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EDITORIAL PREFACE

It is my great pleasure to publish the first issue of Volume three of the Jordan Journal of Biological Sciences (JJBS). JJBS is a refereed, peer reviewed quarterly international journal issued by the Jordanian Ministry of Higher Education and Scientific Research in cooperation with the Hashemite University. The journal covers a wide range of research and development concerning biological sciences. Through the publication, we hope to establish and provide an international platform for information exchange in different fields of biological sciences.

Jordan Journal of Biological Sciences aims to provide a highly readable and valuable addition to the literature, which will serve as an indispensable reference tool for years to come. The coverage of the journal includes all new findings in all aspects of biological sciences and or any closely related fields. The journal also encourages the submission of critical review articles covering advances in recent research of such fields as well as technical notes.

The Editorial Board is very committed to build the Journal as one of the leading international journals in biological sciences in the next few years. With the support of the Ministry of Higher Education and Scientific Research and Jordanian Universities, it is expected that a valuable resource to be channeled into the Journal to establish its international reputation.

I have received a good response to this issue of JJBS from biologists in Jordanian universities. I am pleased by this response and proud to report that JJBS is achieving its mission of promoting research and applications in biological sciences. In this issue, there are Seven interesting papers dealing with various aspects of biological sciences.

JJBS will bring you top quality research papers from an international body of contributors and a team of distinguished editors from the world's leading institutions engaged in all aspects of biological sciences. Now, the JJBS invites contributions from the entire international research community. The new journal will continue to deliver up to date research to a wide range of biological sciences professionals. The JJBS will assure that rapid turnaround and publication of manuscripts will occur within three to six months after submission.

I would like to thank all members of the editorial board and the international advisory board members for their continued support to JJBS with their highly valuable advice. Additionally, I would like to thank the manuscript reviewers for providing valuable comments and suggestions to the authors that helped greatly in improving the quality of the papers. My sincere appreciation goes to all authors and readers of JJBS for their excellent support and timely contribution to this journal.

I would be delighted if the JJBS could deliver valuable and interesting information to the worldwide community of biological sciences. Your cooperation and contribution would be highly appreciated. More information about the JJBS guidelines for preparing and submitting papers may be obtained from www. jjbs.hu.edu.jo

Prof. Naim S. Ismail Editor-
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Please Notice:

The members of the Eidtorial Board are pleased to Announce that the Jordan Journal of Biological Sciences is Accepted to be abstracted by CABI (Global Health Database) as of September 2009.

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ASSESSMENT OF larvicidal PROPERTIES OF AQUEOUS EXTRACTS OF FOUR PLANTS Against *Culex quinquefasciatus* LARVAE

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Abstract

Aqueous extracts of four plants were tested in a laboratory for larvicidal properties against the most serious vector of filariasis and avian malaria, *Culex quinquefasciatus* Say (Diptera: Culicidae). The endeavour is to further explore the use of natural insecticides in integrated vector management programmes to control larvae of *Culex*. Laboratory reared larvae were exposed to 1, 2, 3, 4 and 5 ppm concentrations of the extracts of *Azadirachta indica* Juss., *Gymnema sylvestre* R.Br., *Nerium indicum* Mill and *Datura metel* L. respectively in Zoology research laboratory of D.A-V Degree College, Kanpur, India. Larvicidal assays were conducted according to standard WHO procedure 1981. Result showed that the plant *A. indica* elicited 70-99% mortality, followed by *G. sylvestre* 44-89%, *N. indicum* 41-74% and *D. metel* elicited 19-54% mortality to larvae. The extracts of *A. indica* and *G. sylvestre* were found to be significantly effective in controlling *Culex* larvae. The results indicate that the natural insecticides could be taken in the place of synthetic insecticides and save our environment from chemical hazards.

الملخص

مستخلص 4 نباتات تم دراستها في المختبر لمعرفة تأثيرها على يرقات أخطر بعوض *Culex quinquefasciatus* الناقل الملاريا وداء الخيطيات ، وهذا لمعلاقة فعالية المبيدات الحشرية النباتية للإدماجها في برنامج المكافحة ضد يرقات *Culex*.
تم في المختبر معاملة الطور اليرقي بالتركيز التالية 1، 2 ، 3 ، 4،و 5 ppm بالنسبة لكل من *Azadirachta indica, Gymnema sylvestre*, *Nerium indicum Datura metel* عل التوالي في مختبر جامعة كانبور –الهند ، حيث تمت المعاملة حسب طريقة المنظمة العالمية للصحة (WHO)1981 أظهرت النتائج ان نسب الموت تراوحت بين (70-99 ٪) عند نبات *Azadirachta indica* ، تليها *Gymnema sylvestre* (44-89 ٪) , *Nerium indicum* بـ 41- 74٪، *Datura metel* (19-54 ٪) ، كذلك بينت النتائج ان *Azadirachta indica* أكثر فعالية على يرقات البعوض تليها *Gymnema sylvestre* ثم *Nerium indicum* وفي الأخير *Datura metel*
وتشير النتائج أن استخدام المبيدات الطبيعية فعال، ولهذا يمكن اتخاذها في مكان المبيدات الاصطناعية وإنقاذ بيئتنا من المخاطر الكيميائية.

Keywords: Gymnema sylvestre, Culex quinquefasciatus, malaria, larvicide, LC_{50}.

1. Introduction

Control of mosquito is essential as many species of mosquitoes are vectors of malaria, filariasis, dengue and many other viral diseases and they cause unbearable biting irritations (Curtis, 1994; Collins and Paskewitz, 1995; Gubler, 1998). *Culex quinquefasciatus* is a vector of lymphatic filariasis which is widely distributed tropical disease with around 130 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard et al., 2003)

Control of mosquito larvae frequently depends on continuous use of organophosphates and insect growth regulators (Yang et al., 2002). The organophosphate insecticides target and depress acetylcholinesterase activity in a dose-dependent manner, leading to an excessive acetylcholine output, nerve paralysis and finally death. The acetylcholinesterase inhibition is non-specific, affecting the whole body systems via the cholinergic, muscarinic and nicotinic receptor pathways. Since the body systems affected are the central nervous system, the autonomic nervous system, as well as the peripheral muscular pathways (Robbin, 1991; Hassal, 1982; Purdey, 1994). Therefore the problem can be resolved by developing some new strategies for mosquito control with some less harmful, biodegradable, non toxic larvicidal natural products. Plant extracts may be alternative source to control mosquito larvae, many researchers have reported on the effectiveness of plant extracts or plant oils against mosquito larvae. Secoy & Smith (1983) stated that the roots of Peganum harmala Linn. contain toxic alkaloids for lice and mosquitoes.

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The seed extracts of *Sterculia guttata* Roxb. (Katade et al., 2006b); fruit extract of Balanites aegyptiaca Del. (Wiesman et al., 2006); root extract of Solanum xanthocarpum Schrad and Wendl. (Mohan et al., 2007); leaves of *Artemisia annua* L. and *Azadirachta indica* Juss. (Tonk et al 2006); the acetone extract of *Nerium indicum* Mill. and *Thuja orientelis* L. (Sharma et al., 2005) been tested against the mosquitoes larvae. Oils from 41 plants tested against Aedes larvae, and out of these only 13 plants gave 100% mortality (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum and sandalwood) after 24 hrs. The best oils were tested against third instar larvae of Aedes, Anopheles and Culex in 1, 10, 50, 100 and 500 ppm concentration and found extremely prominent results (Amer and Mehlhorn, 2006).

The Meliaceae plant family is known to contain a variety of compounds that show insecticidal, antifeedant, growth regulating properties (D'Ambrosio and Guerriero, 2002). Azadirachta indica commonly known as Neem, is a deciduous tree native to northwestern India. Its dentate leaves have long been recognized for insecticidal properties. Gymnema sylvestre (Gurmar) belongs to family Asclepiadaceae is an herb native to the southern and central India. The major bioactive constituents of Gymnema sylvestre is a group of oleanane type triterpenoid saponins known as gymnemic acid. Leaves of this species yield acidic glycosides and anthroquinones. Datura metel (Angels trumpet) is perennial shrub belongs to family Solanacae. All parts of plants contain dangerous level of poison; the principal toxic elements are tropane alkaloids. Nerium indicum (Kaner) belongs to family Apocynaceae, it's a green shrub with milky juice. Root, bark and seeds contain cardioactive glycosides. The bark also contains scopoletin and scopoline and small quantity of tannin, found in gangitic plains and Madhya Pradesh, India. The present investigation is conducted to study the larvicidal effect of Azadirachta indica, Gymnema sylvestre, Nerium indicum and Datura metel against larvae of C. quinquefasciatus Say.

2. Materials and Methods

For preparing the plant extracts to be tested, seeds of *A. indica*, leaves of *G. sylvestre*, bark and leaves of *N. indium* and leaves of *D. metel* were collected and their identity were confirmed at Botany department of D.A-V Degree College, Kanpur, India. After that material is cleaned, chopped, dried in shade and ground to fine powder with the help of electric grinder. All plants used in present study were kept safe as voucher specimen in Museum of Botany department. Extraction of all plant parts was carried out in a simplest way, thinking that it could be easy for the local communities to adopt this method. Twenty gram of each powder was placed in separate glass 100 ml of tap water was added and mixed vigorously. The mixture was kept for 24 hours with occasional shaking, after that, mixture was filtered using a fine muslin cloth and the final volume adjusted to 100 ml. A series of concentrations 1,2,3,4 and 5 ppm were prepared by using the stock solution using the tap water.

Culex quinquefasciatus larvae were collected from stagnant surface water of pools with the help of jar and stored in enamel trays containing tap water. They were maintained at 27± 2^0C temperature, 70± 5^0C relative humidity under 12:12 light and dark photo period cycle. The larvae were fed with the fresh food containing finely grounded dog biscuits and yeast extract in a ratio of 3:2. Pupae emerged were transferred to new trays containing tap water and placed in screened cages (30x30x30 cm), where adult emerged. Adult mosquitoes were fed on a 10% sucrose solution and 10% multivitamin syrup and periodically blood fed from fresh blood of rabbit. The egg masses produced due to adults mating were kept to continue next generation.

Laboratory reared IV instar larvae of *Culex quinquefasciatus* were tested with different concentration (1-5 ppm) of selected plants extract in Zoology Research Laboratory, D.A-V Degree College, Kanpur, India during May to July 2009 according to the standard WHO procedure (1981). A total of 25 fourth larvae were introduced in 500 ml glass beaker containing various concentrations of different plant extracts. The treatments were replicated four times, and each replicate set contained one control. Mortalities were reported after 24 hours of the exposure period. Laboratory room temperature was maintained at 27±2^0C during the experiment period. The moribund and dead larvae in four replicates were combined and expressed as percentage mortality for each concentration. Dead larvae were acknowledged when they failed to move after probing with a needle. Moribund larvae were those unable of rising to the surface within reasonable period of time. The percentage mortality was calculated and analysis of data was carried out by employing probit analysis (Finney, 1971) and corrections for mortality if needed were done by using Abbott formula (Abbotts, 1925).

