of the serious risks. The flouroquinolones are one of the main classes of antimicrobial drugs used in the worldwide. The aim of this study was undertaken to investigate antimutagenic effect of vitamin AD3E mixture against the genetic damage induced by ciprofloxacin (CFX) drug. The following genetic endpoints were used: 1- Cytogenetic chromosome analysis in somatic and germ cells, 2- DNA fragmentation assay in mouse spleen cells. The results obtained in this study showed that vitamin AD3E inhibit the DNA damage induced by CFX in dose and time dependent in compared to the CFX alone. The results indicated that vitamin AD3E has antimutagenic effect against genetic damage induced by CFX drug. [Nature and Science 2009; 7(12): 61-71]. (ISSN: 1545-0740).

Key words: Antimutagenicity, Vitamin AD3E, Ciprofloxacin, DNA damage.

Introduction:

Quinolones are currently one of the main classes of antimicrobial used worldwide. The clinical use of quinolones is not restricted to human medicine but is also widely applied in the treatment and prevention of veterinary diseases in food-producing animals, and even as growth-promoting agents **(Greene and Budsberg, 1993; Martinez *et al,* 2006**). Different studies were carried out concerning the genotoxic effect of quinolones. **(McQueen *et al,* 1991; Shimada and Itoh 1996; Enzmann *et al,* 1999).**

Ciprofloxacin (CFX) is an extended spectrum antimicrobial drug belongs to fluroquinolones (**McKellar *et al,* 1999)**. It acts as bactericidal by altering the action of bacterial DNA gyrase, a type II topoisomerase that responsible for supercoiling of bacterial DNA (**Vancutsem *et al,* 1990).** Inhibition of this activity is associated with rapid cell death in bacteria **(Hussy *et al,* 1986).**

CFX is the ultimate reactive metabolite of enrofloxacin (EFX) converted by the cytochrome P-450 enzymes (**Vaccaro *et al,* 2003).** It has shown to be mutagenic in TA102 strain of Salmonella (**Gocke, 1991)**. Positive results of CFX in human and animals *in vitro* (**Curry *et al,* 1996; Itoh *et al,* 2006)** and *in vivo* **(Takayama *et al,* 1995**; **Ikbal *et al,* 2004)** were observed. The cytotoxicity of CFX was evaluated in cultured human peripheral blood lymphocytes in patients treated with the drug *in vivo* (**Ikbal *et al,* 2004). Gürbay *et al,* (2005)** showed that this drug induced cytotoxicity and apoptosis in Hela cells. Also **Gürbay *et al,* (2007)** observed that CFX-induced cytotoxicity in rat astrocytes.

Different studies revealed teratogenic and fetotoxic effects of CFX. **Loebstein *et al* (1998)** observed that women treated with CFX had a tendency for an increased rate of therapeutic abortions. **Channa and Janjua (2003)** strongly suggested that CFX, given during pregnancy, causes sever liver damage in fetuses of Wistar albino rats. This finding was further supported by **Minta *et al* (2005).**

There is general agreement that the Mediterranean diet riches with vitamins. This diet contributes to the prevention of various chronic degenerative diseases such as cardiovascular diseases and cancer **(Zhang *et al,* 2009).** Vitamin AD3E mixture was used in this study as antimutagenic agent against DNA damage induced by CFX drug. Vitamin A (VA) is one of the most important nutrients essential for normal growth and differentiation **(Emura *et al,* 1988**). VA is found in liver, eggs, milk, butter, carrots, vegetables, orange and yellow fruits **(Haslett *et al,* 1999).** It is vital to eye and retina function, regulates multiple biological processes, including cell proliferation, differentiation, and death. So it plays critical roles in embryonic development **(Louis, 1986; Emura *et al,* 1988).**

Table (1): Detailed results of chromosome aberrations induced in mouse bone - marrow treated with CFX and CFX with vitamin AD3E.

Treatment	Doses (mg/kg b.wt.)	Total abnormal aberrations				Types of chromosome aberrations						
		NO	Including gap (mean %±S.E.)	Excluding gaps (mean %±S.E.)	II % (Excluding gaps)	Gaps	Break and// or Frag	Del.	RT.	End.	Poly.	MA.
Control	—	22	4.40±0.24	2.00±0.31		12	7	3	–	–	-	-
MMC	1	141	28.20±0.37**	23.0±0.31**		26	71	5	4	9	6	20
Single AD3E	70	23	4.60±0.53	2.60±0.57								
	140	21	4.20±0.20	2.20±0.58		10	11	1	-	-	-	1
CFX	65	39	7.80±0.37**	4.40±0.40**		10	8	3	–	–	–	-
	130	55	11.00±0.54**	6.60±0.60**		17	18	4	-	-	-	-
	260	68	13.60±0.87**	8.20±0.52**								
CFX+AD3E	260+70	57	11.40±O.60	5.80±0.58♦	29.30	22	24	4	-	3	1	1
	260+140	47	9.40± 0.50♦♦	4.80± 0.55♦♦	41.50							
Repeated 1 week AD3E	70	20	4.00±0.43	2.20±0.50		27	27	5	-	4	1	4
	140	18	3.60±0.30	2.00±0.58		28	24	2	-	1	-	2
CFX	130	80	16.00±0.70**	10.80±0.86**		23	18	2	-	3	-	1
CFX+AD3E	130+70	59	11.80±0.40♦♦	7.00±0.66♦	35.20							
	130+140	52	10.40± 0.52♦♦	6.20± 0.58♦♦	42.60	9	8	1	-	-	-	2
2 weeks AD3E	70	21	4.20±0.48	2.20±0.50		8	8	2	-	-	-	–
	140	22	4.40±0.40	2.00±0.58		26	40	4	1	3	-	6
CFX	130	106	21.20±0.50**	4.20±0.60**								
CFX+AD3E	130+70	69	13.80± 0.58♦♦	7.40± 0.50♦♦	47.90	24	29	2	-	2	-	2
	130+140	55	11.00± 0.54♦♦	6.20± 0.31♦♦	56..30	21	27	2	-	1	-	1
						10	8	1	-	-	-	2
						12	7	2	-	-	-	1
						35	55	4	1	3	1	7

			32 3	27	2	-	4	1
			24 1	23	3	-	2	2

500 metaphases examined in five mice per treatment. RT. = Robertsonian translocation , Del.=Deletion, End.= Endomitosis, Poly.= Polyploidy MA=metaphases with more than one aberrations
**** Highly significant $p < 0.01$ level (t-test) comparing to control.**
♦Significant $p < 0.05$ level ♦♦Highly significant $p < 0.01$ level (t-test) comparing to treatment.

Table (2): Frequency of sister chromatid exchanges (SCEs) in mouse bone-marrow cells treated with CFX.

Treatment and Dose (mg/kg b. wt.)	No. of different types of SCEs/chromosome			Total No. of SCEs	SCEs/Cell Mean%±SE
	S	D	T		
Control	595	49	-	693	4.62±0.40
MMC 1	1521	360	48	2385	15.90±0.72**
CFX					
65	663	66	5	810	5.40±0.53**
130	1027	133	9	1320	8.80±0.25**
260	1272	145	12	1598	10.65±0.53**

The total number of scored metaphases is 150 (5 animals/ group) S= Single D= Double T= Triple
**** Highly significant $p < 0.01$ level (t-test).**

A considerable wealth of research data has been accumulated regarding the efficacy of VA as an antimutagenic **(Antunes *et al,* 2005; Wang *et al,* 2006)** and anticarcinogenic agent **(Toma *et al,* 1998; Simeone *et al,* 2005).**

Vitamin A could have three mechanisms of action. First, an antioxidant action which leads VA to protect the genome against free radicals **(Antunes *et al,* 2005).** Secondly, it has been shown that VA presents a selective inhibition of the mutagen metabolic activation pathway catalyzed predominantly by hepatic microsomal cytochrome P450 dependent monooxygenase system **(Decoudu *et al,* 1992)**.Thirdly, VA may interact with DNA and so could protect the genome towards reactive intermediates (**Decoudu *et al,* 1992**).

Vitamin D_3 (VD3) plays a major role in mammalian calcium and phosphorus homeostasis and bone health. VD_3 exerts pleiotropic effects on cell proliferation, differentiation and the immune system **(DeLuca, 2004).** VD_3 has direct anti-inflammatory properties on microglial cells **(Lefebvre d'Hellencourt *et al,* 2003)**.

The biologically active metabolite of VD (1, $25(OH)_2D_3$) may play an important role in human cancer. Increased risk of breast, prostate and colon cancer have been associated with reduced serum concentration of $1,25(OH)_2D_3$ **(Studzinski and Moore, 1995).** The antimutagenic activity of VD3 was evaluated by several authors (**Sarkar *et al,* 2000; Dusso *et al,* 2004**). The protection effect of VD3 may be attributed to its ability to detoxification of the endo- and xenobiotics (**Kutuzova and DeLuca, 2007).**

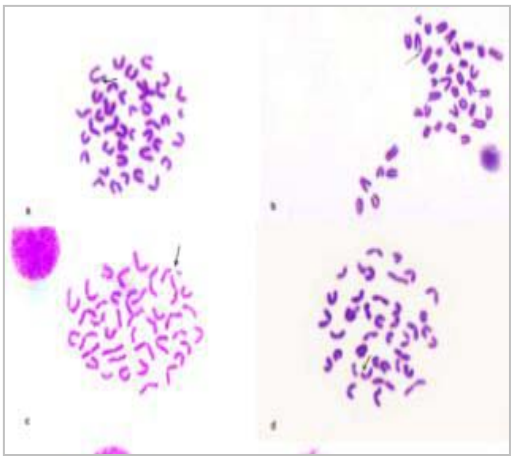

Fig. (1): Metaphase plates from mouse bone marrow cells after treatment with ciprofloxacin showing, a) Gap; b) Fragment; c) Break; d) Deletion.

Vitamin E (VE) is an essential element of human nutrition. Many of its actions are related to its antioxidant properties **(Louis, 1986).** The antioxidant action of VE is also significant to the genetic material stability because autoxidation products of lipids and unsaturated fatty acids are highly toxic mutagenic substances **(Vaca *et al,* 1988).** Many *in vitro* and *in vivo*

studies have indicated a relationship between VE supplementation and reduced risk of cancer **(Albanes *et al,* 2000; Kune and Watson, 2006)** and DNA damage **(Mozdarani and Salimi, 2006**; **Lorenzetti *et al,* 2007).**

The object of the present study was undertaken to evaluate the antimutagenic effect of vitamin AD3E against mutations induction by CFX drug. Taking into account the possible benefit of this therapeutic drug.

Materials and methods

Animals

Male white Swiss mice aged 9–12 weeks were used in all experiments. The animals were obtained from a closed random-bred colony at the National Research Centre. The mice used for any one experiment were selected from mice of similar age (±1 week) and weight (±2 g). Animals were housed in polycarbonate boxes with steel-wire tops (not more than five animals per cage) and bedded with wood shavings. Ambient temperature was controlled at 22±3 ◦C with a relative humidity of 50±15% and a 12-h light/dark photoperiod. Food and water were provided ad libitum. Animals were sacrificed after treatment by cervical dislocation.

Table (3): Number and mean percentage of diakinase metaphase I cells with chromosome aberrations in mouse spermatocytes treated with CFX and CFX with vitamin AD3E.