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

$$\text{Corrected percentage of mortality} = 1 - \frac{n \text{ in T after treatment}}{n \text{ in C after treatment}} \times 100 \quad (2)$$

Where n= number of larvae, T= treatment and C= control

3. Results

The larvicidal activities of aqueous extracts of four plants tested are summarized in Table 1, 2 and Figure 1. It was observed that larvae became slowly inactive within 10 hours and began to fall towards the bottom of the glass beaker. The treated larvae showed curling up, anxiety and vigorous body movements. The larvicidal activity of aqueous extract of *Azadirachta indica* seeds showed 70, 80, 83, 91 and 99% of death with the use of 1, 2, 3, 4 and 5 ppm concentrations, respectively. The 4 ppm concentration killed more than 90% of the larvae. However 99% mortality was observed only at 5 ppm concentration (Fig 1). Aqueous extract of leaves of *Gymnema sylvestre* causes 44, 58, 76, 83 and 89% mortality after 24 hours. The larval mortality was above 50% from 2 ppm concentration, the maximum 89% mortality shown at 5 ppm concentration as mentioned in table 1. Extract of bark and leaves of *Nerium indium* showed greater than 50% mortality when 3 ppm concentration was used, while 5 ppm concentration showed 74% larval mortality. Extract of *Datura metel*

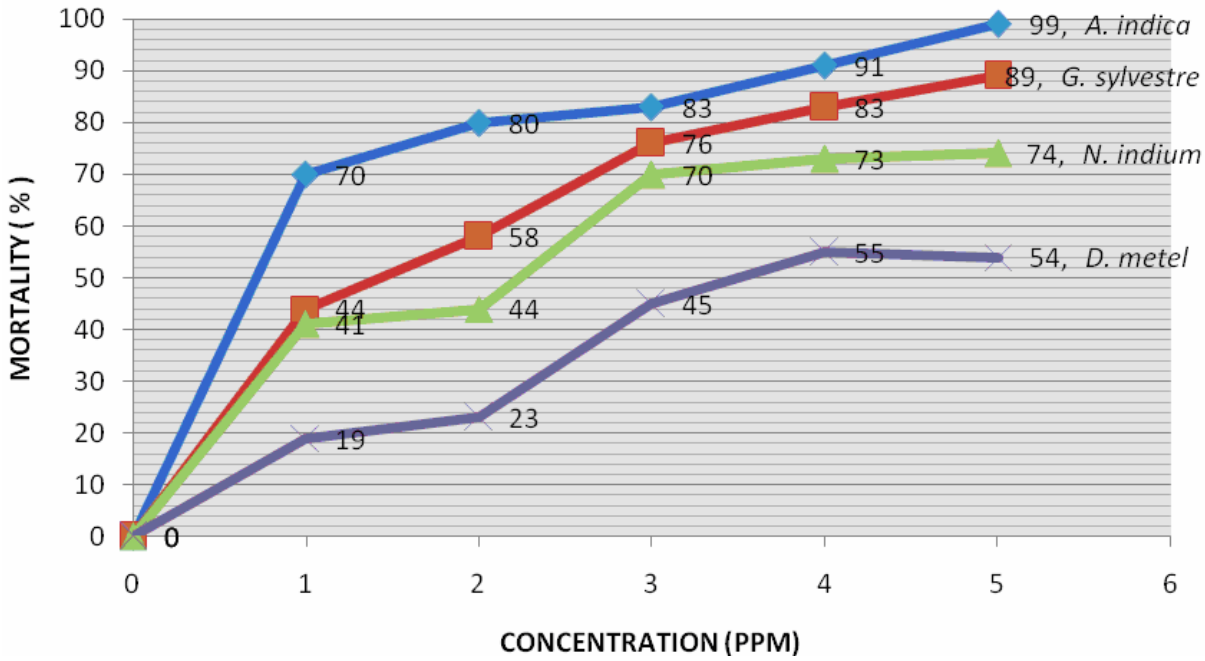

Figure 1. Dose response relationship for selected plant extracts, applied for 24 hours on c.quinquefasciatus Say.

Table 1. Larvicidal activity of various plant extracts to the fourth instar larvae of *C. quinquefasciatus* Say.

Plant Extracts	Observed mortality in percentage after 24 hrs				
	1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
Azadirachta indica	70±2.57	80±1.74	83±0.69	91±1.88	99±2.54
Gymnema sylvestre	44±1.37	58±1.51	76±1.06	83±1.96	89±0.28
Nerium indicum	41±0.38	44±1.96	70±1.85	73±1.47	74±1.88
Datura metel	19±0.58	23±0.51	45±0.37	55±0.92	54±0.68

*Values are the means of 4 (n=4± SE)

Table 2. LC_{50} and LC_{90} with fiducial limits (95%) of tested plant extracts against larvae of *C. quinquefasciatus* Say.

Plant Extracts	Activity (ppm) (95% FL)	
	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)
Azadirachta indica	0.53 (0.25-0.79)	3.423 (2.78-4.65)
Gymnema sylvestre	1.31 (1.02-1.57)	5.95 (4.76-8.41)
Nerium indicum	1.67 (1.25-2.03	12.90 (8.43-28.66)
Datura metel	3.97 (3.35-5.05)	23.85 (14.23-61.25)

FL= Fiducial limits, UCL= upper confidence limit, LCL= lower confidence limit

leaves showed the least effective results, it killed 54% larvae at the 5 ppm concentration.

Among these extracts, seed extract of *A. indica* and leaves extract of *G. Sylvestre* are the most promising ones. More accurate data on the toxicity of the plant extracts were obtained by calculating their LC_{50} & LC_{90} (Table 2). *A. indica* showed high toxicity with a LC_{50} of 0.53 ppm and LC_{90} of 3.42 ppm. *G. Sylvestre* and *N. Indicum* also show LC_{50} values less than 2.00 ppm, while *D. metel* showed 3.97 ppm value for LC_{50}. The LC_{90} value for all extracts ranges between 3.42 ppm to 23.85 ppm. *Datura metel* needed 23.85 ppm concentration to kill 90% larvae where as just 3.42 ppm of *Azadirachta indica* caused 90% mortality. It is clear from figure 1, that the aqueous seed extract of *A. indica* is highly lethal followed by *G. sylvestre*, *N. indium* and *D. metel* respectively.

4. Discussion

Biopesticides may serve as suitable alternative to chemical insecticides in future as they are relatively safe, inexpensive and available everywhere in the world. This work demonstrates the potency of Neem seed extract as an effective larvicide against *C. quinquefasciatus* larvae; it was highly toxic to mosquito larvae. The high rates of larval mortality observed at 3 to 5 ppm within 24 hrs with LC_{50} value 0.53ppm indicate the high toxicity of the extract. Previous studies have shown that *A. indica* extracts possessed significant larvicidal activity. According to Mustafa and Al Khazraji (2008) *Azadirachta excels* Jack showed excellent larvicidal properties at low concentrations against *Culex pipiens molestus*. Its LC_{50} value after 1 day was 62.5µg/mL. Dua et al. (2009) stated that, emulsified concentration of neem oil formulation showed 95.5% reduction in larval population of *C. quinquefasciatus* in one day under field trails and thereafter 80% reduction was achieved up to the third week.

The major bioactive constituents of *Gymnema sylvestre* is triterpenoid saponins (5.50%) and tannins (1.00%). Wiesman et al. (2006) reported that saponin extracted from the fruit of *Balanites aegyptiaca* showed 100% larvicidal activity against *Aedes aegypti* mosquito larvae. Aqueous extract of *Gymnema sylvestre* causes 31, 45, 45, 71 & 100 % mortality to *C. quinquefasciatus* at 1, 2, 3, 4 and 5% concentration respectively (Khanna and Kannabiran, 2007). Results of present study are in line with earlier work done. *Nerium indicum* showed moderate larvicidal properties when compare with other two plants extracts with 74% mortality 5 ppm. Sharma et al. (2005) tested alcoholic and acetone extracts of *N. indium* leaf against *Anopheles stephensi* and found results of LC_{50} at 185.99 ppm after 24 hours of exposure. Srivastava *et al*. (2003) examined the aqueous and methanolic extract of *N. indium* lattices against *Culex quinquefasciatus* and obtained that different dilutions of the lattices delay the post embryonic development of *Culex* larvae, methanolic extract is 1.8 times more toxic than aqueous extract. *Datura metel* showed less activity among the tested plant extracts with 54% mortality at 5 ppm. Mustafa and Al Khazraji (2008) tested the *Datura stramonium* seed extract against larvae of *Culex pipiens* at 20µg/mL, caused very low mortality up till seven days of exposure. These findings have re-emphasised the need to explore the possibility of using plant based larvicide and reduce the chemical hazards in the environment. The seed extract of *A. indica*, leaf extract of *G. sylvestre* and bark & leaf extract of *N. indium* were very promising. Furthermore, all these plant materials can be easily collected from the nature. Therefore, plant originated insecticides can be used as sustainable larvicide in a mosquito control programme.

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Association of hs-CRP with Diabetic and Non-diabetic individuals

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Abstract

الملخص

Association of hs-CRP in diabetic and non – diabetic subjects were studied epidemiologically. The analysis was done with 400 diabetic and 400 non-diabetic individuals. Anthropometric and biochemical parameters were studied to assess the association of hs-CRP with in diabetes mellitus. Type II diabetes Mellitus encompasses -90 % of the diabetic subjects, and it is characterized by insulin resistance often accompanied by obesity and dyslipidemia. hs- CRP, the golden marker of inflammation was analyzed in diabetic and non- diabetic subjects. Anthropometric parameters were found to be high in diabetic subjects compared with non-diabetic subjects. The high hs-CRP levels in diabetic subjects were observed.
The hs- CRP levels were seen in diabetes with insulin resistance. Serum hs- CRP levels were positively related to anthropometric parameters. The relationship of hs-CRP with glycaemic control was studied with HbA1c, and it was positively correlated with hs-CRP. The results concluded that hs-CRP has strong association with diabetic individuals. The significance of hs-CRP in diabetic and non diabetic individuals was discussed.

Keywords: hs-CRP, -Insulin resistance, Diabetes mellitus , biochemical parameters,HbA1C..

1. Introduction

Diabetes is a metabolic -disorder with inappropriate hyperglycemia either due to an absolute or relative deficiency of insulin secretion or reduction in the biologic effectiveness of insulin or both. It is also associated with disturbances concerned with protein, carbohydrate and lipid metabolism. The decreased uptake of glucose into muscle and adipose tissue leads to chronic extra cellular hyperglycemia which results in tissue damage and chronic vascular complications in both type I and II Diabetes Mellitus (Brownlee et al. 1981; Luscher et al. 2003).

Among several markers of inflammation, hs –CRP is found to be significant in people with diabetes. CRP, a pentameric protein produced by the liver has emerged as the ‘ golden marker for inflammation’ . It is a non-immunoglobin protein having five identical sub units. It is a member of pentraxin family proteins. The C-reactive protein derives from the fact that it reacts with capsule polysaccharide of *streptococcus pneumoniae*. It is an acute phase response protein markedly increased in both inflammatory and infectious diseases. It plays an important role in innate immunity. It assists in complement binding to foreign and damaged cells and enhances phagocytosis. It was also noticed that the elevated levels of IL18/IL18BP in plasma during active stages of disease suggest a possible role in the pathogenesis and course of idiopathic thrombocytopenia (ITP) (Shan et al.2009). Hyper glycemia is an associated factor to the increase of serum CRP levels, non-controlled type II diabetic subjects (Martha and Fernando , 1999). Several studies demonstrate that hs-CRP remained a significant predictor of diabetes risk even after adjusting with body mass index, family history of diabetes mellitus, smoking and other factors (Pradhan et al. 2005). In people with diabetes , CRP levels is highest tertile (> 0.28 mg/dl) were associated with a 2 fold increase in CV mortality after adjusting for age , sex and glucose tolerance tests (Chiriboga et al. 2009 ; Jager et al. 1999; Pfutzner and Forst, 2006) . Hypertensive patients with DM2 had higher levels of hs-CRP, a circulating inflammatory marker, than normal subjects. This finding suggests that patients with two associated diseases have a more active inflammatory state (Luciana etal.2007).

Though we have several studies on hs-CRP and diabetes mellitus association with different age groups is limited . So current study focus on association of hs-CRP and diabetes mellitus with different age groups.

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2. Materials and methods

The significance of the hs-CRP levels in different age groups of diabetics, analysis were carried out on various metabolic and biochemical parameters for the 400 healthy non-diabetic subjects and 400 diabetic individuals.

2.1. Anhtropometric measurements:

Weight and height measurements were obtained, using standardized technique as detailed elsewere. (Deepa *et.al.* 2003). BMI was calculated as the weight in kilograms divided by the square of height in meters. Blood pressure was recorded in the sitting position by using the right arm to the nearest 2mm Hg with a mercury Sphygmomano meter.

2.2. Biochemical parameters:

Fasting plasma glucose (glucose –oxidase peroxidise method) , serum cholesterol (cholesterol oxidase – peroxidase-4-amino phenazone method) , serum triglyceride (glycero phosphate oxidase –peroxidase -4-aminophenazone method), and HDL cholesterol (direct method with polyethylene glycol pre treated enzymes) were measured using Hitachi-912 Auto analyzer (Hitachi , Mannheium , Germany). The intra and inter assay coefficient of variation (CV) for the biochemical assays ranged between 3.1 and 7.6%. LDL cholesterol was calculated using the Friedwald et al. (1972) in subjects with triglyceride <400 mgs/ dl.Hs- CRP is estimated quantitatively by means of particle enhanced immuno nephelometry using BN prospectus (Dade behring, Marburg, Germany). Consistent with recent recommendations from the centres for Disease control and prevention, a CRP cut point of 3 mgs/l was used to differentiate high risk and low risk groups.

2.3. Statistical analysis:

One way ANOVA or student ‘ t ’ test as appropriate was used to compare groups for continuous variables. The Chi-square test or Fisher’s extract test as appropriate was used to compare proportions. Pearson’s correlation analysis was done to determine the relation of hs-CRP with other risk variables. All the analyss were done by using the Windows based SPSS statistical Package (Version 10.0 ; SPSS Inc; Chicago,IL , USA) and P- values <0.05 were taken as the level of significance.

3. Results

The clinical and biochemical characteristics in relation to hs-CRP of the study group were shown on the table 1. In comparison with non-diabetic subjects, the diabetic subjects were older (P < 0.001) and had higher body Mass Index (BMI (P<001). They also had higher systolic blood pressure ((P<0.001) and diastolic pressure . Blood pressure (P<0.001) , fasting plasma glucose (P<0.001) , HbA1C % (P<0.001) , fasting insulin (P<0.001) and insulin resistance (P<0.001). The mean hs-CRP value in the whole study population was 1.87 mgs/ l hs- CRP levels were significantly higher among the diabetic subjects with 4.8 mg/l (P<0.001) for non-diabetic subjects with 2.5 mgs/l.

The clinical and biochemical characteristics in normal and abnormal group of study the subjects were shown in table 2.When the study subjects were characterised as high risk using hs-CRP cut –off >3.0 mgs /l , the subjects with abnormal hs-CRP (hs-CRP>3.0 mgs/l) were older (P<0.001) , and also had higher body mass index (P<0.001) systolic pressure (P<0.001) than the subjects with normal hs-CRP (hs-CRP < 3.0 mgs/l).

Fasting plasma glucose (P<0.001) , total cholesterol (P<0.001) , LDL-cholesterol (P<0.001), HbA1C (P<0.001) and insulin resistance(P<0.001) were also higher in subjects with abnormal hs-CRP than the subjects with normal hs-CRP. Table 3 presents the results of the Pearson’s correlation analysis of hs-CRP with high risk variables. hs-CRP showed a significance positive correlation with age (r=0.168, P<0.001) , BMI (r= 0.238, P<0.001), systolic pressure (r=0.161 , P<0.001),fasting plasma glucose (r=0.274, P<0.001) and HbA1c (r=0.307, P<0.001) .

4. Discussion

Diabetic subjects were older with mean age of 51 years than the normal subjects. Diabetic subjects have higher Body Mass Index (BMI) .This was further confirmed by the study done by Ni Mhurchu et al. (2006). Eric and John (2006) and NHANES (2005) report indicates that most adults with diagnosed diabetes were overweight or obese , prevalence of over weight or obesity was 85.2 % and the prevalence of obesity was 54.8% . Cosin Aguilar et al. (2007) from his study state that the obese patients showed higher prevalence of diabetes.

Systolic and diastolic pressure was higher in diabetic subjects. Some studies show that the blood pressure and blood pressure progression were strong and independent predictors of incident Type 2 diabetes among initially healthy women (David and Paul , 2004) .The Third National Health and Nutrition Evaluation Survey (1988-1994) [NHANES] disclosed that 71% of diabetic individuals were found to have hypertension. ((Geiss , et al. 2002). Previous study showed that hypertensive patients have strong association with diabetes mellitus (

Table 1: Clinical and Biochemical characteristics of study subjects

Parameters	Healthy normal subjects n = 400	Type 2 diabetic subjects n = 400	P value
Age (Yrs.)	42 ± 12	51 ± 10	<0.001
Body mass Index (kgs/m²)	23.4 ± 4.5	24.8 ± 4.2	<0.001
Systolic BP(mm Hg)	118 .4 ± 16	129.7 ± 21	<0.001
Diastolic BP (mm Hg)	74.2 ± 10	75.7 ± 11	<0.001
Fasting plasma glucose (mgs/dl)	87 ± 8.1	163 ± 72	<0.001
HbA1c (%)	5.5 ± 0.50	8.7 ± 2.3	<0.001
Insulin reistance (HOMA IR)	1.8 ± 1.27	4.2 ± 2.78	<0.001
Hs-CRP (mgs/l)	2.5 ± 2.9	4.8 ± 3.4	<0.001

Table.2 Clinical and Biochemical characteristics in normal and abnormal hs-CRP levels.

Parameters	Normal hs-CRP (hs-CRP <3.0) (n = 440)	Abnormal hs-CRP (hs-CRP > 3.0) (n = 350)	P value
Age (Yrs.)	45 ± 12.97	49 ± 11.2	<0.001
Body mass Index (kgs/m²)	23.1 ± 4.18	25.3 ± 4.48	<0.001
Systolic BP(mm Hg)	120 .9 ± 18.54	127.9 ± 20.04	<0.001
Diastolic BP (mm Hg)	74.9 ± 10.73	76.9 ± 11.08	0.1
Fasting plasma glucose (mgs/dl)	108 ± 51.68	145 ± 71.68	<0.001
HbA1c (%)	6.4 ± 1.98	7.9 ± 2.39	<0.001
Insulin reistance (HOMA IR)	2.4 ± 2.12	3.6 ± 2.60	<0.001

Table.3 Pearsons correlation analysis of hs-CRP and other risk variables in total subjects.

Parameters	r	P value
Age (Yrs.)	0.168	**<0.001**
Body mass Index (kgs/m²)	0.238	**<0.001**
Systolic BP(mm Hg)	0.161	**<0.001**
Diastolic BP (mm Hg)	0.050	0.055
Fasting plasma glucose (mgs/dl)	0.274	**<0.001**
HbA1c (%)	0.307	**<0.001**
Insulin reistance (HOMA IR)	0.234	<0.001

Coisin Aguilar et al. (2007) ; Eric and John (2006) which support the present study.

Hb1Ac was higher in the diabetic subjects than normal subjects

The present study showed the significance increase of hs-CRP in subjects with Type 2 diabetes. Studies on western populations have shown low grade systemic inflammation to be one of the mechanisms by which known risk factors such as obesity, smoking and Hypertension promote the development of diabetes mellitus (Pradhan et al. 2001; Pfutzner and Forst, 2006). However, there are few studies of hs-CRP in Asian Indians, a very high- risk group for diabetes. (Mohan et al. 2003 ; Mohan et al. 2001 ; Wild et al. 2004). The hs-CRP seems to be strongly associated with diabetes mellitus and insulin resistance. The hs-CRP levels were elevated in diabetic subjects compared with non-diabetic subjects. Several studies have earlier shown that hs-CRP predicts diabetes in western populations (Pradhan et al. (2001), Haffeiner (2003) ; Hanley et al. (2004) as a biomarker of inflammation . The result obtained in this study is comparatively similar to the earlier work that emphasises the prediction of incident Type 2 diabetes by hs-CRP level. (David and Paul (2007) .The present study which showed increased of hs-CRP in diabetes was supported by the previous study results (Li CZ et al.(2004). The hs-CRP levels significantly associated with age in the present study .This was supported by earlier studies (Fransisco et al. 2005; Taniguchi et al. 2002; Chiriboga et al. 2009).

In the present study, Serum hs-CRP levels were positively related to anthropometric variables such as Body Mass Index (BMI) and Systolic and Diastolic blood pressure which is supporting earlier study (Li CZ *et al*. 2004;Francisco *et al.* 2005; Taniguchi *et al.* 2002; Wu *et al*. 2006; Earl and Wayene , 2004) .The correlation of hs-CRP with fasting plasma glucose and HbA1c observed is similar to the previous study (Li CZ , et al. 2004; Pradhan et al. 2001). In the current study, hs- CRP was positively correlated to insulin resistance (HOMA-IR) . Some study have explored the relation of hs-CRP with insulin resistance , which precedes diabetes(Nakanishi et al. ;Wu et al. 2006 ; Taniguchi et al. 2002 ; Chambers et al. 2001 ; Visser et al. 2000).Though the hs-CRP was identified as one of the most sensitive marker of inflammation, there are very studies which have looked at the association of insulin resistance with hs-CRP and none in a high risk Asian Indian population. Another interesting observation was the relationship of hs-CRP with glycemic control could influence inflammation. A prospective study on the Type 2 diabetic subjects suggested a decrease in hs-CRP levels with a decrease in HbA1c (Rodriguez and Guerrero, 1993). In diabetic subjects hs –CRP was positively correlated with HbA1c (Li *et al.* 2004) . hs- CRP was also associated with fasting plasma glucose. A recent population based study showed hs-CRP to be independently associated with fasting plasma glucose. (Aronson *et al*. 2004)

In the present study, subjects with abnormal hs-CRP had higher proportion of Hypertension, obesity, hyper cholesterolemia, (Table 5). Previous reports show that hyper trigyceridemia and Diabetes mellitus are positively associated with CRP levels ((Pick up *et al*. 1997). Previous analysis found high concentration of hs- CRP was significantly associated with Obesity. (Visser *et al*. 2000; Chambers *et al.* 2001; Pradhan *et al*. 2001; Forouchi et al. 2001).This is in contrast to median values in other population based studies of 2.1 mgs/l[(National health and Nutrition Examination Survey , 2. 67 mg/l Cardio Vascular Health Study and 1- 2 mg/l (depending upon age) in Europe (Hutchison et al. 2000). The only population based median (3.49 mg/l) to approach that of the present cohort is for the sub group of 230 women taking unopposed estrogens replacement therapy in the Cardiovascular Health study. (Chushma et al. 1999) .

Possible reasons for the higher CRP values in this population include a very high prevalence of Diabetes Mellitus and obesity since Diabetes and high body mass index are associated with elevated CRP. Similar results are found in earlier study (Barinas *et. al*.2001 ; Frohlich *et al.* 2000). In conclusion , the present study showed that hs-CRP has a strong association with diabetes in Chennai Urban Rural population. It is also concluded that age , body mass index , hyper sensitivity and body weight has strong association with diabetic individuals and high levels of hs-CRP groups predicts the high risk of diabetes mellitus type 2. It is very well understand that the levels of hs-CRP significantly associated with age and positively related to insulin resistance, BMI, systolic and diastolic pressure. Similarly, low HbA1c strongly related to negative hs-CRP levels. It is also observed that hs-CRP levels are the ensitive marker forinflammation. Moreover this study also concludes that elevated hs-CRP level significantly different with different age groups of diabetes mellitus individuals.

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The effect of environmental conditions on the start of dawn singing of blackbirds (*Turdus merula*) and Bulbuls (*Pycnonotidae*)

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Abstract

Environmental pollution is known to influence bird behavior in many ways. In this study, the effect of noise, street lighting, moon light and weather conditions on the start of dawn singing of the two most common bird species in the area, bulbuls (Pycnonotus barbatus) and blackbirds (Turdus merula) is studied in a filed environment over a period of three autumn months. Results show that streetand moon lighting and the call for prayers by mosque loud speakers did not affect the start of singing compared to sunrise. Weather conditions (wind and rain) are the main factors that influenced the onset of singing in these two species. A relationship between sunrise and start of singing is established for blackbirds and bulbuls. Results show that sunlight,but not moonlight, affected the onset of dawn chorus in bulbuls and blackbirds. This study could serve as a record for similar future investigations on the influence of environmental conditions on birds behavior in the region.

الملخص

يتأثر سلوك الطيور بالتغيير البيئي والمناخي بعدة طرق منها التغيير في بداية وقت الغناء الصباحي . لقد تم في هذه الدراسة الميدانية تتبع وقت الغناء الصباحي لكل من طائر البلبل وطائر السود ودراسة تأثير الصوت العالي / الضجيج، الإضاءة الصناعية، ضوء القمر والعوامل المناخية مثل المطر و الرياح لمدة 3 أشهر في نهاية الصيف وبداية الخريف . عند مقارنة بدء الغناء مع وقت شروق الشمس فانه لم يظهر أي فرق بين الطيور التي تعيش في منطقة مضاءة صناعيا (أضواء الشوارع) وبين المناطق غير المضاءة. كذلك لم يلاحظ آي تغيير في وقت الغناء عندما يكون القمر كاملا. إن نداء صلاة الفجر الأول و الثاني لم يظهر إنهما يوقظان هذه الطيور ولم تغن أي منها بعد الأذان مباشرة أو خلال فترة وجيزة. إن العوامل الوحيدة التي أحدثت تغييرا هي هطول الأمطار الغزيرة أو الرياح الشديدة. تكمن أهمية هذه الدراسة و أية دراسات مشابهة في مناطق أخرى من الشرق الأوسط في أنها ستحدد نمط سلوك طيور المنطقة بحيث يمكن الرجوع لهذه النتائج مستقبلا وسيكون هذا مرجعا للجميع وإذا كان هناك تغييرات في هذا النمط فسيدل ذلك على تغيير في سلوك الطيور وبالتالي على التغيير البيئي والمناخي

Keywords: Bulbuls, Blackbirds, Sunrise, Noise, Light, Weather, Singing.