Treatment	Doses (mg/kg b.wt.)	Total abnormal aberrations			Types of chromosome aberrations				
		NO	Mean %±S.E.	Inhibitory Index %	X-Yuniv Chain (IV)	AU	X-Yuniv + AU	Frag or Break	Frag or Break + X-Yuniv
Control	—	15	3.00±0.31		11	3	1	–	–
MMC	1	108	21.6±1.16**		52	29	10	6	3
Single AD3E	70	15	3.00±0.53						
	140	16	3.20±0.20		10	5	-	-	-
CFX	65	19	3.80±0.37		10	6	-	–	–
	130	26	5.20±0.24**		14	5	-	–	-
	260	31	6.20±0.37**		18	7	-	1	-
CFX+AD3E	260+70	25	5.00±O.44	19.40	19	8	1	1	1
	260+140	23	4.60± 0.24♦	25.80	16	6	1	1	1
Repeated 1 week AD3E	70	17	3.40±0.40		17	5	-	1	-
	140	16	3.20±0.38						
CFX	130	48	9.60±0.50**		9	8	-	-	-
CFX+AD3E	130+70	30	6.00±0.54♦♦	37.50					
	130+140	24.	4.80± 0.42♦♦	50.00					
2 weeks									

AD3E	70	15	3.00±0.40		13	3	-	–	–	–
	140	17	3.40±0.42		30	15	-	3	-	-
CFX	130	59	11.80±0.58**		21	6	1	1	-	1
CFX+AD3E	130+70	35	7.00± 0.51♦♦	40.70	17	4	-	2	1	-
	130+140	27	5.40± 0.50♦♦	54.20	10	5	-	-	-	-
					12	5	-	–	–	–
					33	16	5	2	1	2
					23	12	-	-	-	-
					14	13	-	-	-	-

500 metaphases examined in five mice per treatment. X-Y univ. = X-Y univalent , A.U.= Autosomal univalent , Frag.= Fragment
**** Highly significant $p < 0.01$ level (t-test) comparing to control**
♦Significant $p < 0.05$ level ♦♦Highly significant $p < 0.01$ level (t-test) comparing to treatment.

Table (4): Percentage of sperm abnormalities induced in male mice after oral treatment with different doses of CFX.

Treatment and doses (mg/kg bw)	No of examined sperm	Abnormal sperm		Number of sperm head abnormalities					Coiled tail
		No	Mean %±S.E.	Amorphous Big	Without hook	Triangle	Banana	Small	
Control	5092	79	1.55± 0.40	25	12	28	2	1	11
MMC 1	5017	784	15.60± 0.51**	185	145	177	25	9	237
CFX									
65	5252	110	2.10± 0.31						34
130	5350	243	4.50± 0.27**	27	13	33	3	-	88
260	5292	281	5.30 ± 0.31**	42	31	70	8	3	86
				53	40	84	10	6	

**** Highly significant $p < 0.01$ level**.

Chemicals

Ciprofloxacin (CFX) was purchased from Amoun Pharmaceutical Co., Egypt and vitamin AD3E mixture was purchased from Alwatanya Co., Egypt.

Treatment and cytological preparations

Chromosome aberrations in bone marrow cells.

For analysis of bone marrow cells mice were orally treated (using a stomach tube) with a single dose of CFX at doses of 65,130(therapeutic dose) and 260 mg/kg b.wt. Other groups of mice were given 70 and 140 mg/kg b.wt.AD3E, simultaneously with the highest dose of CFX. Samples were taken 24 h after treatment.

For the repeated dose experiment, mice received daily oral doses of 130 mg /kg b.wt. CFX for 1 and 2 weeks (7 and 14 days). Samples were taken 24 h after the last treatment. In the repeated dose treatments, other groups of mice were given 70 and 140 mg/kg b.wt.AD3E, simultaneously with the CFX. A negative (non-treated) and positive with 1 mg/kg b.wt. mitomycin C groups of mice was tested. In addition, another group of mice was given the oral doses of AD3E (70 and 140 mg/kg b.wt.) for 2 weeks.

Bone marrow preparations were made according to the technique described by **Yosida and Amano (1965).** A group of five mice was used for each treatment and 100 well-spread metaphases were analyzed per animal for scoring of different kinds of abnormalities including gaps, breaks, fragments, deletions, Robertsonian translocations , endomitosis and polyploidy metaphases.

Sister chromatid exchanges (SCEs).

For analysis of SCE's, mice were orally treated with a single dose of CFX at doses of 65,130 and 260 mg/kg b.wt.. Another group of mice used as control. Samples were taken 24 h after treatment. The method described by **Allen (1982)**, for conducting *in vivo* SCE's induction analysis in mice was applied with some modifications. The fluorescence-photolysis Giemsa technique was used **(Perry and Wolff, 1974)**. The frequency of SCE's was recorded for each animal in 30 well spread metaphases for SCE's /cell.

Chromosome aberrations in spermatocytes.

For analysis of spermatocytes mice were treated as in chromosome aberrations in bone marrow cells above. Chromosomal preparations from testes were made according to the technique developed by **Evans *et al* (1964**) and 100 well-spread diakinesis metaphase-I cells were analyzed per animal to assess abnormalities in five mice per group. Metaphases with univalents, chromosome breaks and/or fragments and chain (IV) were recorded.

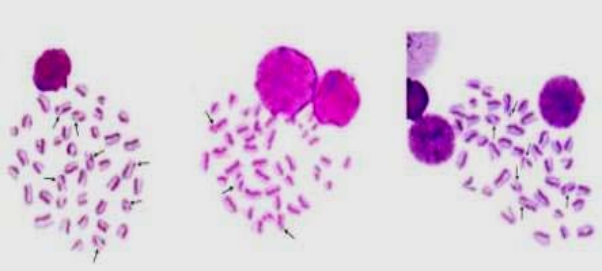

Fig (2): Metaphase plates with sister chromatid exchanges from mouse bone marrow cells after treatment with ciprofloxacin.

Sperm-shape abnormalities

Groups of five mice were orally treated with CFX daily for five consecutive days at dose levels of 65,130 and 260 mg/kg b.wt. Animals were sacrificed 35 days after the first treatment by cervical dislocation. Sperm from negative (non-treated) and positive with 1 mg/kg b.wt. mitomycin C was tested. Sperm were prepared according to the recommended method of **Wyrobek and Bruce (1978**). The epididymides were excised and minced in 2ml physiological saline, dispersed and filtered to remove large tissue fragments. Smears were prepared and stained with 1% Eosin Y.

DNA Fragmentation Assay.

For DNA fragmentation assay CFX with the doses 130 and 260 mg/kg b.wt. were used as single doses and 130 mg/kg b.wt. for 7 and 14 days as repeated treatment. Vitamin AD3E at 140 mg/kg b.wt. was used with the highest single dose and repeated dose for 14 days of CFX.

1- DNA Fragmentation % (DPA Assay).

The colorimetric estimation of DNA content was detected according to **Perandones *et al* (1993**) with some modifications. Both supernatant and the pellet were used for DPA assay after acid extraction of DNA. The percentage of DNA fragmentation was expressed by the formula: % DNA fragmentation

$$= \frac{\text{O.D. of supernatant}}{\text{O.D. of supernatant + O.D. of pellet}} \times 100$$

2- DNA fragmentation (agarose gel electrophoresis).

The method of DNA fragmentation was carried out according to **Perandones *et al* (1993).**

Statistical analysis.

The significance of the difference between groups and negative control and between CFX with AD3E against CFX alone was calculated using the t-test.

Results

Chromosome aberrations in bone marrow cells. Table (1) and Fig.(1) present chromosomal aberrations induced in bone marrow cells after single and repeated oral treatments with different doses of CFX. The results showed that the tested doses of CFX induced a statistically significant increase in the percentage of chromosomal aberrations even after excluding gaps. Such percentage was found to be dose- and time-dependent. The results in Table 1 also demonstrate that the percentage of chromosomal aberrations in bone marrow cells was significantly reduced in all groups of mice treated simultaneously with AD_3E at 70 and 140 mg/kg b.wt. and CFX at the tested dose levels.

Table (5): Mean percentage of DNA fragmentation induced in mouse spleen cells after concurrent treatment with CFX and CFX plus vitamin AD_3E

Treatment	Doses (mg/kg b.wt.)	DNA fragmentation Mean% ±S.E.	DNA fragmentation Inhibition %
Control (Non-treated)	-	3.12±0.28	
MMC (positive control)	1	15.33±0.47**	
Single dose AD_3E	140	2.75±0.45	
CFX	130	6.20±0.28**	
	260	7.12±0.32**	
$CFX+AD_3E$	260+140	3.97±0.21♦♦	44.20
Repeated dose AD_3E	140×14 days	2.95±0.27	
CFX	130×7 days	7.78±0.30**	
	130×14 days	8.48±0.38**	
$CFX+AD_3E$	130×14days+140	4.4±0.24♦♦	48.10

No of animal= 5 animal/group.
**** Significant at 0.01 level (t-test) comparing to control (non-treated).**
♦♦ Significant at 0.01 level (t-test)

Table 1 show that successive treatment of mice for 14 days with CFX and vitamin AD_3E significantly reduced the percentage of chromosomal aberrations. It reached 47.90 and 56.30 % reduction in the percentage of chromosome damage after treatment with the two doses of AD_3E respectively.

Sister chromatid exchanges (SCEs).

Table (2) and Fig. (2) showed a detailed study of the effect of single oral treatment with different doses of CFX (65, 130 and 260 mg/kg b.wt.) on the induction of sister chromatid exchanges in mouse bone marrow cells 24 h. after treatment. The percentage of SCE's increased with increasing the dose of the drug. It reached 8.80±0.25 and 10.65±0.53/cell ($P<0.01$) after treatment with 130 and 260 mg/kg b.wt. respectively compared with 4.62 ± 0.40 /cell for control (Table 2).

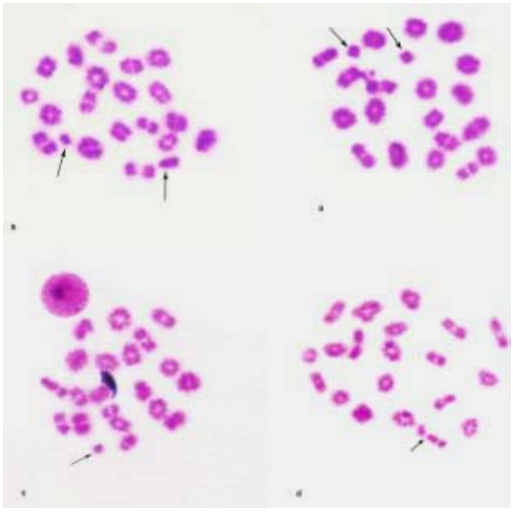

Fig (3): Diakinesis-metaphase I plates of meiosis after treatment with ciprofloxacin showing, a) X-Y univalent; b)Autosomal univalent; c) Fragment; d) Break.

Chromosome aberrations in spermatocytes.

CFX at the tested doses induced a significant percentage of chromosomal abnormalities in mouse spermatocytes (Table 3 and Fig. 3). This percentage increased with increasing dose and with longer duration of treatment, and it reached a maximum of 11.80±0.58 after repeated treatments for 2 weeks with the tested dose (130 mg/kg b.wt.). It decreased to 7.00± 0.51 and 5.40± 0.50 in the CFX -treated groups that also received AD_3E at doses 70 and 140 mg/kg b.wt. respectively.

Morphological sperm abnormalities.

The percentage of sperm abnormalities reached 2.10, 4.50 and 5.30% after treatment with the three tested doses respectively compared with 1.55% for the control group (Table 4). Table (4) and Fig. (4) also illustrates the number and different types of sperm abnormalities after oral treatment with different doses of CFX. The dominant abnormalities found were amorphous, triangle head and coiled tail.

DNA Fragmentation assay.

1- DNA Fragmentation % (DPA Assay).

Table (5) demonstrated the mean percentage of DNA fragmentation induced in mouse spleen cells after concurrent administration of antibiotic drug and antibiotic drug with vitamin. Administration of AD_3E (140 mg/kg b.wt.) decreased the percentage of DNA fragmentation induced by the highest single dose of CFX reached 3.97 % compared to 7.12% for the group treated only with CFX. For repeated dose treatment for 2 weeks the percentage of DNA fragmentation was decreased to 4.40% ($P<0.01$) after concurrent treatment of CFX with AD_3E compared to 8.48% for CFX alone (Table 5).