1. Introduction

Environmental pollutants can trigger responses that might affect many animals behavior in a way that may enhance or harm their chances of survival and reproduction. Artificial lighting is considered as an environmental pollutant that might disrupt biological clocks that evolved to natural patterns of light and dark (Longcore and Rich 2004). Artificial light can cause disorientation and trapping of birds (Odgen 1996, Rich and Longcore 2006). Environmental noise was shown to affect birds singing. High daytime *noise* levels in urban areas have caused *robins* to *sing* at night (Fuller et al. 2007) and great tits (Parus major) to sing in noisy areas at a higher pitch to avoid the detrimental effect of auditory masking (Slabbekoorn and Peet 2003 and Brumm 2004).

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Bird's biological clock is adjusted to photoperiod. In the wild, animals rely on the cycling of the sun, and the seasons to adjust their biological clocks and metabolism (Thrush 1999). Birds have a highly developed sense of light. Their biological clock depends on the sun and changes in the quality of light and length of the day, which sets the stage for breeding, migration, molting, and daily behavior patterns. It is believed that relatively larger eyed birds start singing earlier (Berg et al. 2006)

The two bird species investigated in this study are the two most common resident bird species in the area. Both start singing before sunrise, singing throughout the day and stop singing just after sunset but do not sing during the night. It is thought that the increase of light intensity around sunrise makes the birds start singing. The onset of birds singing varies among species. Some start singing as early as one hour before sunrise such as robins (Erithacus rubecula) while others start around few minutes before sunrise like common house sparrow *(*Passer domesticus*)* . Northern mockingbirds (Mimus polyglottos) start singing 0.5 to 1 hour before sunrise and continue singing

throughout the day (Hill et al. 2005). The activity of birds can also be influenced by the intensity of moonlight (Wilson and Watts 2006).

In this study, the effect of moonlighting and other environmental factors such as noise, street lighting, and changing weather conditions on the start of singing of these two species is monitored in their natural habitat. It is expected that these birds will have a constant pattern of singing in future years and any change in this pattern might indicate a change in environmental conditions that lead to behavioral change.

2. Methods

In this study, we investigated free Bulbuls (*Pycnonotidae*) and blackbirds **(***Turdus merula***)** in their natural habitat without any human interference .The timing of first singing is recorded on a calendar that shows the daily sunrise / sunset and the five daily Moslem prayers in two adjacent locations; one is in front of a group of houses alongside a street where street lighting is installed and the other is in the back of the same houses about one hundred meters away where there is no artificial lighting or traffic. Street lights were switched on 15 minutes after sunset and switched off 40 minutes before sunrise. Tables and graphs showing singing times and sunrise times for the months of October, November and December 2008 are determined. October is early autumn in Middle East where days are still warm and some nights start to cool down a bit. Days also get shorter almost everyday which allows for comparing singing times with steady changes in sun photoperiod. This period also allows for monitoring of the effect of moon lighting on start of singing during full lunar months since the start of October coincided with the start of a lunar month. Nights are generally clear without clouds. The situation is very similar in November and December, but nights are usually colder than in October with the chance of having cloudy, windy or rainy nights. The study site is a hill at the outskirts of Tulkarem town in the West Bank, Palestinian Authority Territory. This is a very quiet location with few houses that have large gardens, and there are no main roads and no heavy traffic. It is very rare to have vehicles passing by between 10:00PM and 6:00AM. The number of birds investigated is not exactly known, but it can be assumed that there were 5-10 birds that started singing immediately after the first singer.

3. Results:

The start of singing of blackbirds and bulbuls changed with the time of sunrise. Birds commenced singing within one minute on both locations. This indicates that street lighting is not a determinant factor in birds singing in this study. Tables 1, 2 and 3 show the start of singing and sunrise times for the months of October, November and December 2008, consecutively. Graphs in figure 1, 2 and 3 show the time difference between start of singing and sunrise for the months of October, November and December consecutively. Blackbirds start singing earlier than bulbuls. In bulbuls the onset of singing was more regular than in blackbirds, though both species showed a

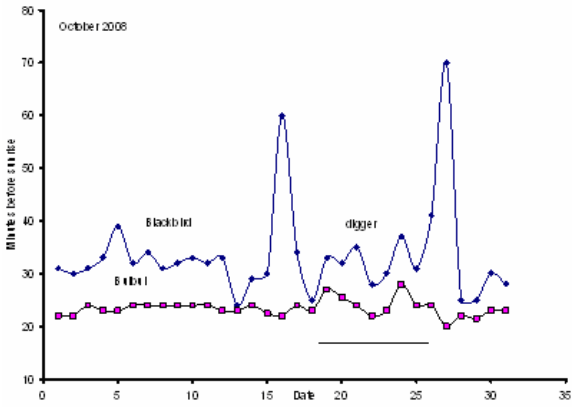

Figure 1. Difference between start of singing and sunrise for October 2008

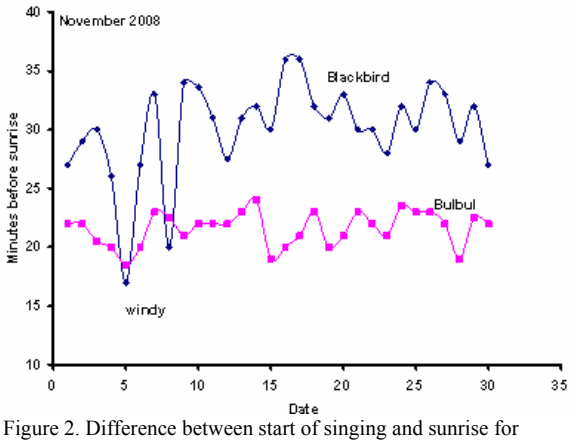

Figure 2. Difference between start of singing and sunrise for November 2008

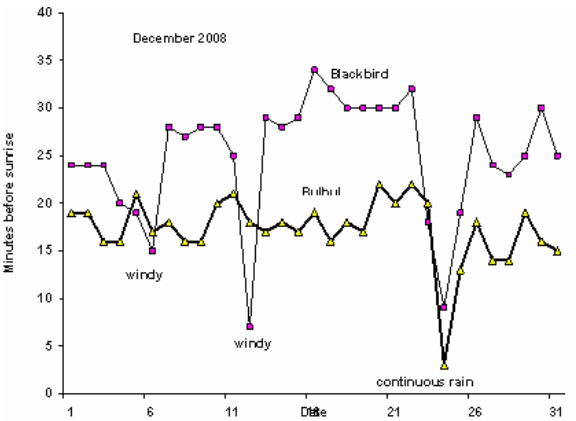

Figure 3. Difference between start of singing and sunrise for December 2008

similar pattern. Moonlight had no effect on the start of singing of both birds. As mentioned earlier, the start of October coincided with the start of a lunar month. Therefore, full moon was around the 15th of the month. Data and figures do not show any obvious variations in the start of singing of both these birds at this time or around it for three consecutive months. Moderate to strong wind and continuous moderate to heavy rain affected blackbirds and bulbuls considerably. Light drizzle a short time (15 min) before or at the time of singing has no effect. Blackbirds were more influenced than bulbuls. They started singing later than usual even later than bulbuls in some occasions. The first call for the dawn prayer by mosques loudspeakers is usually around 90 minutes before sunrise while the

Table 1. Start of singing and time difference before sunrise for October 2008

	Sunrise	Start of singing		difference (min)	
October	hr.min	Blackbird	Bulbul	blackbird	Bulbul
1	5.31	5.00	5.08	31	22
2	5.32	5.02	5.10	30	22
3	5.32	5.01	5.08	31	24
4	5.33	5.00	5.10	33	23
5	5.34	4.55	5.11	39	23
6	5.34	5.01	5.10	32	24
7	5.35	5.01	5.10	34	24
8	5.36	5.05	5.12	31	24
9	5.36	5.04	5.12	32	24
10	5.37	5.04	5.13	33	24
11	5.38	5.06	5.14	32	24
12	5.38	5.05	5.15	33	23
13	5.39	5.15	5.16	24	23
14	5.40	5.11	5.16	29	24
15	5.40	5.10	5.175	30	22.5
16	5.41	4.40	5.19	60	22
17	5.42	5.08	5.18	34	24
18	5.43	5.18	5.2	25	23
*19	5.43	5.10	5.16	33	27
20	5.44	5.12	5.185	32	25.5
21	5.45	5.10	5.21	35	24
*22	5.46	5.18	5.24	28	22
23	5.47	5.17	5.24	30	23
*24	5.47	5.10	5.19	37	28
25	5.48	5.17	5.24	31	24
26	5.49	5.08	5.25	41	24
*27	5.49	4.40	5.29	70	20
28	5.50	5.25	5.28	25	22
29	5.51	5.26	5.295	25	21.5
30	5.52	5.22	5.29	30	23
31	5.53	5.25	5.3	28	23
* digger			average	33.48387	23.43548

Table 2. Start of singing and time difference before sunrise for November 2008

	Sunrise	Start singing		difference (min)	
November	hr.min	Blackbird	Bulbul	blackbird	Bulbul
1	5.53	5.26	5.31	27	22
2	5.54	5.25	5.32	29	22
3	5.55	5.25	5.34.5	30	20.5
4	5.56	5.30	5.36	26	20
*5	5.57	5.40	5.39	17	18
6	5.57	5.30	5.37	27	20
7	5.58	5.25	5.35	33	23
*8	5.59	5.39	5.36.5	20	22.5
9	6.00	5.26	5.39	34	21
10	6.01	5.275	5.39	33.6	22
11	6.02	5.31	5.40	31	22
12	6.02	5.35	5.40	28	22
13	6.03	5.32	5.40	31	23
14	6.04	5.32	5.40	32	24
15	6.05	5.35	5.46	30	19
16	6.06	5.30	5.46	36	20
17	6.07	5.31	5.46	36	21
18	6.07	5.35	5.44	32	23
19	6.08	5.37	5.48	31	20
20	6.09	5.36	5.48	33	21
21	6.10	5.40	5.47	30	23
22	6.11	5.41	5.49	30	22
23	6.12	5.44	5.51	28	21
24	6.12	5.4	5.49	32	24
25	6.13	5.43	5.5	30	23
26	6.14	5.40	5.51	34	23
27	6.15	5.42	5.53	33	22
28	6.16	5.47	5.57	29	19
29	6.17	5.45	5.55	32	23
30	6.17	5.50	5.55	27	22
* light-moderate wind			average	30.03667	21.58333

second call for dawn prayer is around 75 minutes before sunrise. These calls do not seem to waken up these birds since blackbirds started singing around 30 minutes before sunrise while bulbuls started at around 20 minutes before sunrise. The disturbance shown between the 19th and the 25th of October might be due to noise caused by a digger that was doing some construction work in the neighborhood (Fig 1). There is no explanation for the big change (about one hour) on the days of the 16th and 27th of October (Fig 1,).

4. Discussion:

The start of bird singing is used as a marker to investigate the effect of light, noise and changing weather conditions on birds' behavior. The start of singing varies between different species of birds.

For example, mockingbirds occasionally sing more than one hour before sunrise (Merritt 1984) and this species are known to sing also in moonlit nights (Hill et al. 2005, Allard 1930). This study shows that blackbirds and bulbuls started singing about 30 min and 20 min respectively before sunrise. Street lights and moonlighting had no effect on the start of singing of the two birds while noise caused by diggers but not by mosques loudspeakers delayed the onset of dawn chorus. It was shown (Wilson and Watts, 2006) that moonlighting influenced the start of birds singing. This is in contrast to our findings.

The most prominent factor that affected the start of singing in blackbirds and bulbuls were weather conditions. It is known that weather has an influence on birds songs in that both cool and hot weather will decrease the amount of singing, as do rain and wind (Catchpole & Slater 2008).

Table 3. Start of singing and time difference before sunrise for December 2008

	sunrise	Start singing		difference (min)	
December	Hr.min	blackbird	bulbul	blackbird	bulbul
1	6,18	5,54	5,59	24	19
2	6,19	5,55	6,00	24	19
3	6,20	5,56	6,04	24	16
4	6,20	6,00	6,04	20	16
*5	6,21	6,02	6,00	19	21
*6	6,22	6,07	6,05	15	17
7	6,23	5,55	6,05	28	18
8	6,23	5,56	6,07	27	16
9	6,24	5,56	6,08	28	16
10	6,25	5,57	6,05	28	20
11	6,26	6,01	6,05	25	21
*12	6,26	6,19	6,08	7	18
13	6,27	5,58	6,10	29	17
14	6,28	6,00	6,10	28	18
15	6,28	5,59	6,11	29	17
16	6,29	5,55	6,10	34	19
17	6,29	5,57	6,11	32	16
18	6,30	6,00	6,12	30	18
19	6,30	6,00	6,13	30	17
20	6,32	6,02	6,10	30	22
21	6,32	6,02	6,08	30	20
22	6,32	6,00	6,10	32	22
23	6,32	6,04	6,12	18	20
**24	6,33	6,24	6,30	9	3
***25	6,33	6,14	6,20	19	13
26	6,34	6,05	6,16	29	18
27	6,34	6,10	6,20	24	14
28	6,34	6,11	6,20	23	14
29	6,35	6,10	6,16	25	19
30	6,35	6,05	6,19	30	16
#31	6,35	6,10	6,20	25	15
* moderate wind			average	25	17.25806

**medium rain all night, first real winter day in season

***light rain #light drizzle during night

The amount of clouds in the skies does not affect the start of singing in this study although it is reported that cloudiness in the morning will delay singing (Hill et al. 2005). The amount of sun light rather than moonlight and the time of day determined the beginning and end of singing. Most birds show a seasonal variation that is mainly correlated with breeding activities and hormone production. In winter months these birds start singing closer to sunrise, but the time difference is not too big, about 10 minutes only. For most species, hormones, stimulated by photoperiod, probably play a dominant role in determining the time of year a bird sings. The injection of male hormones into male birds in mid-winter will start them singing (Wilson and Watts 2006). Dawn chorus is also shown to be a reliable indicator of male quality and social interactions (Amrhein & Erne 2006). Harsh weather conditions can cause stress in birds. Birds produce cortisone in response to this stress that might trigger physiological and behavioral changes to ameliorate these effects (Romeo et al. 2000).

Finally, this study clearly shows that moonlight and street lighting had no effect on start of dawn singing and it establishes a pattern for the two main singing birds in the region with a clear correlation with sunrise. In comparison to other cities where similar studies were undertaken (Fuller et al. 2007) the anthropogenic impact on birdsong seems to be small.

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Functional response of the predator *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) feeding on the aphid *Brachycaudus helichrysi* (Kaltenbach) infesting chrysanthemum in the Laboratory

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Abstract

Functional response of the predatory coccinellid *Hippodamia variegata* (Goeze) to the density of *Brachycaudus helichrysi* (Kaltenbach) aphid infesting chrysanthemum was studied under laboratory conditions at 25 ˚C. A single adult female predator on isolated areas with different prey densities was used. Response pattern of the six-day old predator to the various prey densities was found. Predator exhibited a decelerating curvilinear rise to a plateau (type II) response in which the proportion of prey consumed declines monotonically with the initial number of prey offered. The mortality curve of the prey at different densities due to the predator was also evaluated. The mortality first increases with prey increasing density, and then declines. The predator functional response can be simulated by Holling disc equation and expressed as: (H*a* = 0.97525 H / 1 + 0.0066317 H) and by the reciprocal linear transformation of Holling's equation as: (y = 0.5127 x + 0.0068). The estimated coefficient of attack rate (a) is 1.9505 and the handling time (T*h*) is 0.0034.

الملخص

تمت دراسة الاستجابة الوظيفية للمفترس هيبوداميا فاريجيتا بالنسبة لكثافة من الاقحوان تحت ظروف المختبر على درجة حرارة 25 ْم. و قد وضعت الحشرات الكاملة لانثى المفترس فرادى مع عدة كثافات من الفريسة لهذه الغاية. و قد وجد نمط الاستجابة لاناث المفترس ذات العمر ستة ايام لعدة كثافات من الفريسة حيث اظهر المفترس استجابة من النوع الثاني بزيادة بخط منحني بسرعة متناقصة حتى مرحلة الاستقرار حيث أن نسبة الفريسة المستهلكة تراجعت على نفس الوتيرة بالنسبة للعدد الاولي للفريسة المقدمة. كما و تم تقييم منحنى وفيات الفريسة بكثافاتها المختلفة تحت تأثير حشرات المفترس. في البداية، زادت الوفيات بزيادة فة الفريكنة و من ثم بدأت الوفيات بالانحدار. و قد أمكن اظهار و التعبير عن الاستجابة الوظيفية للمفترس باستخدام معادلة هولنج القرصية ب (Ha= 0.97525 H/ 1+ 0.0066317 H) و باستخدام التحويل الخطي ذو العلاقة المتبادلة لمعادلة هولنج على النحو التالي: (Y= 0.5127 X + 0.0068) و قد قدر معامل معدل الهجوم ب (1.9505) و قدر زمن المناولة ب (0.0034).

Keywords: *Brachycaudus helichrysi*, functional response, *Hippodamia variegata*, predator-prey interaction .

1. Introduction

The relationship between prey density and a consumption rate is known as the functional response (Abrams and Ginzburg, 2000; Jeschke *et al*., 2002). It is an important phenomenon, it describes the rate at which a predator kills its prey at different prey densities and can thus determine the efficiency of a predator in regulating prey populations (Murdoch and Oaten, 1975). Four fundamental types of functional response curves are described (Holling, 1959, Jervis and Kidd, 1996); linear rise to a plateau (type I), negatively accelerated rise to a plateau (type II), S-shaped rise to a plateau (type III) and dome-shaped type IV. This could further be simplified in terms of density dependence. That is, they result in a constant (type I), decreasing (type II) and increasing (type III) rate of prey killing and yield density-dependent, negatively density dependent and positively density dependent prey mortality, respectively (Pervez and Omkar, 2005). Predators that impose positively density dependence prey mortality (type III) are supposed to potentially manage the prey population and could be considered as efficient biological control agents (Fernandez-Arhex and Corley, 2003). However, certain

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predators exhibiting type II response have been successfully established and managed prey populations (Hughes *et al*., 1992; Fernandez-Arhex and Corley, 2003). Functional response, though an important tool, but cannot only be attributed to reported success and failures in biological control programs. For instance, other factors, such as intrinsic growth rates, host patchiness, predation and competition, host traits, and environmental complexities also have a major influence on the efficiency of the predator (Pervez and Omkar, 2005). The functional response curves can be differentiated by evaluating the functional response parameters, coefficient of attack rate and handling time.

The plum leaf-curl aphid, *Brachycaudus helichrysi* (Kaltenbach) (Hemiptera: Aphididae) is a cosmopolitan insect that known to be the most serious and economic pest of chrysanthemum in glass houses (Wyatt and Brown, 1977). Coccinellids are one of the important groups of predatory insects that have immense biological control potential (Omkar and Pervez, 2003). Many experiments about predaceous insects in agricultural systems have evaluated diverse aspects of the functional response; Veeravel and Baskaran (1997) and Vieira *et al*. (1997) with coccinelids. The originally Palaearctic but now widespread beetle *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is an important predator of twelve different aphid species, particularly *B. helichrysi* (Franzmann, 2002). It is native to Europe but introduced to North America.

The present study was carried out to determine how *H. variegata* will respond to changing prey density under simplified experimental conditions

2. Materials and Methods

2.1. Insect's culture & maintenance and experimental design:

B. helichrysi aphid was obtained from infested chrysanthemum plants grown in the plastic houses of Al-Balqa' Applied University/ in Al-Salt City and cultured inside cages (2x2x2 m) on chrysanthemum seedlings maintained at 25 ± 2˚C, 65% RH and in 16: 8 light: dark. Adults of the predator *H. variegata* were collected from the same location as the prey and brought to the laboratory in Al-Balqa' Applied University and reared inside chambers. Coccinellids were kept in pairs inside cages (2x2x2 m) containing chrysanthemum seedlings infested with aphids for oviposition. Seedlings held at the previously mentioned environmental conditions. Leaves with eggs were isolated individually in Petri dishes (9 cm in diameter) to obtain six-day old virgin adult females to be used in experiments. Tests were undertaken on chrysanthemum leaves cut so that they retained two cm of petiole length covered with wet cotton wool and placed upside down in Petri dishes. Females of the predator were kept without food for 12 hrs to standardize their hunger before use. Thereafter, they were kept separately at different densities 10, 20, 40, 60, 70, 100, 120 and 140 of the aphid after it settled down, in 15 replicates. After 12 hrs, the females removed and the number of aphids left was recorded but consumed prey were not replaced during the experiment. Then, we calculated the number of aphids preyed in each dish.

2.2. Data Analysis:

A logistic regression model was used to determine the shape of the functional response by taking into consideration the proportion of the prey eaten (H*a*/ H) as a function of prey offered (H) (Juliano, 2001). The data were fitted to a polynomial function that describes the relationship between H*a*/H and H:

$$\frac{Ha}{H} = \frac{\exp\left(L_o + L_1 H + L_2 H^2 + L_3 H^3\right)}{1 + \exp\left(L_0 + L_1 H + L_2 H^2 + L_3 H^3\right)}$$

With L0, L1, L2 and L3 being the intercept, linear, quadratic and cubic coefficients, respectively, estimated using the method of maximum likelihood. If L1 < zero, describing a type II functional response (Juliano, 2001). After the determination of the functional response type, the handling time (T*h*) and the attack constant (a) were estimated using Holling's disc equation modified by reciprocal linear transformation (Livdahl and Stiven, 1983). The modified equation is as follows:

$$\frac{1}{Ha} = \frac{1}{a}.\frac{1}{H.T} + \frac{Th}{T}$$

where $\frac{1}{Ha}$ represents y, $\frac{1}{a}$ represents α, $\frac{1}{H.T}$ represents x and $\frac{Th}{T}$ represents ß. The linear regression form becomes y = α x + ß. The maximum number of consumed prey per predator (asymptote), Ha_{max} = T/T*h* was found.

3. Results

Predation potential of *H. variegata* was evaluated across eight prey densities of *B. helichrysi* to asses the form of its functional response. Figure 1 shows the graph of the predator functional response that corresponds to Holling disc equation. The functional response curve obtained is belonging to Holling's type II. Most of the values of L1 were negative; confirming the functional response to be of type II (Table 1). The number of prey killed approaches the asymptote hyperbolically as prey density increases. This corresponds to an asymptotically declining proportion of prey killed (Figure 2). The highest percentage of death was recorded at the aphid density of 20 and then the curve goes down with increasing prey density.

The linear regression of the predator as shown in Figure 3 can be represented by the equation: y = 0.5127 x + 0.0068. From the coefficients of the linear regression, the instantaneous search rate (a) was estimated to be 1.9505 and the handling time (T*h*) was 0.0034. The calculated maximum number of consumed prey per predator was 147.06.

Table 1: The experimental data for the functional response parameters of the predator *Hippodamia variegata* feeding on the *Brachycaudus helichrysi* aphid infesting chrysanthemum plants

Prey density (H)	Total prey killed for the 15 repl.	% Prey killed	Average no. prey killed (Ha)	1/Ha	1/(H.T)	Death (d) = Ha/H	Linear coefficient (L1)
10	133	88.7	8.867	0.113	0.20	0.887	- 0.90334
20	288	96.0	19.20	0.052	0.10	0.960	Reference value
40	540	90.0	36.0	0.0278	0.05	0.900	0.44794
60	660	73.3	44.0	0.0227	0.033	0.733	- 0.03419
70	705	67.1	47.0	0.0213	0.029	0.671	- 0.35861
100	785	52.3	52.33	0.0191	0.02	0.523	- 0.80835
120	825	45.8	55.0	0.0182	0.017	0.458	0.22760
140	870	41.4	58.0	0.0172	0.014	0.414	- 0.28905

*The duration of the experiment (T) = 12 hrs

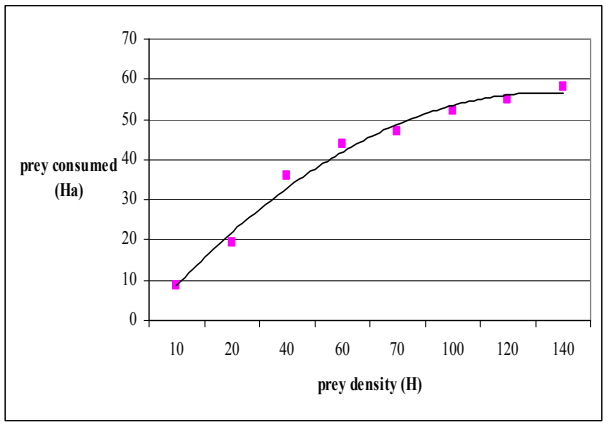

Figure 1: Type II functional response of the predatory coccinellid *Hippodamia variegata* feeding on different densities of the aphid *Brachycaudus helichrysi*.