2-DNA fragmentation (agarose gel electrophoresis).

DNA fragmentation induced by CFX assessed by agarose gel electrophoresis was decreased after simultaneous treatment with the dose 140 mg AD_3E/kg b.wt compared to that treated with 260 mg CFX/kg b.wt. as single dose and 130 mg CFX/kg b.wt. as repeated dose for 14 days (Fig. 5) **.**

Discussion

The fluoroquinolones are a class of compounds that comprise large and expanding groups of synthetic antimicrobial agents (**Van Bambeke *et al,* 2005).** CFX drug belongs to flouroquinolone and is the main active metabolite of EFX **(Vaccaro *et al,* 2003)**. In this study CFX induced elevation in the chromosomal aberrations in bone marrow cells and spermatocytes in dose and time dependent, comparing to the negative control. These finding run in agreement with results of **Mukherjee *et al* (1993)** who observed that doses of 0.6, 6.0 and 20 mg CFX/kg b.wt. given intraperitoneally induced a positive dose-dependent chromosomal aberrations in mouse bone marrow cells. **Basaran *et al* (1993)** observed that administration of 20 and 200 mg CFX/kg b.wt. to rats significantly induced chromatid breakage in a dose-dependent manner. Also, CFX exerted cytotoxic effects in human fibroblast cells depending mainly on the concentration and the duration of exposure.

Also our results showed that CFX induced a significant and dose dependent elevation of SCE's in mouse bone marrow cells. These results are in agreement with those reported by **Mukherjee *et al* (1993)** and **Ikbal *et al* (2004)**. They found that CFX has the ability to induce SCE's in mouse and human lymphocytes respectively in dose dependent manner.

According to the present study, the mean percentage of sperm shape abnormalities increased by increasing the dose of CFX. **Merino and Carranza-Lira (1995)** observed that treatment of patient with CFX did not reduce sperm quality but modified the accessory gland function. On the other hand, **King *et al* (1997)** found that this drug may decrease human sperm hyperactivation, adversely affect sperm motility and decrease rapid progression. Also, CFX at 150 mg/kg/day for 10 days induced decrease in testicular volume and sperm concentration in rats (**Demir *et al,* 2007)**.

The present work has shown CFX induced extensive damage in DNA of mouse spleen cells as determined by the DNA fragmentation assay. This damage was observed dose- and time-dependent. The resistance of DNA damage in spleen cells up to 14 days may be due to inhibition of Bcl-2 gene which act as antiapoptotic **(Gürbay *et al,* 2006)** and/or activation of some genes such as P53, Bax and caspase, which accelerates apoptosis **(Herold *et al,* 2002).** Our results are supported with **(Herold *et al,* 2002; Gürbay *et al,* 2006; Lim *et al,* 2008).** They observed that CFX induced inhibition of cell proliferation, DNA synthesis and apoptosis in mammalian cells in dose and time dependent manner.

DNA damage induced by CFX may be attributed to its ability to releasing oxygen free radicals **(Gürbay *et al,* 2006).** Oxygen free radicals attack DNA causing mutations **(Arriaga-alba *et al,* 2000).** In a trial to minimize the genotoxicity of CFX in somatic and germ cells of mice, vitamin AD_3E mixture was administered simultaneously with CFX. Our results showed that vitamin AD_3E inhibited DNA damage induced by CFX in all experimental tests. Many authors reported that vitamins A, D_3 and E have the ability to inhibit the mutagenicity or carcinogenicity induced by mutagens and/or carcinogens (**Ouanes *et al,* 2003, 2005**; **Gürbay *et al,* 2006, 2007**; **Arriaga-Alba *et al,* 2008)**.

The protective effect of vitamin AD_3E against DNA damage induced by CFX raises a question whether the AD_3E effect interfere with the CFX efficacy as bactericidal. **Arriaga-alba *et al*., (2000)** observed that the *in vitro* bactericidal effect of quinolones was not altered by β-carotene, which is free oxygen radical scavenger. However, the bactericidal effect of quinolones was due to inhibition of DNA gyrase enzyme (responsible for supercoiling of bacterial DNA) (**Vancutsem *et al*., 1990)**. According to the observation of **Arriaga-alba *et al*., (2000)**, we can presume that using vitamin AD_3E in this study, which is a free oxygen radical scavenger and/or detoxification of endo- and xenobiotics **(Louis, 1986; De Flora *et al*., 1999;**

Kutuzova and Deluca, 2007) may inhibit the power of CFX to induce genetic damage without interfering with its capacity as bactericidal.

In conclusion, the present work indicated that the antimicrobial drug CFX has a mutagenic effect in somatic and germ cells of mice. Vitamin AD_3E mixture might be a good alternative to reduce genotoxic risk associated with quinolones therapy. Further studies need to be conducted in order to determine if the vitamin AD3E can effectively inhibit genetic damage induced by CFX drug without effect on its action as bactericidal.

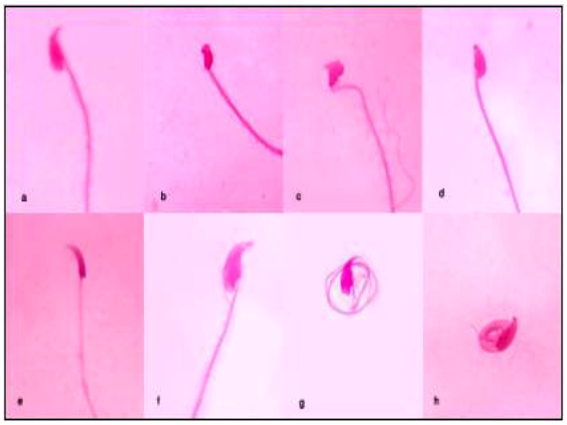

Fig (4): Sperm abnormalities induced in male mice after treatment with ciprofloxacin showing, a) normal; b) amorphous; c) Triangle; d) Without hook; e) Banana; f) Big head; g,h) Coiled tail.

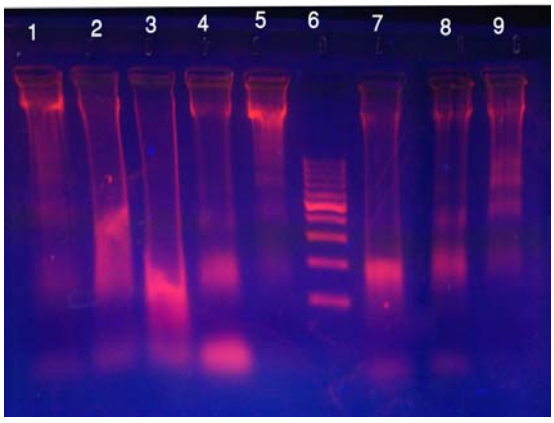

Fig. (5): Relationship between the DNA fragmentation induced in mouse spleen cells by ciprofloxacin and the protective effect of vitamin AD3E against ciprofloxacin : [**Lane 1:** Control (non-treated); **Lane 2:** Positive control (Mitomycin C); **Lanes 3,4:** Cells treated with 130x14d and 130x7d mg ciprofloxacin/kg b.wt. respectively; **Lane 5:** Cells treated with 130x14d.,mg ciprofloxacin/kg b.wt. with 140 mg AD3E/kg b.wt ; **Lane 6:** 100 b.p. DNA ladder; **Lanes 7,8:** Cells treated with 260 and 130 mg ciprofloxacin/kg b.wt. respectively**; Lane 9:** Cells treated with 260 mg ciprofloxacin/kg b.wt. with 140 mg AD3E/kg b.wt].

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The Anti-mutagenic Activity of Piperine against Mitomycine C induced Sister Chromatid Exchanges and Chromosomal Aberrations in Mice

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Abstract: Piperine is a major pungent substance and active component of black pepper (*Piper nigrum* Linn.) and long pepper (*Piper longum* Linn.). Regarding the variable biological activities of piperine, it is important to study its antimutagenic activity in somatic and germ cells *in vivo*. Swiss albino male mice were orally administered piperine at the doses of 5, 10 and 15mg/kg b. wt. for three consecutive days then treated with mitomycin C (MMC) interaperitonealy (i.p.) at a dose of 1mg/kg b. wt. Twenty-four hours thereafter, all animals were sacrificed and samples were collected from somatic and germ cells for chromosomal aberrations (CA) and sister chromatid exchanges (SCEs). Piperine inhibited the frequency of SCEs induced by MMC in bone marrow cells. This inhibition reached to 41.82% with piperine (15mg /kg b.wt.). The number of chromosomal aberrations induced by MMC in mouse splenocytes and spermatocytes decreased gradually with increasing the dose of piperine. The percentage of inhibition of chromosomal aberrations was 50% and 40.78% in splenocytes and spermatocytes respectively. In conclusion, the results of this *in vivo* study show that piperine has antimutagenic potential against carcinogens. Further investigations are required now to underlie the molecular mechanisms of piperine bioactivity. [Nature and Science 2009; 7(12): 72-78]. (ISSN: 1545-0740).

Keywords: Piperine, MMC, Chromosomal aberrations, SCEs, Spermatocytes, Mice.

1. Introduction

Piperine is an alkaloid found naturally in plants belonging to the Piperacese family, such as Piper nigrum Linn, known as black pepper, and Piper longum Linn, known as long pepper. It has been used extensively as a condiment and flavoring for all types of savory dishes (**Govindarajan, 1977**). Piper species have been used in folklore medicine for the treatment of various diseases, including seizure disorders (**Pei, 1983; Singh, 1992; D'Hooge *et al.*, 1996**). Regarding its structure (Figure 1), piperine contains pentacyclic oxindole group which is effective for immunomodulation (**Reinhart and Uncaria 1999; Pathak and Khandelwal, 2008**).

Piperine is known to exhibit a variety of biological activities which include anti-pyretic (**Parmar *et al.*, 1997**), anti-metastatic (**Pradeep and Kuttan 2002**), antithyroid (**Panda and Kar 2003**) and antidepressant (**Lee *et al.*, 2005**). Piperine exhibits a toxic effect against hepatocytes (**Koul and Kapil, 1993**) and cultured hippocampal neurons (**Unchern *et al.*, 1997**). Simultaneous supplementation with black pepper or piperine in rats fed high fat diet lowered thiobarbituric acid reactive substances (TBARS) and conjugated dienes levels and maintained superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S- transferase (GST) and glutathione (GSH) levels close to controls in rats (**Vijayakumar *et al.*, 2004**). **Selvendiran *et al.* (2005a, b**) observed that supplementation of piperine caused inhibition of Phase I and II enzymes, elevation of glutathione metabolizing enzymes, reduction in DNA damage and DNA protein cross-links in benzo (a) pyrene induced lung carcinogenesis in mice.

The anti-apoptotic efficacy of piperine has also been demonstrated by **Choi *et al.* (2007)** against cisplatin induced apoptosis via heme oxygenase-1 induction in auditory cells. In another study, **Li *et al.* (2007)** showed that piperine could reverse the corticosterone induced reduction of brain derived neurotrophic factor (BDNF) mRNA expression in cultured hippocampal neurons. Also piperine has high immunomodulatory and antitumor activity (**Sunila and Kuttan, 2004**). This immunomodulation activity is due to its multi-faceted activities such as anti-oxidative (**Mittal and Gupta, 2000; Pathak and Khandelwal, 2008**), anti-apoptotic and restorative ability against cell proliferative mitogenic response; thymic and splenic cell population and cytokine release (**Pathak and Khandelwal, 2008**).

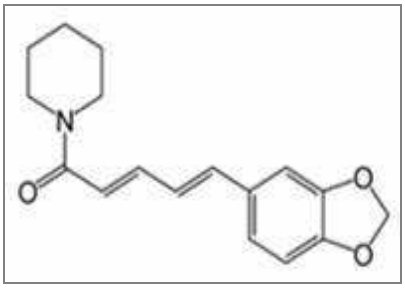

Figure (1): Piperine

Table 1: Frequency of sister chromatid exchanges (SCEs) in mouse bone-marrow cells treated with MMC and piperine with MMC.

Dose (mg/kg b. wt.)	No. of different types of SCEs/chromosome			Total No. of SCEs[a]	SCEs/Cell[b] Mean ±SE	Inhibitory Index (%)
	Single	Double	Triple			
I. Control	627	62	3	760	3.80±0.45	-
II. Piperine						
5mg	626	65	4	768	3.84±0.48	-
10mg	698	68	4	846	4.23±0.51	-
15mg	766	69	6	922	4.61±0.57	-
III. MMC						
1mg	4522	361	42	5370	26.85±0.53**	-
IV. piperine+MMC						
5mg +1mg	3784	352	38	4602	23.01±0.64•	16.65
10mg +1mg	3275	340	31	4048	20.24±0.58••	28.67
15mg +1mg	2854	333	28	3604	18.02±0.71••	38.30

a. The total number of chromosomes is 8000
b. The total number of scored cells is 200 (5 animals/ group)
**** p<0.01: Significance compared to control; • p<0.05, •• p<0.01: Significance compared to treatment (t- test).**

Mitomycine C (MMC) is one of the powerful mutagenic agents which have the ability to induce genotoxicity in a proliferating cell population such as mice bone marrow cells from the first division after treatment. Thus, it is widely used as positive control agent in genotoxic tests, both in laboratory animals or in cell cultures (**Russo *et al.*, 1992**). It damages chromosomes through generating of free radicals and alkaylating DNA thereby producing mutation (**Brookes, 1990**). Also, it has been demonstrated to inhibit mammalian DNA topo II (**Minford *et al.*, 1986**).

Considering all the above bioactivities of piperine, it was very important to assess the non toxic effect of piperine on somatic and germ cells *in vivo*. Also, this study was performed to determine the role of piperine in the inhibition of the mutagenic effects which could be induced by MMC in mice somatic and germ cells using two main cytogenetic parameters , i.e. chromosomal aberration and sister chromatid exchanges.

2. Materials and Methods

2.1. Animals:

Laboratory-bred strain Swiss albino male mice of 10-12 weeks old with an average weight of 27.5±2.5 g obtained from the National Research Center, Cairo, Egypt, were used. Animals were housed in groups (5 animals/ group) and maintained under standard conditions of temperature, humidity and light. The animals were given standard food and water *ad libitum*.

2.2. Chemicals:

Piperine and mitomycin C were purchased from Sigma, USA. All other chemicals used were of analytical grade.

2.3. Doses:

Animals were divided into 8 groups of 5 animals each. Group I were used as negative control. Group II as positive control, were treated with mitomycin C (MMC) at 1mg/kg b.wt. intraperitonealy (i.p.). Groups III, IV and V were treated orally with 5, 10 and 15mg piperine/ kg b.wt. for 3 consecutive days, respectively. Groups VI, VII and VIII were treated orally with 5, 10 and 15mg/kg b.wt. of piperine, respectively, for 3 consecutive days and with MMC 24h after the last dose of piperine for a single dose . Animals were sacrificed 24h after the last treatments.

2.4. Sister chromatid exchanges (SCEs):

The method described by **Allen (1982)**, for conducting *in vivo* SCE's induction analysis in mice was applied with some modifications. Approximately 55mg 5'-Bromodeoxyuridine (BrdU, Fluka AG, Buchs SG) tablets were inserted in mice subcutaneously (s.c.) 21-23h before sacrifice. Mice were injected intraperitonealy with colchicine at a final concentration of 3mg/kg body wt. 2hrs before sacrifice. Bone-marrow cells from both femurs were collected. The fluorescence-photolysis Giemsa technique was used **(Perry and Wolff, 1974)**. 40 well spread metaphases were analyzed per mouse to determine the frequency of SCEs/cell .

Table 2: Number and mean percentage of chromosomal aberrations in mouse splenocytes after treatment with MMC and piperine with MMC.

Dose (mg/kg b. wt.)	No. of different types of chromosomal aberrations					Total aberrations (without gaps)		Inhibitory Index (%)
	Gaps	Fragment and/or Break	Deletion	Robertosonian translocation	Polyploidy	No.	(%)	
I. Control	4	12	1	-	-	13	2.60	-
II. Piperine								
5mg	5	11	3	-	-	14	2.80	-
10mg	3	12	3	-	-	15	3.00	-
15mg	6	10	4	-	-	14	2.80	-
III. MMC								
1mg	21	67	25	8	3	103***	20.60	-
IV. piperine+MMC								
5mg +1mg	18	50	23	7	3	83 n.s.	16.60	22.22
10mg +1mg	17	44	23	4	2	73••	14.60	33.33
15mg +1mg	18	40	13	4	1	58•••	11.60	50.00

The total number of scored cells is 500 (5 animals/ group)
***** p<0.001: Significance compared to control**
n.s.: not significant; •• p<0.01, ••• p<0.001: Significance compared to treatment (X^2- test).

2.5. Chromosome abnormalities in somatic and germ cells:

For somatic and germ cells preparations, animals from the different groups were injected i.p. with colchicines, 2-3h before sacrifice. Chromosome preparations from splenocytes (somatic cells) carried out according to the method of **Yosida and Amano (1965)**. 100 well spread metaphases were analyzed per mouse. Metaphases with gaps, chromosome or chromatid breakage, fragments, deletions, Robertsonian translocation as well as numerical aberrations (polyploidy) were recorded. Chromosome preparations from spermatocytes (germ cells) were made according to the technique of **Evans *et al.* (1964)**. 100 well spread diakinase-metaphase I cells were analyzed per animal for chromosomal aberrations. Metaphases with univalents and chromosome translocations were recorded.

Evaluation of the activity of piperine to reduce SCEs and chromosomal aberrations induced by MMC was carried out according to **Madrigal-Bujaidar *et al.* (1998)** formula as follows:
Inhibitory index (II) = [1- (piperine and MMC – control)/ (MMC- control)] X100

2.6. Statistical analysis:

The significance of the results from the negative control data and between piperine with MMC comparing to MMC alone was calculated using t-test for SCEs and Chi-square test (2X2 contingency table) for chromosomal aberrations.

3. Results

3.1. Sister chromatid exchanges:

Sister chromatid exchanges analysis is a rapid objective method of observing reciprocal exchanges between sister chromatids. In the present study, the frequencies of SCEs/cell induced with the different doses of piperine were not significant in comparing to control group. When mice treated with piperine three days prior to MMC, all piperine doses decreased the mean percentage of SCEs/cell induced by MMC alone. The mean percentage of SCEs/cell was reduced from 26.85±0.53 with 1mg/kg b.wt. MMC to 23.01±0.64, 20.24±0.58 and 18.02±0.71 after pretreatment with 5, 10 and 15mg piperine/kg b.wt. respectively. The percentage of inhibitory index increased from 16.65% with low dose to 38.30% with the high dose of piperine. The number and percentage of the different types of SCEs/ chromosome were recorded (Table 1 and Figure 2 a, b).

3.2. Chromosomal aberrations:

3.2.1. In somatic cells:

Table (2) and Figure (2c, d) show the number and percentage of the chromosomal aberrations induced in control and treated animals after three consecutive days of piperine. The percentages of aberrant cells in animals treated with piperine were statistically not significant in comparing to the control group. Piperine reduced the number of the chromosomal aberrations when administered before the positive control MMC in a dose dependent manner. This reduction of

Table 3: Number and mean percentage of diakinase metaphase I cells with chromosomal aberrations in mouse spermatocytes after treatment with MMC and piperine with MMC.

Dose (mg/kg b. wt.)	No. of different types of chromosomal aberrations					Total Aberrations		Inhibitory Index (%)
	XY univalent	Autosomal univalent	XY+ Autosomal univalent	Frag.	Chain (IV)	No.	(%)	
I. Control	8	7	-	-	-	15	3.00	-
II. Piperine								
5mg	10	7	-	-	-	17	3.40	-
10mg	13	6	-	-	-	19	3.80	-
15mg	12	8	-	-	-	20	4.00	-
III. MMC								
1mg	50	31	3	3	4	91***	18.20	-
IV. piperine+MMC								
5mg +1mg	41	30	1	2	2	$76^{n.s.}$	15.20	19.73
10mg +1mg	44	25	-	1	-	$70^{n.s.}$	14.00	27.63
15mg +1mg	40	18	1	-	1	60••	12.00	40.78

The total number of scored cells is 500 (5 animals/ group); Frag. : Fragment
***** p<0.001: Significance compared to control**
n.s.: not significant; •• p<0.01: Significance compared to treatment (X^2- test).

chromosomal abnormalities excluding gaps reached 50% which was highly significant (p<0.001) with 15mg piperine/kg b.wt. in comparing to MMC alone.

3.2.2. In germ cells:

There were no significant differences between the animals treated with piperine alone and the control group. The mean percentage of diakinesis metaphase I cells was 18.2% (p<0.001) with 1mg MMC/kg b.wt. compared to the control. This percentage was decreased gradually parallel to pretreatment with the different doses of piperine and decreased to 12.0% (p<0.01) with 15mg piperine/kg b.wt. (Table 3). Also, Table (3) illustrates the protective effect of piperine in reducing the different types of aberrations. The different types of chromosomal aberrations such as XY-univalents and /or autosomal univalents were recorded (Figure 2e, f).

4. Discussion

The relationships between food, nutrition and cancer and the knowledge that cancer may be a preventable disease has resulted in an increased interest in studying the mutagenic or antimutagenic potential of some dietary constituents (**Azevedo *et al.*, 2003**). Also, considerable emphasis has been laid down on the use of dietary constituents to prevent the mutagen induced mutation and/or chromosomal damage due to their relative non-toxic effects (**Wongpa *et al.*, 2007)**. Depending on these ideas, it was very important to study the cytogenetic effects induced by piperine in animal model and its bioactivity against MMC as a mutagen.

In the present study, the incidences of the cytogenetic parameters (SCEs and chromosomal aberrations in somatic and germ cells) were not significant when animals were treated with the different doses of piperine alone compared with the control group. This result is confirmation to the other studies which demonstrated that piperine appears to be a non-genotoxic chemical (**Singh *et al.*, 1994; Karekar *et al.*, 1996)**. **Karekar *et al.* (1996)** studied the genotoxic potential of piperine using four different test systems, namely, Ames test using *Salmonella typhimurium*, micronucleus test, sperm shape abnormality test and dominant lethal test using Swiss albino mice. In the Ames test, six different doses of piperine, in the range of 0.005-10 mumol/plate, did not induce his+ revertants, with or without metabolic activation, indicating its nonmutagenic nature.

In the bone marrow micronucleus test using two doses in the range of therapeutic usage (10 and 20 mg/kg b. wt.), piperine itself was non-mutagenic. Like in somatic cells, piperine (10 and 50 mg/kg b. wt.) failed to induce mutations in male germ cells of mouse as assessed by using the sperm shape abnormality and dominant lethal tests.

The frequency of SCEs in bone marrow cells induced by MMC was reduced significantly when animals were pretreated with piperine. **Singh *et al.* (1994)** studied the effect of piperine on the cytotoxicity and genotoxicity of aflatoxin B1 (AFB1) in rat hepatoma cells H4IIEC3/G-(H4IIE) using cellular growth and formation of micronuclei as endpoints. They demonstrated that piperine reduced the AFB1-induced formation of micronuclei in a concentration-dependent manner. They also suggested that piperine is capable of counteracting AFB1 toxicity by suppressing cytochromes P-450 mediated bioactivation of the mycotoxin and markedly reduced the toxicity of the mycotoxin. **Reen *et al.* (1997)** investigated the potential of piperine for inhibiting the activity of cytochrome P4502B1 and protecting against aflatoxin B1 in V79MZr2B1 (r2B1) cells. They demonstrated that piperine was a potent inhibitor of rat CYP4502B1 activity, and piperine counteracted CYP4502B1 mediated toxicity of AFB1 in the cells offered a potent chemopreventive effect against procarcinogens activated by CYP4502B1.