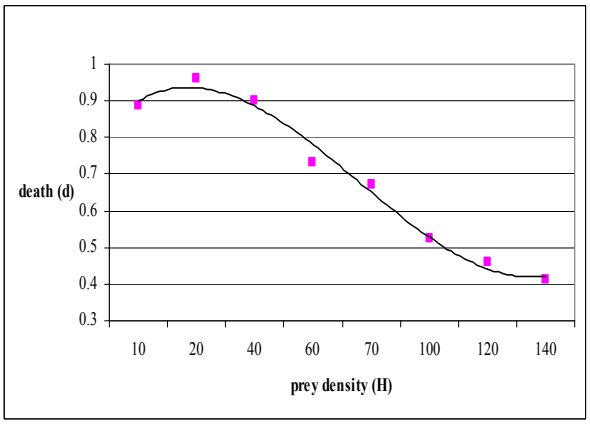

Figure 2: Prey mortality curve of the chrysanthemum aphid *Brachycaudus helichrysi* due to the predator *Hippodamia variegata*.

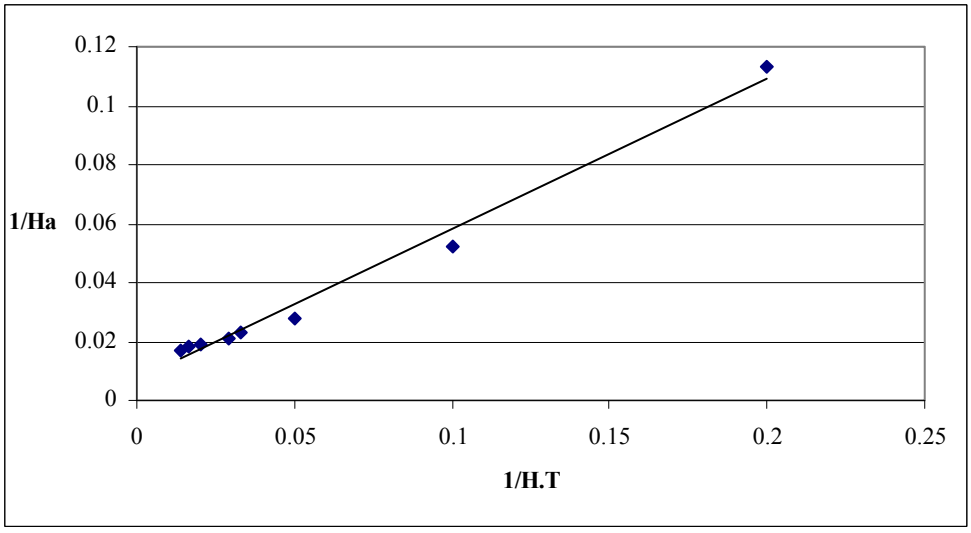

Figure 3: The linear regression for parameters estimation of *Hippodamia variegata* feeding on *Brachycaudus helichrysi* aphid (y = 0.5127 x + 0.0068.

4. Discussion

In this study, the type II functional response model obtained gave a satisfactory fit to the data of *H. variegata* preying on *B. helichrysi*; which being the most frequently observed type for a wide variety of predators including insects (Aukema and Raffa, 2004; Begon *et al*., 1996). A logistic regression model of proportions of prey killed (H*a*/H) versus (H) was also used to confirm the correctness of the functional response type obtained (Figure 2), because it provides a more powerful and accurate means of distinguishing between type II and type III (Trexler *et al*., 1988). Moreover, the resulted negative values for the linear coefficient in Table 1 (L1 < zero) were also confirmed the obtained type. The predator shows a negative density dependence of the proportion of the prey killed (H*a*/H) as the density increases (Table 1). The predator shows a decreasing consumption rate with increasing prey density. Our finding coincides with the results found by Pervez and Omkar (2003) who mentioned that a high rate of prey consumption at higher densities is not a feature of aphidophagous coccinellid predators.

The obtained functional response parameters (a) and (T*h*) can be used to determine the simulated prey consumed (H*a*) at any wanted prey density (H) using the obtained equation: H*a* = 0.97525 H / (1 + 0.0066317 H). This will lead to minimizing the future efforts needed to generate voluminous empirical data at different aphid densities in the laboratory.

Our data provide information and idea as to how this predator responds to a change in prey density on single and isolated patches under laboratory conditions. These patches are small leaves, confining the predator and the prey movement to a few square centimeters. For conclusive estimation of its control potential, field data are essential to complete the laboratory results, since in natural conditions other variables can interfere in predator behavior; introducing modifications in functional response components.

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No significant Cytogenetic Effects in Cultured Human Lymphocytes Exposed to Cell Phones Radiofrequencies (900MHz and 1800MHz)

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Abstract

The aim of this study isto examine whether radiofrequency (RF) used in cellular phone communications at a specific absorption rate (SAR) less than 1.2 w/kg could increase the spontaneous rate of sister chromatid exchanges (SCE) or elicit alterations both in the cell replication index (CRI) and mitotic index (MI) in human peripheral blood lymphocytes. Whole blood samples were obtained from twenty six healthy donors (male nonsmokers). Cultures were placed in sterile T-25 tissue flasks and sham-exposed or RF-exposed to 900 MHz or 1800 MHz radiation for 1 h, then incubated for 72 h at 37 °C. The mean SCE values of both RF exposure groups slightly increased, as compared with the sham-exposure group. However, there was no significant difference between the RF exposure groups and sham-RF exposure group. Compared with the negative control, both CRI and MI were non-significantly elevated after exposure to both frequencies, but to a lesser extent in case of 1800 MHz exposure. In view of the present guidelines of mutagenicity, which necessitates at least two-fold increase in SCE rate, RF is not considered as mutagenic.

الملخص

هدفت هذه الدراسة الى تقصي ما اذا كانت الموجات الراديوية المستخدمة في الهاتف النقال، عند معدل امتصاص محدد (اقل من 1,2 واط لكل كيلو غرام)، تزيد المعدل الطبيعي للتبادلات شق- الصبغية والى تغيير المستويات العادية لمعاملي تكاثر و انقسام الخلا يا اللمفية البشرية. و قد اخذت ت دم ستة وعشرين متطوعا من الرجال غير المدخنين. وتم تعريض مزارع الخلايا لمستويين من موجات الهاتف النقال(900 و 1800 ميغا هيرتز) لمدة ساعة واحدة. و بعد ذلك، اكملت مدة الزراعة ال اثنتين و سبعين ساعة على درجة حرارة 37 سيلسيس. و اظهرت نتائج البحث زيادة ضئيلة، ليست ذات دلالة احصائية، في معدلات لالتبادشق- الصبغية في الخلايا المتعرضة لأي من الترددين، مقارنة بالخلايا الضابطة, كما بينت الدراسة زيادة ملحوظة، لكنها ليست ذا معنى احصائي، في معاملي تكاثر و انقسام الخلايا، بالمقارنة مع المزارع الضابطة. وكانت هذه الزيادة اكثر و ضوحا عند مستوى التردد الا على . و في ضوء التعليمات العالمية الحالية المتعلقة بالسمية الوراثية، فانه يمكن الاستنتاج ان التعرض للمستويات المدروسة من اشعاعات الهاتف النقال لا تؤ ثر سلبا في المادة الوراثية.

Keywords: Human Lymphocytes; Sister-Chromatid Exchanges; Cell Replication Index; Mitotic Index; Cell Phone; Radio frequency Radiation..

1. Introduction

Cellular phones have been one of the fastest growing industries in modern history. The number of mobile phone users worldwide soared to over 3.3 billion by the end of 2007, a total penetration rate of 49 percent. Since their introduction in 1983, there have been remarkable changes in the cellular industry. The first generation mobile phone systems were analog and used 450 and 900 MHz portion of the RF spectrum. The second generation is digital and operates at frequencies 900 MHz and 1800 MHz. For example; the Global System for Mobile Communication (GSM) operates at 900MHz and 1800 MHz with digital modulation scheme and Time Division Multiple Access (TDMA). Recently, the third generation system such as the Universal Mobile Telecommunication System has been introduced and operates in the 1900-2200 MHZ range and uses digital modulation with Code Division Multiple Access (CDMA) (Moulder *et al*., 2005).

A great concern has been raised about the biological effects in persons using these devices. The relatively fixed position of the antenna to the head causes a repeated irradiation of a more or less fixed amount of electromagnetic (EM) energy (Radon *et al*., 2006). Results from present studies on use of mobile phones for 10 or more years give a consistent pattern of increased risk for

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acoustic neuroma and glioma. The risk is highest for ipsilateral exposure (Hardell *et al*., 2007). Furthermore, a statistically significant association between mobile usage and parotid gland (salivary gland) tumors has been reported (Sadetzki *et al*., 2008).

Children's brains absorb a greater proportion of the radiation emitted by a mobile phone (Christ and Kuster 2005; De Salles *et al*., 2006) and they are also likely to be at a greater risk due to their higher rate of cell division (than adults). Since normal cell growth is controlled by the genetic material (DNA), any change in DNA would be reflected on the general health of the living organism. In this regard, analysis on whole-genome cDNA arrays showed alterations in gene expression after various RF exposure conditions using different cell types, but no consistent RF- signature such as stress response could be identified (Remondini *et al*., 2006). In addition, modulation in gene regulation after RF fields exposure at SAR of 1.5 W/kg in p53-deficient embryonic stem cells has been described (Nikolova *et al*., 2005).

It has been reported that exposure of Molt-4 T-lymphoblastoid cells to the cell phone frequency of 836 MHz, consistently resulted in an observable variation of DNA damage (Phillips *et al*., 1998). Furthermore, DNA strand breaks were reported in human diploid fibroblasts and cultured rat granulosa cells (Diem *et al*., 2005) as well as in embryonic stem cell--derived neural progenitor cells (D'Ambrosio *et al*., 2002) after RF field exposure. In individuals, who used digital mobile phones for at least 2 years, with uplink frequencies at 935-960 MHz, there was a significant increase in SCE, but there was no change in cell cycle progression (Gadhia *et al*., 2003). Recently, a number of in vivo experiments have found mobile phone or simulated mobile phone radiation exposure can cause cell damage, reactive oxygen species (ROS) formation (which are the primary cause of DNA strand breaks), and cell death (Oktem *et al*., 2005; Ferreira *et al*., 2006; Oral *et al*., 2006; Panagopoulos *et al*., 2007). In vitro experiments have found an association between RF exposure and ROS production, and then subsequent DNA single and double strand breaks (SSB and DSB) (Nikolova *et al*., 2005; Friedman *et al*., 2007; Yao *et al*., 2008). Also, there have been a number of other cellular effects found, from cell mutations such micronuclei (MN) formation and cellular aneuploidy, following exposure to RF fields (Bisht *et al*., 2002; D'Ambrosio *et al*., 2002; Mazor *et al*., 2008; Schwarz *et al*., 2008) and even impaired cell repair or cell death (Joubert *et al*., 2008; Manti *et al*., 2008; Palumbo *et al*., 2008). Exposure to 900 MHz GSM radiation induced changes in gene expression, but not DNA DSB, in rat brain cells (Belyaev *et al*., 2006).

On the other hand, in vitro exposure of human blood leukocytes and lymphocytes to RF signals emitted by cellular telephones for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes, nor did exposure for 3 h induce a significant increase in micronucleated cells among lymphocytes (Tice *et al*., 2002). However, these researchers found that exposure of lymphocytes for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated cells. In cultured human blood cells, others (Zeni *et al*., 2005; Stronati *et al*., 2006) reported results that do not support evidence for any DNA damage [SCE, chromosome aberrations (CA), and MN] induced by extremely low frequency magnetic fields (ELFMF) exposure. Using the same cell system used by Diem *et al*. 2005), Speit *et al*. (2007) obtained clearly negative results. Their finding that RF radiations at cellular phone level do not induce DNA/ genetic damage has been recently confirmed (Tiwari *et al*., 2008). Furthermore, some papers could not find an association between RF exposure and ROS production as well as subsequent DNA SSB and DSB (Lantow *et al*., 2006; Valbonesi *et al*., 2008). No statistically significant difference in the level of DNA damage was observed between sham-treated Molt-4 cells and cells exposed to RF radiation for any frequency, modulation or exposure time (Hook *et al*., 2004). In mammalian cells, no direct cytogenetic effect of 835-MHz RF-EM field was found in the in vitro CA test (Kim *et al*., 2008).