Chromosomal aberrations assay was proved to be sensitive indicator for monitoring the genotoxicity of environmental chemicals (**Dulout *et al.*, 1983; Tucker and Preston, 1996**).Thus using MMC as a genotoxic agent was important to detect the protective effect of piperine in reducing the number of chromosomal aberration induced by MMC in mouse splenocytes and spermatocytes *in vivo*. In the present study piperine reduced the percentage of chromosomal abnormalities induced by MMC in somatic and germ cells significantly ($p<0.01$) in a dose dependent manner.

Our results agreed with the recent study by **Selvendiran and his co-workers (2005c).** They demonstrated that a significant suppression (26.7-72.5%) and (33.9-66.5%) in the micronuclei formation induced by cyclophosphamide (CP) and benzo(a)pyrene respectively were reduced following oral administration of piperine at the doses of 25, 50 and 75 mg/kg b. wt. in mice. Also, **Wongpa *et al.* (2007)** observed that oral administration of piperine significantly reduced chromosomal aberrations induced by CP in rat bone marrow cells.

The mechanism of action of piperine may involve scavenging potentially toxic mutagenic electrophiles and free radicals. Moreover, the modification of phase II enzymes and the enhancing of detoxification pathways can be involved **(Reen *et al.*, 1996; Selvendiran *et al.*, 2003).**

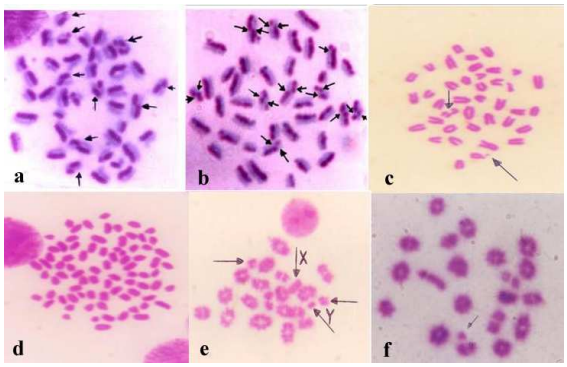

Figure (2): Metaphases from mice treated with piperine and mitomycin C showing (a, b) sister chromatid exchanges from mouse bone marrow cells; (c, d) metaphases from mouse splenocytes showing (c) break and fragment, (d) polyploidy; (e, f) metaphases from mouse spermatocyte cells showing (e) XY univalent and autosomal univalent , (f) fragment.

In conclusion, this study is a complementary survey to the biochemical and immunomodulatory studies and the few cytogenetic studies which were carried out upon piperine to investigate its cytogenetic activity directly on chromosomes by two main cytogenetic biomarkers chromosomal aberrations and sister chromatid exchanges. Using of MMC as a free radical generator and DNA alkylating agent makes severe mutations in chromosomes gives the ability to fairly judge on the cytogenetic activity of piperine in mice *in vivo*. This work explores the antigenotoxic activity of piperine, and its antimutagenic effects in reducing and preventing the DNA damages which can be induced by carcinogens in somatic and germ cells. The underlying molecular mechanisms now require attention.

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Structure of Understorey Vegetation in Native and Exotic Plantations of Semi-Arid Regions of Punjab, India.

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Abstract: In the present study we have compared the native *Dalbergia sissoo* and exotic *Eucalyptus* hybrid plantations of semi-arid regions of Hoshiarpur. Total 37 plants were reported from both the sites. Exotic plantation sites reported higher number of species, genera and Shannon-Wiener's diversity. These sites were dominated by exotic weeds like *Lantana camara*, *Parthenium hysterophorus*, *Murraya koenigii* etc., which were using most of the resources. Native plantation sites on the other hand were dominated by *Cannabis sativa* and a good number of saplings (390 individuals ha^{-1}) and seedling (1000 individuals ha^{-1}) of native tree species. [Nature and Science 2009; 7(12):79-85]. (ISSN: 1545-0740).

Keywords: Understorey vegetation, diversity, semi-arid regions, Punjab, *Lantana camara.*

1. Introduction

An ecosystem occupies a volume, and distribution of individuals varies within this volume. Volume spacing of individuals of different species provides an ecosystem its structure. This structure is evolved on account of interactions of individuals with environmental factors, and is an essential tool to be familiar with the health status of any ecosystem. Biodiversity is an important constituent of structure of an ecosystem and is vital for human survival and economic well being, as it enhances productivity and stability of ecosystem (Tilman et al., 1996).

Plantations, as part of terrestrial ecosystems, are of immense value for their production and protection potential. They usually include exotic species, non-local native species, or native species typically forming extensive pure stands. There is a common belief that the managed forests negatively influence biodiversity, but recent studies (Parrotta, 1995; Otsoma, 2000; Viisteensari et al., 2000) have shown that they can help in enhancing the recruitment, establishment and succession of native woody species by functioning as foster ecosystem, as they stabilizes the soil and create conditions favourable for native animals and plants to re-colonize (Parrotta, 1995; Yirdaw, 2002).

Over the last twenty years the Forest Department on an average has been planting between 15-25 million plants per annum in Government/Private areas. But, without proper care and management, most of these plantations represent a highly disturbed fragment of vegetation. They are mainly explored for fuel-wood and cattle grazing. In the present study, understorey vegetation of *Eucalyptus* hybrid and *Dalbergia sissoo* were studied with an objective of knowing the dominance and diversity status of plant species under these plantations.

2. Material and Methods

2.1 Study site

Present study site is located between 30° 90' and 32° 05' N latitude and 75° 32' and 76° 12' E longitude. It has an average elevation of 296 metres (971 feet). May and June are the hottest months of the year during which the temperature rises to 45 °C. The average annual rainfall for the last five years is 832.32 mm. Hoshiarpur falls into two nearly equal portions of hill and plain country. Its eastern face consists of the westward slope of the Solar Singhi Hills; parallel with that ridge, a line of lower heights belonging to the Siwalik Range traverses the district from south to north, while between the two chains stretches a valley of uneven width, known as the Jaswan Dun. Its upper portion is crossed by the Sohan torrent, while the Sutlej sweeps into its lower end through a break in the hills, and flows in a southerly direction until it turns the flank of the central range, and debouches westwards upon the plains.

2.2 Methodology

Thirty quadrats each of 1m x 1m sized were plotted in all the plantation sites. Analytic and synthetic characteristics like frequency, density, abundance and

basal cover, relative frequency, relative density, relative dominance and importance value index of plant species were calculated using formulas given by Misra (1968). The data collected from the quadrats was further analyzed for the alpha and other diversity indices, and evenness. Various formulas used for the calculation of diversity indices are as follows:

Shannon-Wiener diversity index (Shannon and Wiener, 1963) was calculated from the importance values using the formula as given in Magurran (1988):

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

where

H' is Shannon-Wiener Index of species diversity,

pi is the proportion of *i*th species and

s is the number of individuals of all the species.

Concentration of dominance (C_d) was calculated for the observation of strongest control of species over space in different sites (Simpson, 1949).

$$C_d = \sum_{i=1}^{s} (pi)^2$$

where

pi is the proportion of *i*th species, and s is the number of individuals of all the species.

Evenness was calculated following Pielou (1966), which reads;

$$J = \frac{H'}{\ln s}$$

where

H' is the Shannon-Wiener diversity index, and s is the number of species.

2.3 Statistical Analysis

Student's t-test was applied to analyze significance of the data. SPSS was used for the statistical analysis.

3. Results

3.1 Taxonomic and life-form diversity

Total 37 plant species and 34 genera belonging to 21 families were found in the two plantations (Table 1). *Eucalyptus* hybrid plantation reported higher number of species and genera than *Dalbergia sissoo* plantation. *D. sissoo* reported higher number of families. Other than herbs, all the life-forms reported higher richness in *E.* hybrid plantation. Equal numbers of tree saplings (four) were present in both the plantations, whereas tree seedlings were present in native plantation only.

3.2 Density and total basal area

Density of shrub species in *Eucalyptus hybrid* plantation forest of Dholbah varied between 30 and 720 individuals ha^{-1} (Table 2). Contribution of top three species, all weeds, was 77.19%. Total basal area was the maximum for *Lantana camara* (0.950 m^2 ha^{-1}) and minimum for *Crataeva nurvala* (0.029 m^2 ha^{-1}). In herb layer, *Setaria glauca* was the most densely (17000 individuals ha^{-1}) distributed species, and maximum basal area (0.923 m^2 ha^{-1}) was recorded for *Dactyloctenum aegypticum*. In Nara (Table 3), only two shrubs (both exotic weeds) were present in the understorey of *E.* hybrid plantation forest. *L. camara* with 93.02% density and 83.91% basal area was the more represented species. Density of herb layer ranged between 100 and 9750 individuals ha^{-1}. Top five species, mostly weeds, contributed 81.48% to the total density of the stand.

Table 1. Taxonomical and life-form diversity under *Eucalyptus* hybrid and *Dalbergia sissoo* plantations

Parameters	Total	*E.* hybrid	*D. sissoo*	Percentage gain or loss (*E.* hybrid over *D. sissoo*)
Species	37	27	21**	22.22
Genera	34	25	20**	20.00
Families	21	15	16^{ns}	- 6.67
Herbs	10	6	8*	- 33.33
Shrubs	10	8	6^{ns}	25.00
Tree (seedlings)	1	0	1^{ns}	-
Tree (saplings)	6	4	4^{ns}	0.00
Grasses	8	7	4^{ns}	42.86
Sedges	1	1	0^{ns}	100.00
Climbers	1	1	0^{ns}	100.00

* & ** are significant changes in values at P<0.05 and 0.01, respectively & ns- non significant for two sample t-test.

Table 2. Density, total basal area (TBA), relative frequency, density and dominance, and importance values (IVI) of

understorey vegetation in *Eucalyptus* hybrid plantation of Dholbah, Hoshiarpur.

Name of Plant Species	Density (individuals/ ha)	TBA (m^2)	Relative Frequency	Relative Density	Relative Dominance	IVI
Shrub Layer						
Lantana camara Linn.	720	0.950	32.00	42.11	38.64	112.75
Murraya koenigii (Linn.) Spreng	270	0.448	16.00	15.79	18.24	50.03
Parthenium hysterophorus Linn.	330	0.457	8.00	19.30	18.58	45.88
Casearia tomentosa Roxb.	180	0.312	16.00	10.53	12.69	39.22
Butea monosperma (Lamk.) Taub.	60	0.118	8.00	3.51	4.79	16.30
Carissa cavandas Linn.	60	0.054	8.00	3.51	2.21	13.72
Holarrhena antidysenterica Wall.	30	0.054	4.00	1.75	2.21	7.96
Cassia fistula Linn.	30	0.036	4.00	1.75	1.46	7.21
Crataeva religiosa Forst.	30	0.029	4.00	1.75	1.17	6.93
Herb Layer						
Dactyloctenum aegyptium (Linn.) P. Beau.	814	0.923	5.17	10.47	34.00	49.64
Trumfetta rhomboidea Jacq.	10500	0.464	12.07	14.85	17.08	44.00
Setaria glauca (Linn.) P. Beauv.	17000	0.163	12.07	24.05	6.02	42.14
Sida cordifolia Linn.	9000	0.298	15.52	12.73	11.00	39.25
Poa annua. Linn.	10000	0.049	10.34	14.14	1.81	26.30
Setaria verticillata (Linn.) P. Beauv.	4100	0.322	5.17	5.80	11.86	22.83
Cynodon dactylon (Linn.) P. Beauv.	4800	0.094	3.45	6.79	3.47	13.71
Commelina benghalensis Linn.	1300	0.102	6.90	1.84	3.76	12.50
Ichnocarpus frutescens Br.	500	0.039	6.90	0.71	1.45	9.05
Oxalis corniculata Linn.	1200	0.034	5.17	1.70	1.25	8.12
Desmodium concinuum DC.	800	0.040	5.17	1.13	1.48	7.78
Bidens bipinnata Linn.	900	0.057	3.45	1.27	2.11	6.83
Setaria intermedia Roem. and Schult.	900	0.045	3.45	1.27	1.67	6.39
Cyperus niveus Retz.	1700	0.048	1.72	2.40	1.77	5.90
Aerva sanguinolenta (Linn.) Blume	500	0.025	1.72	0.71	0.93	3.36
Tribulus terrestris Linn.	100	0.009	1.72	0.14	0.35	2.22

Table 3. Density, total basal area (TBA), relative frequency, density and dominance, and importance values (IVI) of understorey vegetation in *Eucalyptus* hybrid plantation of Nara, Hoshiarpur.

Name of Plant Species	Density (individuals/ ha)	TBA (m^2)	Relative Frequency	Relative Density	Relative Dominance	IVI
Shrub Layer						
Lantana camara Linn.	1220	1.107	66.67	93.02	83.91	243.60
Parthenium hysterophorus Linn.	100	0.212	33.33	6.98	16.09	56.40
Herb Layer						
Achyranthes aspera Linn.	9750	0.765	11.76	24.22	50.17	86.16
Trumfetta rhomboidea Jacq.	2400	0.188	13.73	14.91	12.35	40.98
Bidens bipinnata Linn.	1900	0.149	13.73	11.80	9.78	35.30
Aerva sanguinolenta (Linn.) Blume	2400	0.092	13.73	14.91	6.05	34.68
Setaria verticillata (Linn.) P. Beauv.	2600	0.033	11.76	16.15	2.14	30.05
Poa annua. Linn.	400	0.166	5.88	2.48	10.89	19.25
Sida cordifolia Linn.	900	0.057	9.80	5.59	3.75	19.15
Cynodon dactylon (Linn.) P. Beauv.	600	0.012	5.88	3.73	0.77	10.38
Commelina benghalensis Linn.	500	0.057	3.92	3.11	3.70	10.73
Ichnocarpus frutescens Br.	200	0.004	3.92	1.24	0.26	5.42

Cyperus niveus Retz.	200	0.001	3.92	1.24	0.09	5.26
Digitaria bifasciculata	100	0.001	1.96	0.62	0.05	2.63

In *D. sissoo* plantation forest of Chaksadhu, *Cannabis sativa* was the main contributor towards density (83.46%) and basal area (66.57%) of shrub layer. *Setaria verticillata* (density, 10400 individuals ha^{-1}) and *Achyranthes aspera* (basal area, 0.502 m^2 ha^{-1}) were the main herbs (Table 4). In case of Nara (Table 5), again *C. sativa* with 565 individuals ha^{-1} (73.86%) and 0.600 m^2 ha^{-1} (91.47%) density and basal area, respectively was the most represented shrub. In herb layer, maximum density was recorded for *Cynodon dactylon* (7200 individuals ha^{-1}) and maximum basal area for *A. aspera* (0.789 m^2 ha^{-1}).

The density of shrub and herb layers of *E.* hybrid and *D. sissoo* plantation was significant different whereas total basal area did not varied significantly.

Table 4. Density, total basal area (TBA), relative frequency, density and dominance, and importance values (IVI) of understorey vegetation in *Dalbergia sissoo* plantation of Chaksadhu, Hoshiarpur.

Name of Plant Species	Density (individuals/ ha)	TBA (m^2)	Relative Frequency	Relative Density	Relative Dominance	IVI
Shrub Layer						
Cannabis sativa Linn.	1110	0.258	33.33	83.46	66.57	183.36
Dalbergia sissoo Roxb.	180	0.119	44.44	13.53	30.69	88.67
Ziziphus mauritiana Lamk.	20	0.009	11.11	1.50	2.34	14.95
Murraya koenigii (Linn.) Spreng	20	0.002	11.11	1.50	0.41	13.02
Herb Layer						
Setaria verticillata (Linn.) P. Beauv.	10400	0.131	20.00	34.55	9.72	64.27
Achyranthes aspera Linn.	2500	0.502	10.00	8.31	37.37	55.68
Peristrophe paniculata	4800	0.305	15.00	15.95	22.70	53.65
Perilla frutescens (Linn.) Brittom	2300	0.116	12.50	7.64	8.60	28.74
Setaria glauca (Linn.) P. Beauv.	3800	0.075	10.00	12.62	5.55	28.17
Sida cordifolia Linn.	1200	0.076	12.50	3.99	5.68	22.16
Oplismenus compositus (Linn.) P. Beauv.	3100	0.061	5.00	10.30	4.53	19.82
Aerva sanguinolenta (Linn.) Blume	900	0.057	10.00	2.99	4.26	17.25
Euphorbia hirta Linn.	1100	0.022	5.00	3.65	1.61	10.26

3.3 Dominance

In *Eucalyptus* hybrid plantation of Dholbah (Table 1), *Lantana camara* (IVI, 112.75) was the most dominant shrub. *Murraya koenigii, Parthenium hysterophorus* and seedlings of *Casearia tomentosa* with importance values 50.03, 45.88 and 39.22, respectively were the co-dominants, and *Crataeva nurvala* was the least dominant species. Herb layer was dominated by *Dactyloctenum aegypticum* with 49.64 importance value closely followed by *Trumfetta rhomboidea* and *Setaria glauca* with 44.00 and 42.14 importance values, respectively. *Aerva javanica* and *Tribulus terrestris* were the rare species having less than 1% of the total importance value. Similar to Dholbah, *Lantana camara* (IVI, 243.60) was the most dominant shrub in *E.* hybrid plantation site of Nara also, whereas *Achyranthes aspera* was the most dominant herb. *Digitaria bifasciculata* was the least dominant species (Table 2).

In case of *D. sissoo* plantation in Chaksadhu, *Cannabis sativa* was the most dominant and *Murraya koenigii* the least dominant shrubs (Table 3). Herb layer was dominated by *Setaria verticillata* and co-dominated by *A. aspera* and *Peristrophe* paricularis. In Nara, the understorey shrub layer of *D. sissoo* was again dominated by *C. sativus*. *A. aspera*, with 63.21 importance value, was the most dominant and *Setaria glauca* (IVI, 9.60) was the least dominant herbs (Table 4).

Table 5. Density, total basal area (TBA), relative frequency, density and dominance, and importance values (IVI) of

understorey vegetation in *Dalbergia sissoo* plantation of Nara, Hoshiarpur.

Name of Plant Species	Density (individuals/ ha)	TBA (m^2)	Relative Frequency	Relative Density	Relative Dominance	IVI
Shrub Layer						
Cannabis sativa Linn.	565	0.600	33.33	73.86	91.47	198.66
Cassia tora Linn.	170	0.013	25.00	11.11	2.04	38.15
Ziziphus mauritiana Lamk.	120	0.009	12.50	7.84	1.44	21.78
Casearia tomentosa Roxb.	20	0.014	8.33	1.31	2.16	11.80
Lantana camara Linn.	30	0.008	8.33	1.96	1.16	11.46
Crataeva religiosa Forst.	30	0.007	8.33	1.96	1.04	11.33
Trumfetta rhomboidea Jacq.	30	0.005	4.17	1.96	0.70	6.83
Herb Layert						
Achyranthes aspera Linn.	3100	0.789	17.07	11.61	34.52	63.21
Cynodon dactylon (Linn.) P. Beauv.	7200	0.565	2.44	26.97	24.75	54.15
Setaria verticillata (Linn.) P. Beauv.	6900	0.135	19.51	25.84	5.93	51.28
Sida cordifolia Linn.	1600	0.102	17.07	5.99	4.45	27.52
Ageratum conyzoides Linn.	2800	0.108	12.20	10.49	4.72	27.40
Perilla frutescens (Linn.) Brittom	1300	0.102	9.76	4.87	4.47	19.09
Commelina benghalensis Linn.	1200	0.212	4.88	4.49	9.28	18.65
Bauhenia variegata Linn.	1000	0.201	4.88	3.75	8.80	17.42
Aerva sanguinolenta (Linn.) Blume	500	0.057	7.32	1.87	2.47	11.66
Setaria glauca (Linn.) P. Beauv.	1100	0.014	4.88	4.12	0.60	9.60

3.4 Diversity

Species richness and Shannon-Wiener's diversity index of shrub and herb layers was more in exotic (*E.* hybrid) than native (*D. sissoo*) plantation sites (Table 6). The values of Simpson's dominance index were slightly higher (statistically non-significant) in *E.* hybrid plantation forest. Evenness of shrub layer was higher in *E. hybrid* plantation and of herb layer was higher in *D. sissoo* plantation forest. Species richness of herb layer and Shannon-Wiener's diversity index of both shrub and herb layers were significantly different among exotic and native plantation forests.

Table 6. Species richness, diversity indices and evenness of vegetation under exotic and native plantations

Richness/Diversity parameters	*E.* hybrid plantation		*D. sissoo* plantation		t-test	P value
	Dholbah	Nara	Chaksadhu	Nara		
Shrub Layer						
Species richness	9	2	4	7		ns
Shannon-Wiener's index	1.80	0.54	0.65	1.19	1.72	0.043
Simpson's dominance index	0.22	0.69	0.47	0.46		ns
Pielou's evenness	0.82	0.72	0.68	0.61		ns
Herb Layer						
Species richness	16	12	9	10	3.18	< 0.001
Shannon-Wiener's index	2.43	2.13	2.05	2.13	1.77	0.038
Simpson's dominance	0.11	0.15	0.14	0.14		ns
Pielou's evenness	0.88	0.86	0.93	0.92		ns

4. Discussion

It is widely thought that plantation forests are, on average, less favourable as habitat for a wide range of taxa, particularly in the case of even aged, single species stands involving exotic species (Hartley, 2002). But, in the semi-arid conditions of Punjab, where anthropogenic disturbances have destroyed almost all the natural forests, plantation forests can be very fruitful in reviving the biodiversity of indigenous flora.

Floristic diversity of present plantation forests is less than earlier studies of plantations in India by Pande et al. (1988) and Dogra et al. (2009). This may be due to

soil moisture limitations and anthropogenic disturbances. Dominance of therophytes and grasses in these plantations also supports the drought like conditions prevailing in these plantations.

Species richness of both shrub and herb layers was higher in exotic *E.* hybrid plantation sites than native *D. sissoo* plantation sites. These results are different from various earlier studies (Kadavul and Parthasarthy, 1999; Martin, 1999; Christer et al., 2008; Dogra et al., 2009), which reported higher diversity in native plantation sites. The reason for this may be invasion of shade intolerant (heliophytes) weeds like, *Lantana camara, Parthenium hysterophorus, Bidens bipinnata, Trumfetta rhomboidea, Cyperus niveus, Murraya koenigii, Crataeva nurvala* etc. in the open conditions. In case of *D. sissoo* plantation sites, after the development of canopies shade loving species (sciophytes) like *Cannabis sativa*, *Commelina benghalensis, Oplismenus compositus* etc. started dominating the sites and due to this successional change the native plantation sites reported low species richness.

Although, species richness was higher in *E.* hybrid plantation sites but, they were full of weeds. *L. camara*, an obnoxious weed, was the most dominant shrub of the *E.* hybrid plantation forests. Domination of *L. camara* may be ascribed to its control at the first stage of succession (Molina Colon and Lugo, 2006), which is also supported by its exclusion from native forest.