Several studies investigated the influence of RF fields on cell cycle kinetics, but in the majority of the investigations no effects were detected (Capriet *et a*l., 2004; Zeni *et al* ., 2005; Lantow *et al*., 2006; Stronati *et al*., 2006; Chauhan *et al*., 2007; Huang *et al*., 2008). Similarly, no detectable changes in cell viability or incidence of apoptosis were observed in any of RF-field-exposed groups in a series of human-derived cell lines (Capri *et al*., 2004; Hook *et al*., 2004; Caraglia *et al*., 2005; Lantow *et al*., 2006; Chauhan *et al*., 2007; Huang *et al*., 2008), as well as in proliferating or differentiated murine neuroblastoma cells (Moquet *et al*., 2008). Alteration in cell proliferation was described only in a few reports (Pacini *et al*., 2002; Capri *et al*., 2004).

As stated by Özyalçin *et al.* (2002), one major reason for obtaining contradictory results is basically using ill-established experimental set up. Therefore, this study represents an attempt to further investigate the cytogenetic effects of RF field under specific experimental conditions which simulate the actual radiated power from GSM mobile phones; particularly applying the field at two frequencies, with fixed intensity, and vertical orientation of this field to cultured human lymphocytes.

2. Materials and Methods

Twenty six men (average age, 30 years) participated in this study. Each person completed a questionnaire to obtain relevant health history. All the subjects reported neither exposure to diagnostic x-ray nor treatment with drugs during the last three months before blood collection. Every man donated 1.0 ml of venous blood collected in heparinized tubes. Another 1.0 ml of blood was obtained from each of 6 randomly selected men of the study group to be used in control cultures.

A standard whole blood culture was prepared with slight modifications as reported previously (Khalil *et al*., 1993). Briefly, 0.5 ml of blood was added to 4.5 ml of complete RPMI 1640 culture medium (Sigma, USA) in a T25 polystyrene tissue culture flask. The medium was supplemented with 10 % new born calf serum (Eurolon, USA), 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml fungizone (Eurolon, USA). The culture medium was buffered with 15 mM HEPES at pH 7.2.

Two types of culture from each individual were made. The first was exposed for 1 h RF radiation at a frequency

of 900 MHz, while the second was exposed to radiation at a frequency of 1800 MHz. The exposure system consisted of GSM signal generator DLW-3000 xp which generates either 900 MHz or 1800 MHz. The signal generator was located at about 40 cm below the culture flasks. The Narda model 8616 and detachable probe (Model 8623D) were used extensively to measure the electric field and adjusted to be 24 V/m. This simulates the actual exposure levels from the available GSM mobile phones to the direction of the human tissues. Figure (1) shows the uniformity of the electric field distribution within the area where the cultures being located. This figure was obtained by simulating the exposure system using the HFSS software (HFSS, 2007). It is worth noting that the value of 24 V/m is equivalent to SAR value of 1.0 and 1.2 watts/kg for 900 MHz and 1800 MHz, respectively (Gandhi *et al*., 1996). In the two experimental protocols, the irradiation was performed while the flasks were kept in the incubator in a horizontal position at 37 °C in a humidified 5 ± 0.1 % CO2 atmosphere.

After the 1 h exposure to RF, the cultures were initiated by addition of 30 µg/ml bromodeoxyuridine (ACROS Organization, USA) and 5.0 µg/ml phytohemagglutinin-M (Sigma). Two additional sets of exactly matching cultures, except RF exposure, were included. The first consisted of six cultures with only culture medium added and served as negative control. The other, which was also of six cultures, constituted the positive control. To each of the positive cultures, 50 ng/ml of a chemical DNA damaging agent; mitomycin C (MMC, Janssen Chemical, Geel, Belgium) were administered. All cultures were incubated for a total of 72 h excluding the 1 h RF-exposure or sham -exposure period. During the last 3 h of the culture period, 10 µg/ml of colchicine (Park Scientific, Northampton, UK) were included.

Immediately following termination of cultures, cells were harvested and chromosome spreads were prepared, differentially stained and cytogenetically analyzed according to Khalil and Qassem (1991). The slides were coded and blindly scored for the three end points under investigation; sister chromatid exchanges (SCE), cell replication index (CRI) and mitotic index (MI). From each culture, the mean SCE frequency was determined in 25 to 30 well-differentiated second metaphases (each with at least 44 ± 2 chromosome complement). From the same preparations, 200 cells were classified as first (M1), second (M2), and third or further (M3) dividing cells. The CRI was calculated by multiplying the % of M1, M2 and M3 by 1, 2, or 3, respectively, then dividing the sum by 100. The proportion of mitotic nuclei was found in a total of 2000 nuclei per culture to figure out the MI.

Duplicates of each indicated experiment were performed.

3. Statistical analysis

ANOVA test was used to evaluate the collected data. Furthermore, a multiple comparison (LSD; Exact Fisher) test was performed to evaluate the differences between the 900 MHz or the 1800 MHz and the negative control values. To compare the effects of the 900 MHz and 1800 MHz treatments on the MI data, the independent *t*-test was employed.

4. Results

Descriptive statistics demonstrated no large variability within the data gathered from any group. Therefore, corresponding data for individuals of each group were pooled (Table 1, figures 2-4). When the cells were exposed either to 900 MHz or 1800 MHz, the individual SCE means were normally distributed as shown by the Kolmogorov-Smirnov (K-S) analysis. Thus, ANOVA test was used for the statistical analysis. The SCE means under exposure conditions were not significantly elevated in comparison to the negative control values ($p \leq 0.05$). A multiple comparison (LSD; Exact Fisher) test was performed to evaluate the differences between the 900 MHz or the 1800 MHz and the negative control values. Similarly, when the data obtained after using 900 MHz or 1800 MHz were analyzed, no statistical differences were observed under the two exposure conditions.

Also, ANOVA demonstrated clearly higher CRI values in both cases of exposure relative to the negative control. Further analysis did not show any significant difference between 900 MHz and 1800 MHz CRI data.

When ANOVA was applied to the MI data, obvious increases in MI values were detected under exposure conditions either to 1800 MHz or 900 MHz frequency over the negative control. To compare the effects of the 900 MHz and 1800 MHz treatments on the MI data, the independent *t*-test was employed. The mean MI values were not significantly different ($P \leq 0.5$) in cultures exposed to either frequency.

Finally, it should be reported that MMC (the positive control), at 50 ng/ml, resulted in significantly higher SCE levels, but significantly lower CRI and MI values than those of the experimental and the negative control ($P \geq 0.01$).

5. Discussion

Effects of RF fields on different biological systems have been investigated. Although the majority of studies have found no evidence of genotoxic effects, there are a few positive findings that should be followed up. Vijayalaxmi and Obe (2004) reviewed large number of investigations conducted during the years 1990–2003 using rodents, cultured rodent and human cells, and freshly collected human blood lymphocytes to determine the genotoxic potential of exposure to RF radiation. The results of most of these studies (58%) did not indicate increased damage to the genetic material (assessed from DNA strand breaks, incidence of CA, MN and SCE) in cells exposed to RF radiation compared to sham-exposed and/or unexposed cells. Some investigations (23%) reported an increase in such damage in cells exposed to RF radiation. Positive results means that epidemiological studies of people exposed to EM radiation are likely to show increased cancer, miscarriage and reproductive adverse effects. In fact many epidemiological studies have shown these effects (Szmigielski, 1996; Goldsmith, 1997; Sadetzki *et al*., 2008). The observations from other studies (19%) were inconclusive.

It is possible that certain cellular constituents altered by exposure to EMF, such as free radicals, indirectly affect DNA. Recently, Mcnamee and Bellier (Mcnamee and

Table 1: The effects of cellular phone radiofrequency fields; 900 MHz and 1800 MHZ on sister chromatid exchanges (SCE), cell replication index (CRI) and mitotic index (MI) in cultured human lymphocytes. Culture medium, negative control; mitomycin C (MMC), positive control (Mean ± standard error of the mean; SEM)

Treatment	SCE		CRI		MI	
	Mean	SEM	Mean	SEM	Mean	SEM
900 MHz	4.94	0.3	2.85	0.1	0.247	0.0
1800 MHz	5.35	0.2	2.56	0.3	0.235	0.0
Culture Medium	3.28	0.5	2.20	0.2	0.215	0.0
MMC	21.20	2.0	1.60	0.1	0.155	0.0

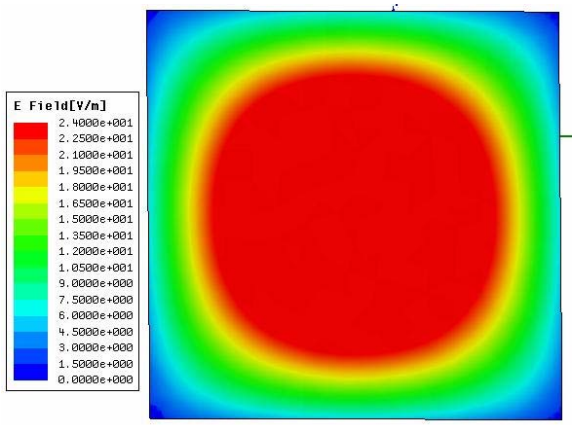

Fig.1.The electric field distribution within the culture area.

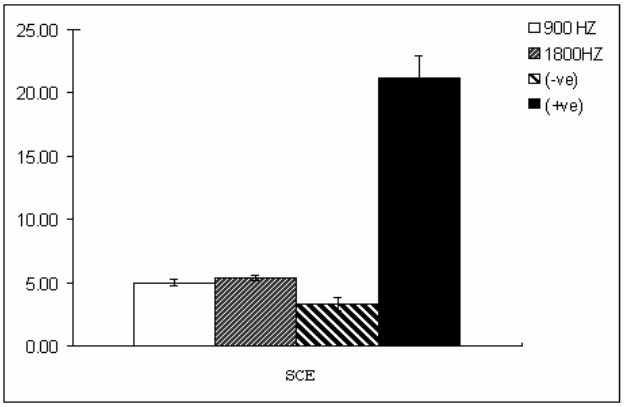

Fig.2 The effects of cellular phone radiofrequency fields at 900 MHz and 1800 MHz on sister chromatid exchange (SCE) in cultured human lymphocytes.

- VE culture was treated with culture medium only. + VE culture was treated with 50 ng/ml mitomycin C (MMC).

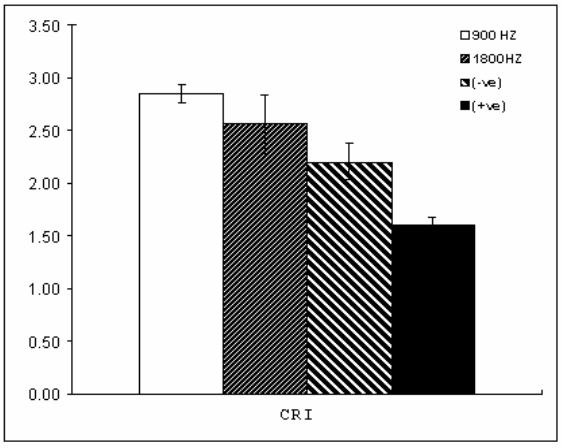

Fig.3 The effects of cellular phone radiofrequency fields at 900 MHz and 1800 MHz on cell replication index (CRI) in cultured human lymphocytes.- VE culture was treated with culture medium only. + VE culture was treated with 50 ng/ml mitomycin C (MMC).

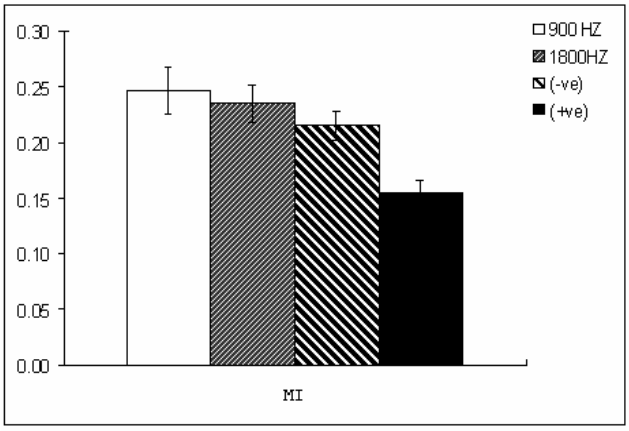

Fig.4 The effects of cellular phone radio frequency fields at 900 MHz and 1800 MHz on mitotic index (MI) in cultured human lymphocytes.- VE culture was treated with culture medium only. + VE culture was treated with 50 ng/ml mitomycin C (MMC).