In tropical forests, values of species diversity (H′) vary between 5.06 and 5.40 (Knight, 1975). In Indian forests however, H′ varies between 0.00 and 4.21 (Bisht and Sharma, 1987; Parthasarathy et al., 1992; Visalakshi, 1995; Pande, 1999; Gautam et al., 2008). The values of H′ reported in the present study (0.54 to 2.43) are well within these limits. Pielou's evenness of shrub layer was higher in exotic than native plantation sites and *vice versa* in herb layer. This represents greater dominance by few species in exotic sites than in native sites where the species were distributed more evenly, similar to results from other studies (Ramjohn, 2001; Molina Colon and Lugo, 2006).

Often the role of exotic trees in the recovery of degraded ecosystems has been ignored, especially in tropical dry forests due to the dramatic changes that come from a long history of anthropogenic abuses. They protect against erosion and fires caused by humans and also may serve to rehabilitate ecosystem properties when natives are not capable of recolonizing immediately. Their high growth rate and productivity is likely to replenish environmental conditions that can improve the conditions for establishment of native flora (Martínez, 2007). In the present study also, exotic plantation sites were by no means lesser than the native plantations. Although, the dominant taxa was exotic weeds but during successional development, if anthropogenic disturbances decrease, the saplings of native tree species present in these sites will increase the canopy cover and eliminate them (weeds) from these ecosystems.

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Community Development Programmes in Malaysia

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Abstract: This study looks at the philosophy, policy development and practices of community development as well as rural development programmes in Malaysia. The history of community development and its policy is important for provide community development processes to date. Since independence, the Malaysian government has introduced various types of community development programmes through its development policies, with the main of improving the economic, social and cultural conditions of the people. Understanding of these programmes can help to community leaders and stakeholders to achieve future programmes for realize community goals. [Nature and Science. 2009;7(12):86-89]. (ISSN: 1545-0740).

Keywords: Community Development, Participation, Malaysia Plan

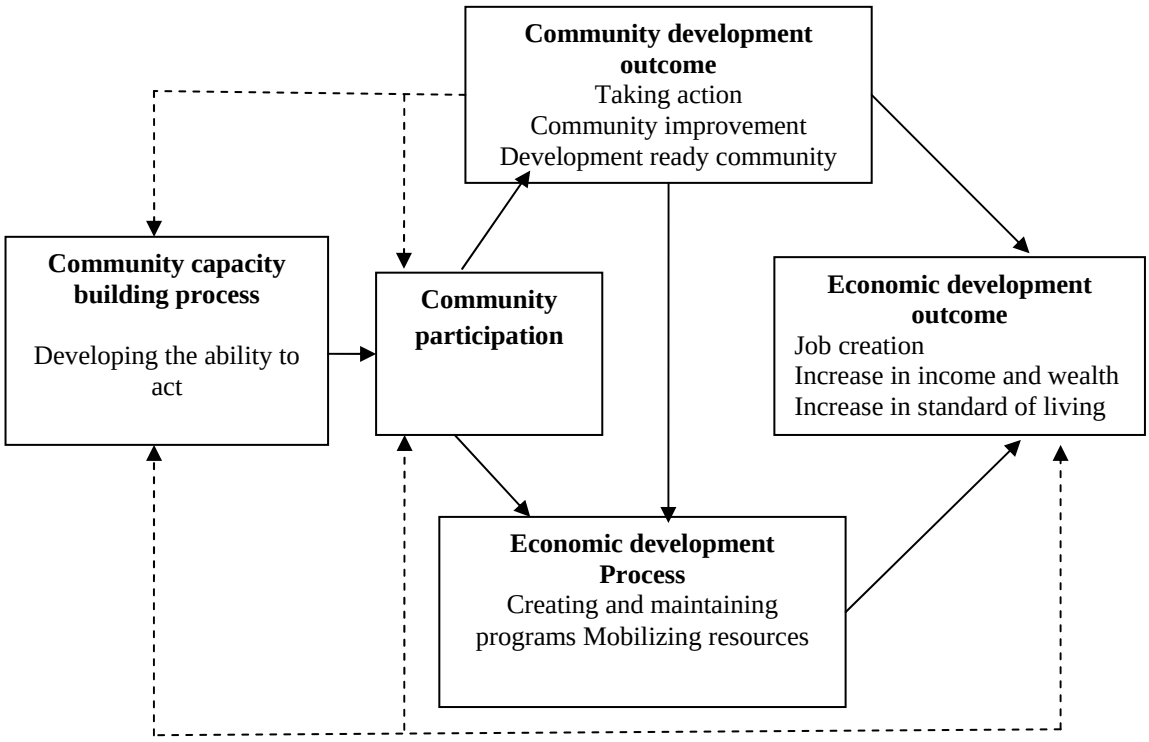
1. Introduction

The concept of community development has a longer history (Kleiner et al., 2004). The concept of community development is explored in terms of community participation, community empowerment and community capacity (Singh, Timothy, & Dowling, 2003). Community development can be seen as building social capital for collective benefits. It uses skill and knowledge and essential strategy in their practice (Gilchrist, 2004). The key phase that links both government efforts and the contribution of the nation's progress is people participation. Hence it is important to note that to understand the present community development programme, is necessary to trace community development history. For understanding community development history in Malaysia, this study provides a framework for community development programmes in local communities of Malaysia.

2. Literature Review

Community development aims to increase residents' participation in their community. In community development, emphasis is placed on community as a social network, bounded by geographical location or common interest (Talbot & Verrinder, 2005). According to Frank & Smith (1999) community development is the planned evaluation of all aspects of community well-being. According to Gilchrist (2004) community development helps local community residents to identify unmet needs. It seeks to build capacity by improving skill and knowledge for individuals and the community as a whole (Gilchrist, 2004). Community development is viewed as the best way to build the capacity of community residents to engage with each other and find solutions to issues that affect their community. Community development holds potential to build community cohesion by facilitating a community's capacity to engage connections between individuals, organizations and local groups (Chaskin et al., 2001). Helping a community to build its capacity for development is a primary goal of community development (Ife, 2002). Community development is a process that allows community residents to come together to plan, generate solutions and take action developing the evolution of social, economic, environmental and cultural aspects of community (Hackett, 2004). Community development emphasizes the importance of participation as a means of strengthening local communities (Kuponiyi, 2008). Community development builds peoples' skills for community issue. Hence it is vital to the survival of local communities (Talbot & Verrinder, 2005). Community development has evolved over the past few decades into a recognized discipline of interest to both practitioners and academicians. However, community development is defined in many different ways. Most practitioners think of community development as an outcome – physical, social, and economic improvement in a community (Phillips & Pittman, 2008). Figure 1 depicts the community development chain. The figure shows

that progress in the outcome of community development also contributed to developing community capacity building as well as economic development in local communities. This figure also emphasized on community participation as important key for community development.



Source: Adapted from Phillips & Pittman (2008)

Figure 1: Community Development Chain

3. Methodology

The research was performed as a qualitative library in which the researcher had to refer to relevant and related sources. Secondary sources such as official reports, community development guideline, programme books, and working papers were used as a source of data in this study. The sources that we used to collect needed information about community development in Malaysia were also obtained through the Agriculture Department and Ministry of National and Rural Development, Federal Land Development Authority, and United Malays National Organization.

4. Community Development Programmes

Community development is the basic policy formulated since independence to the present day, and it is stressed that people should take the opportunities provided by the state to participate in the government sponsored imitative. In its five years master plan, since the first Malaya plan (1956-1960) until the sixth Malaysian plan (1991-1995) community development has been as a philosophy of the development which underlies all government agencies policy. From 1951-1961, Rural and industrial Development Authority played its major role at the forefront of the community development programme. However, not much had been reported on its success to mobilise local participation. In community development, except that based on the loans given by Rural and industrial Development Authority to sponsor small scale activities such as fish ponds, cottage industries, animals and agriculture (Ness, 1967). The success of community development

programmes during this period was not particularly significant. It was reported that poor public participation and poor condition between canter and state government concerning the projects implemented contributed to this failure. Land development schemes were the government strategy for development among rural people. There were two such schemes , the first was carried out by the federal autonomous bodies such as federal land Development Authority, Federal land Consolidation and Rehabilitation, and the second by the state government. However the government support was limited to providing community infrastructures and subsistence allowance to the settlers for the first two years from the day they joined the scheme.

The second Malaya plan was since independence (1961-1965). In this plan two main strategies were embedded in the policy, the first was a continuation of the land development programme, and the second was the politico-administration improvement, meant enrich of the local participation in the development programme. In the first strategy, the land development scheme became the primary approach of the government to provide land for the landless and create job opportunities for the needy. The second strategy was concerned with improving human resource support and involvement, and to achieve this aim the government introduced a decentralized community development policy. With this policy, the decision making process was partially transferred from the central to the lower level within the state politico-bureaucratic administration system.

In The first Malaysia plan (1966-1970) to second Malaysia plan (1971-1975) the government discovered that although the development cover crops had achieved its target, it had filed to bring comprehensive community development. Participation from community masses was still low and poverty was still underlying problem in local communities. It was felt that people should be educated to enable them to fully utilise the amenities and programmes provided, as sponsored and initiated by the state. The government deep concern about the importance of people's participation in community development can be seen through the responsive strategy adopted beginning with the first Malaysia plan until the first half of the second Malaysia plan. For nearly ten years this strategy had been the dominant community development approach to mobilize local people to support the government effort in the processes of bringing about social change.

In the third (1976-1980) there was not new community development programme propagated by the government. In the fourth Malaysia plan (1981-1985), the importance of people participation was rested by the government, which said "in the national development effort, the people must respond positively to the challenge and be prepared to play their part" (FMP 1981 paragraph 1097). And the main objective of community development programmes were to inculcate in community values oriented towards development and self-reliance. In relation to this policy, a new approach to village and community development was introduced by the prime minster in mid 1984. The main objectives of this programme were to tackle the backwardness and poverty as the classic problem among rural Malays, especially within the peasant community (Asnarulkhadi, 1996)

The fifth and sixth Malaysia plan were carried out in (1986-1990) and (1991-1995). In this plan the initiative popularized by the Agriculture Department and Ministry of National and Rural Development known as Village Revolution that was announced in 1986. Literally, the concept used was rhetorical rather than practical. This is because the aims were similar to the philosophy of operation movement in the 60's. In practice this campaigning slogan was to support the new approach to village and rural development policy, which aimed at improving standards of living, and increasing the levels of productivity. Increased productivity remained the primary goal of the community development programmes portrayed in both five year master plans. Although there was no specific clause calling for people's participation in this master plan, it is understood that without participation from the local people, all development programmes initiated and sponsored by the government aimed at increased productivity and community development were impossible to achieve.

In summary community development, however has different meanings but community development in Malaysia can be explained in two levels. At the policy level community development means the programmes inspired by the government aimed at improving and developing the masses which turn enables them to contribute to national development. At the implementation level, to achieve the programmes' objectives, community development is an approach used by the government to encourage people's participation in those initiated and inspired programmes. In both situations community development is the state induced planned change programme for people to participate together. The community development programmes in Malaysia is strongly related to the political processes and events (Shamsul, 1986).

5. Conclusion

This study attempted to discuss the policy and implementation of community development programmes in Malaysia. It is showed that the philosophy and principals of Malaysian community development programmes concentrate mainly on upgrading living standards and tackling poverty, especially among rural Malays. It is assumed by the government that by providing basic amenities and other social programmes, people could cooperatively contribute by participating in those activities towards achieving the community goals which leads to economic growth and national progress. However the top bottom strategy of community development programmes initiated by the government was not an easy process. In fact, the process of mobilizing people through the responsive strategy advocated by the government to promote and enhance community participation in development programmes was not thoroughly successful. It is expected that the findings of this study could be utilized by the community developers for their future follow-up studies and reassessment of people's participation for community development.

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Biochemical and Immunological studies in Tilapia Zilli exposed to lead pollution and climate change

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Abstract: Heavy metals are persistent contaminants in the environment causing serious illness in fish, animals and human**.** Lead represents the main toxic element in nature. Lead has a tendency to accumulate in tissue and organs of exposed fish. The present study aimed to investigate the effect of lead pollution on fish with special reference to the hematological, immunological, serum biochemical parameters, where fifty healthy Tilapia Zilli fish were divided into 3 groups. Fish of gp1 served as a control. Fish of gp. 2 & 3 were used for the determination of acute lethal concentration dose and the pathological effect of lead on the exposed Nile tilipia. Blood samples were collected to obtain serum for biochemical studies and heparinized blood for hematological investigations. RBCs, Hb, HCt, and MCHC showed significant elevations, the serum GPT and GOT were increased significantly. L.D.H, glucose and cortisol were elevated, while serum cholesterol concentration was reduced significantly in high tem30^{o}C. [Nature and Science. 2009;7(12):90-93]. (ISSN: 1545-0740).

Key words: Lead pollution, Tilapia Zilli, Biochemical changes

1. Introduction

Lead occurs naturally in the environment. However, most lead concentrations that are found in the environment are a result of human activities. Due to the application of lead in gasoline, an unnatural lead-cycle has consisted. In car engines lead is burned, so that lead salts (chlorines, bromines, oxides) will originate. These lead salts enter the environment through the exhausts of cars. The larger particles will drop to the ground immediately and pollute soils or surface waters, the smaller particles will travel long distances through air and remain in the atmosphere. Part of this lead will fall back on earth when it is raining. This lead-cycle caused by human production is much more extended than the natural lead-cycle, and has caused lead pollution to be a worldwide issue [1].

The high level of lead could be also due to the industrial discharges from superphosphate factories, traffics of high way or motor vehicles as well as the extensive use of agrochemicals such as fertilizers, pesticides and growth promotors [2].

Lead can enter the human body through uptake of food (65%), Water (20%) and air (15%) and cause several unwanted effects, such as: Disruption the biosynthesis of haemoglobin and anaemia, a rise in blood pressure,kidneydamageand Miscarriages and subtle abortions, disruption of nervous systems, brain damage, declined fertility of men through sperm damage and diminished learning abilities of children and behavioural disruptions of children, such as aggression, impulsive behavior and hyperactivity. Lead can enter a foetus through the placenta of the mother. Because of this, it can cause serious damage to the nervous system and the brains of unborn children [3].

Industrial and agricultural discharges are considered the primary source of metal poisoning to fish in Egypt [4]. Lead has a tendency to accumulate in tissue and organs of exposed fish resulting in hepatic and renal dysfunction with growth retardation [5]. Consequently, it could induce alterations in hematological and serum biochemical parameters [6] as well as pathological changes in most body organs [7].

The present study aimed to investigate the effect of lead pollution on fish with special reference to the haematological, immunological, serum biochemical parameters.

Material and Methods:

1- Fish:

Fifty healthy tilapia fish of both sexes and 150 $\pm$ 50 gm body weight, were obtained alive and transported immediately to the laboratory. They were kept in 5 glass aquaria (100 X 30 X 50 cm) that provided daily with a tap water and continuously with filtered air. The water temperature was adjusted at

30°C along the period of experiment using thermostatic heater. The fish were fed a balanced ration daily using the formula suggested by *Ahmed and Matty*, 1988 [8]. Fish were kept under observation for 2 weeks.

Fish were divided into 3 groups (gps). Fish of gp1 (10) served as a control with no treatment. Fish of gp. 2 & 3 (20, each) were used for the determination of acute lethal concentration dose (LD_{50} /72 hr, gp2) and to investigate the pathological effect of lead on the exposed fish (gp3).

2-Experiments:

A- Determination of acute lethal concentration dose:

To determine lethal concentration dose, fish of gp. 2 were subdivided into 5 equal subgroups. Subgroup 1 served as a control. Other 4 subgroups exposed to 35, 75, 150 and 300 mg/L of lead acetate; respectively. Each dose was dissolved in the distal water of each aquarium. The number of dead fish was recorded within 72 hrs post-exposure and the acute lethal concentration dose was calculated according to the formula of *Brown, 1980* [9].

B- long term exposure:

Fish of gp3 were exposed to 1/100 of LD_{50} /72 hr (1.5 mg/L) of lead acetate for 2 weeks according to *Taylor et al., 1985* [10]**.** The excreta were removed regularly and the water was replaced within 4 days interval. Fish were kept under observation along the 14 days of exposure.

3- Sampling:

Blood sample were collected from the caudal vein after 3, 7, 14 days of exposure, part of blood was left to clot and then centrifuged at 3000 r.p.m. to obtain serum for biochemical studies, the other part was heparinized for hematological investigations using the methods of *Drabkin, 1949* [11]**.**

4-Haematological examinations:-

The erythrocytic indices (RBCs, Hb, HCt & MCHC) were estimated according to *Schalm, 1986* [12]**.**

5-Serum biochemical analysis:

Kits Biomericux France were used for the determination of serum glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), lactate dehydragenase (LDH), alkaline phosphatase (AP), serum glucose, serum cholestrol, and total protein. Serum cortisol hormone was analyzed by means of a gemmacoat 125-cortisol radio-imumnassay kit (Diagnostic corporation, USA). Serum Ig M was also measured according to *Fuda, et al., 1991* [13]**.**

6-Statistical analysis:

The obtained data were statistically analysed according to *Snedecor and Cochran,1969* by T test [14].

3. Results

A-Determination of acute lethal concentration dose:

Experiment 1 revealed that, the acute lethal concentration dose was 150 mg/L during the 1st 72 hrs post-exposure.

B- Long term exposure:

The effect of lead acetate exposure on RBC's count, Hb level, HCt and MCHC values of exposed tilipia fish were recorded in Table (1). Polycythemia was observed on the 14 day ($p<0.01$). Blood Hb, HCt, and MCHC showed a significant elevation by 14 days of experiment.

Table (2) revealed the changes of some biochemical constituents in the blood of tilipia fish due to lead acetate exposure. The obtained data revealed that serum GPT activity increased significantly by 14 days of exposure. A significant elevation in serum GOT activity was also observed on the 14th day ($p<0.01$). L.D.H serum activity was elevated along the whole period of experiment especially on the day 14th. Hyperglycemia was constant findings from the beginning of the experiment until the end of the experiment. Serum cholesterol concentration was increased, on the 3rd day and the 7thday and was reduced significantly, on the day 14thday. Cortisol hormone was elevated along the whole period of experiment especially on day 14th.

Discussion:

Regarding the impact of lead on the hematological profile of Nile tilipia, polycythemia accompanied by elevated hemoglobin level, HCt value and MCHC were observed. Similar findings were reported by *Mckim et al.*[15], *Hilmy et al.*[[16] and *Taylor et al.*[[10] recorded polycythemia in *rasy barb*. But in contrary to our finding Hb level and MCHC were reduced in *Clarias lazera* exposed to copper [17]**.** The increased RBCs count may be due to stimulation of erythropoietin by elevated demands for O_2 or Co_2 transport as a result of increased metabolic activity or distruction of gill membranes causing faulty gaseous exchange. The increase Hb content could be explained as a process where the

body tries to replace the oxidized denatured Hb [18]. The increase of HCt value and MCHC may be attributed to swelling of RBCs due to increased Co_2 in blood, hypoxia or stressful procedures [19] and [20].

Exposure of Nile tilipia to sublethal concentration (1.5 mg/L) of lead acetate for 14 days resulted in a marked increase in the activities of serum GPT, GOT, LDH and ALP. The present findings agree with our microscopic findings, which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to the liver injury [21].

Serum cholesterol level, in the present study, showed a significant reduction that could be due to greater level of utilization of cholesterol during corticosteroidogenesis, as it is the precursor for steroid hormones [22]. In addition, they reported a rise in the blood protein resulted in a high density of lipoprotein in the serum and was suggested to be the cause of hypocholesterolemia in exposed fish.

Our results showed similar findings as that of *Gill et al.,* [6] and *Snieszko* [23], who reported that, exposure of fish to lead had no significant increase on blood glucose of *salmo gairnei*. The blood glucose level reflected the changes in carbohydrate metabolism under hypoxia and stress conditions. Rise of glucose level indicated the presence of stressful stimuli eliciting rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue and accompined by cortisol elevation [24]. Concerning serum protein level, a significant increase was noted 14 days postexposure to lead. The elevated protein concentration may be due to the induction of protein synthesis in liver.

The serum Ig. M was determined to find out information about fish immune system which was previously investigated in different species by many authors as *Fuda et al.,* [13] *O`Neill* [25], in this work, the purified Ig. M was revealed a single perception against specific polyvalent antiserum to tilipia fish Ig, similar results was obtained by *Bagee et al.,* [26] who found that, *Coho salmon* Ig was detected by specific anti Ig 14. Our study revealed a significant decrease in Ig. M level in fish exposed to lead pollution if compared within control groups. *Anderson et al.,* [27] found a relation between cortisol and IgM as when cortisol increased IgM decrease. The significant increase in cortisol level in fish exposed to lead could be attributed to stress factors and the intoxication of fish [28].

Table 1: Effect of lead on some haematological parameters of tilapia fish along the period of experiment (Mean±S.E.)

Parameter	3days		7days		14days	
	Control	Exp.	Control	Exp.	Control	Exp.
RBCs 10^6/mm^3	3.4 ± 0.23	3.4 ± 0.61	3.3 ± 0.40	4.1 ± 0.84	3.7 ± 0.69	4.8 ± 0.78*
HB g/dl	7.30 ± 0.54	8.3 ± 0.20	7.1 ± 0.16	8.9 ± 0.7.	7.3 ± 0.23	9.4 ± 0.64**
H.Ct%	19.80 ± 1.20	22.9 ± 1.24	19.95 ± 0.29	27.4 ± 1.54	21.7 ± 1.64	29.7 ± 1.94**
MCHc%	33.70 ± 1.90	34.8 ± 1.40	32.52 ± 0.94	37.52 ± 1.26	31.3 ± 1.42	41.6 ± 1.17**

Exp: experimental * Significant at p<0.01. ** Non-significant

Table 2: Effect of lead on the serum biochemical parameters of tilapia fish along the period of experiment (Mean±S.E.)

Parameter	3days		7days		14days	
	Control	Exp.	Control	Exp.	Control	Exp.
SGPT (I.U/L)	29.2 ± 1.3	52.3 ± 044	31.3 ± 064	44.5 ± 1.29	33.0 ± 1.11	49.9 ± 2.68*
SGOt (I.U/L)	39.3 ± 2.0	41.7 ± 3.0	41.8 ± 1.2	48.9 ± 2.48	39.32 ± 1.0	55.40 ± 3.74**
L.D.H (I.U/L)	192 ± 3.3	192 ± 3.94	192 ± 4.3	192 ± 5.23	195 ± 3.40	199 ± 5.28*
A.L.P (U/L)	3.80 ± 1.3	3.1 ± 1.64	3.2 ± 1.84	4.8 ± 1.90	2.82 ± 1.60	5.94±2.25**
Glucose (mg / dl)	29.24 ± 2.0	33.68 ± 1.2	29.82 ± 1.2	42.20 ± 2.8	30.64 ± 1.8	62.8 ± 2.78**
Total protein (g/dl)	2.27 ± 0.32	2.42 ± 0.12	2.60 ± 0.64	3.0 ± 0.29	2.6 ± 0.55	4.89 ± 0.94*
Cholesterol (ng/dl)	121.4±3.2	127.6 ± 2.9	127 ± 3.0	173 ± 3.23	122.9 ± 2.4	199.4 ± 4.3*
Ig. M (ng/ml)	1.9 ± 0.13	1.75 ± 0.24	1.91 ± 0.42	1.53 ± 0.92	1.85 ± 0.12	0.3 ± 0.075*
Cortisol (ng/ml)	0.85 ± 0.23	1.43 ± 0.07	0.99 ± 1.23	1.87 ± 1.20	0.85 ± 0.72	1.94 ± 1.64*

Exp: experimental * Significant at p<0.01. ** Non-significant

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