Bellier, 1997) evaluated the biological and/or health effects of thermalizing and non-thermalizing RF radiation exposures and concluded that the scientific literature on this subject is full of conflicting results and the question of whether RF radiation exposure can contribute to cancer risk remains unresolved. Even with using the same experimental system, the genotoxic effects of exposure to RF-EM fields were not reproducible (Bisht *et al*., 2002).

Some in vitro studies including the present one provide evidence that gene expression is affected at RF exposure close to the guidelines. If these studies are confirmed they will be important for a mechanistic understanding of the interaction of RF fields with cellular tissue. While it seems appropriate to perform experimental studies using pure experimental RF fields, it may be needed to emulate the complex modulation patterns and intensity variations typical to real mobile phone use in future studies. This way data can be obtained which are better suited for comparison to epidemiologic studies.

Two plausible biological mechanisms involving free radicals are involved in this effect. The first involves increased free radical activity and genetic damage as a response to exposure. The second involves increased free radical activity and genetic damage because of an induced reduction of a free radical scavenger, e.g. reduced melatonin (Reiter, 1994). It is clear however, that both mechanisms have the same effect of damaging the DNA and chromosomes. Another established biological mechanism, EMR-induced alteration of cellular calcium ion homeostasis (Blackman, 1990) is also involved in cell regulation, cell survival and apoptosis, DNA synthesis and melatonin regulation.

The response is not likely to be linear with respect to the intensity of the radiation as it is seen from the present findings. Other parameters of RF radiation exposure, such as frequency, duration, waveform, and amplitude-modulation, etc, are also important determinants of biological responses and affect the shape of the dose (intensity)-response relationship. In this regard, the impact on calcium ions, which are important in maintaining normal health functions in brain tissues, was found in experiments. An exposure level of 30 mW / cm^2 is usually able to slightly raise the temperature over an hour. The Blackman's experiment was undertaken under isothermal conditions, with samples being kept within 0.4 ºC of 22 ºC.

The reductions in the MI reported in this study may be related to the impaired cell repair or cell death observed recently by other workers (Panagopoulos *et al*., 2007; Joubert *et al*., 2008; Palumbo *et al*., 2008). Furthermore, the present data that RF exposure results in depressions in the CRI is in agreement with some previous studies (Pacini et al.2002; Carpi *et al*., 2004), but not with others (Huang *et al*., 2008; Moquet *et al*., 2008).

According to the United Kingdom Environmental Mutagen Society (UKEMS) guidelines (UKEMS, 1990), at least a doubling in SCE frequency or a clear dose-response relationship should be accepted as a positive response. Thus, based on the data obtained from the present study and in reference to the UKEMES guidelines, it can be concluded that the 900 MHz and 1800 MHz exposure does not double the SCE frequency. However, the possibility that RF fields are weak mutagens can not be ruled out. Future studies in our laboratory are going to be carried out to examine the immunocytogenetic effect of RF-field exposure in both in vitro human and in vivo rodent model systems.

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Exposure to potassium carbonate emulsion induced nephro-toxicity in experimental animals.

SHORT COMMUNICATIONS

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Abstract

الملخص

The study investigated the possible toxic effects of potassium carbonate emulsion on some biomarkers of tissue damage in rabbits. Exposure of rabbits to potassium carbonate (K_2C0_3) emulsion at 50mg/L and 100mg/L via oral drinking for 14 consecutive days caused significant increase in the creatinine and uric acid at 100mg/L by 48.6% and 126.3% respectively. Also, potassium carbonate (K_2C0_3) emulsion significantly increased serum blood urea nitrogen (BUN) at 50mg/L and 100mg/L concentration. The results however suggested that potassium carbonate emulsion exposure via oral drinking could precipitate kidney damage.

Keywords: Potassium carbonate emulsion, nephro-toxicity, serum metabolites.

1. Introduction

Potassium is an essential dietary mineral and electrolyte. Normal body function depends on tight regulation of potassium concentrations both inside and outside of cells (Peterson, 1997). A limited number of enzymes require the presence of potassium for their activity. The activation of sodium, potassium-ATPase requires the presence of sodium and potassium. The presence of potassium is also required for the activity of pyruvate kinase, an important enzyme in carbohydrate metabolism (Sheng, 2000). Severe hypokalemia may result in muscular paralysis or abnormal heart rhythms (cardiac arrhythmias) that can be fatal (Sheng, 2000; Food and Nutrition Board, 2004). The richest sources of potassium are fruits and vegetables. People who eat large amounts of fruits and vegetables have a high potassium intake (8-11 grams/day) (Sheng, 2000; Food and Nutrition Board, 2004). A recent dietary survey in the U.S. indicated that the average dietary potassium intake is about 2,300 mg/day for adult women and 3,100 mg/day for adult men (Hajjar et al., 2001). The use of potent potassium supplements in potassium deficiency requires close monitoring of serum potassium concentrations. Potassium supplements are available as a number of different salts, including potassium chloride, citrate, gluconate, bicarbonate, aspartate and orotate (Hendler & Rorvik, 2001). Potassium carbonate (Pearl ash or potash) is not used as potassium supplement. It is strongly alkaline and it is mainly used in the production of soap and glass. Abnormally elevated serum potassium concentrations are referred to as hyperkalemia. Hyperkalemia occurs when potassium intake exceeds the capacity of the kidneys to eliminate it. The most serious complication of hyperkalemia is the development of an abnormal heart rhythm (cardiac arrhythmia), which can lead to cardiac arrest (Mandal, 1997). The present study was undertaken to elucidate toxicity associated with exposure to potassium carbonate emulsion.

2. Materials and methods

Twelve California rabbits (males) weighing 1.05kg – 1.65kg were purchased from the animal house of the college of animal Science University of Agriculture, Abeokuta, Ogun State, Nigeria, housed in cages and exposed to 12hr light/ dark photoperiod cycle . The animals were left to acclimatize for two weeks prior to the administration of the emulsion. They had free access to food and water *ad libitum.*

3. Exposure study

The experimental animals were divided into three groups of four. Groups I and II received 50mg/L and 100mg/L of potassium carbonate (K_2Co_3) emulsion respectively for two weeks and group III was administered with physiological saline for the same period.

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3.1. Blood Sample Collection

The animals were sacrificed by cervical dislocation after a 12hour fasting period. In all cases, blood samples were collected by cardiac puncture into clean dry centrifuge tubes and allowed to coagulate by standing for 30minutes. The blood samples were then centrifuged for 10minutes at 3000g using a bench centrifuge. Plasma aliquots were collected and preserved at 70^0C for biochemical assays.

3.2. Clinical chemistry

Serum total protein, Albumin and potassium ion (K^+) as well as biomarkers of nephro-toxicity such as urea, creatinine and uric acid were determined. The total protein was determined by Lowry et al (1951). Albumin concentrations were measured by Sigma bromocresol purple (BCP) as described by (Beale and croft, 1961). Serum K^+ was determined by flame emission spectrophotometer using UNICAL 1300. The estimation of urea was measured by the method of coloumbe and Farreaus (Hervey, 1953). Creatinine was determined by the method of Eichhorn et al (1961). Uric acid was estimated using commercial kit (Randox) according to manufacturer's protocol.

4. Statistical Analysis

Treated groups were compared to control group by student's t- test using Microsoft excel. All data were expressed as mean ± S.D (n = 4). A value of $P < 0.05$ was considered to indicate a significant difference.

5. Results

The result of effects of potassium carbonate emulsion on plasma albumin, plasma protein and blood K+ ion are presented in Table 1. Treatment with the emulsion of carbonate resulted in significant increase in serum potassium ion (K+) and albumin.

Table 1: The effects of potassium carbonate emulsion on plasma proteins and electrolytes.

Parameters	Albumin (g/dL)	Total protein (mg/dL)	Blood K^+ ion (mmol/L)
Control	3.64±0.03	9.73±0.08	0.78±0.01
50mg/L	4.07±0.32* (11.8)**	8.31±1.16 (14.6)**	2.99±1.84* (283.3)**
100mg/L	4.95±0.75* (36.0)**	8.39±0.94* (13.8)**	2.34±2.06* (200.0)**

Values are expresses as mean±S.D (n=5)

*Values differ significantly from control ($P < 0.05$)

** Percentage change compared with the control

Potassium carbonate emulsion in rabbits caused significant increase in total albumin in concentration dependent manner when compared with the control. The levels of total protein decreased significantly but not in a concentration dependent manner when compared with the control. Table 2 shows the result potassium carbonate emulsion on uric acid, creatinine and urea. The emulsion significantly increased uric acid, creatinine and urea by 126.3%, 48.6% and 458.8% respectively at highest concentration (100mg/L) when compared with the control ($P<0.05$). As shown in table 2.0, the results of urea, creatinine and uric acid showed that oral administration of the potassium carbonate emulsion significantly increased at concentration 50mg/L by 253.8%, 38.6% and 88.8% respectively when compared with the control.

Table 2: The effects of potassium carbonate emulsion on plasma metabolites.

Parameters	Urea (mg/dL)	Creatinine (mmol/L)	Uric acid(mmol/L)
Control	0.80±0.01	0.70±0.09	0.80±0.21
50mg/L	2.83±0.25*(253.8)**	0.97±0.37(38.6)**	1.51±0.73(88.8)**
100mg/L	4.47±0.85*(458.8)**	1.04±0.16*(48.6)**	1.81±0.21*(126.3)**

Values are expresses as mean±S.D (n=5)

*Values differ significantly from control ($P < 0.05$)

** Percentage change compared with the control

6. Discussions

Civilization and technological advancement had created a great harm to human health through the introduction of food additives as chemical preservatives, flavours, seasonings, modification of food texture, nutritional quality or colorants (Timothy and Peckham, 1978). Similarly, there have been increasing efforts in agricultural activities particularly in preservation of farm products which is a result of the ever-increasing human population as well as food losses to pests and disease both on the field and in storage (Sheng, 2000; Food and Nutrition Board, 2004). Some of these activities and chemical preservatives have resulted in food poisoning and becoming unsafe for human consumption with significant public health implications while some are carcinogenic inducers e.g. potassium bromate (Hajjar et al., 2001; Hendler & Rorvik, 2001; Mandal, 1997).

In the present study rabbits were orally exposed to different concentrations of potassium carbonate emulsion. Early works on the study of potassium carbonate emulsion had established that preservatives are dose-dependent. It was observed that sodium and potassium salts of benzoic acid have been found to cause no deleterious effect when used in small quantity (Morris et al., 1986; Milks, 1991). Also, potassium carbonate before meals promotes the secretion of gastric juice but large doses neutralized the free hydrochloric acid (HCL) in the stomach and render

the chyme neutral or alkaline, this interferes with the secretions from the pancreas, liver and intestines and hindered digestion (Mikkelsen, 1984). It was investigated that potassium ion causes depression of the central peripheral nervous system by depressing the reflexes and paralyses by higher concentration (Welt et al., 1960; Perkins, 1984; Weisburg, 1999). It was also noted that salt poisoning causes progressive muscular weakness, inflammation of the gut, dark colour liver, inability to stand, convulsion, excessive thirst and eventually death with haemorrhage and severe congestion in gastro intestinal (GIT) tract, liver, muscle and kidney (Shahr and John, 1991).

In acute or chronic renal failure, the use of potassium-sparing diuretics and insufficient aldosterone secretion (hypoaldosteronism) may result in the accumulation of excess potassium due to decreased urinary potassium excretion. This suggests that the emulsion of the carbonate might cause kidney damage. The significant decrease in protein synthesis as recorded in the study indicated possible damages to liver causing reducing protein synthesis. The rabbits treated with 100mg/L of the emulsion showed symptoms such as withdrawal from food, excessive thirst, drowsiness (weakness, reduced irritability, and polyuria, an indication of potassium acute toxicity (Mandal, 1997; Liu et al., 2000).

In conclusion, this study shows that high exposure to emulsion of potassium carbonate (K_2CO_3) may precipitate nephro-toxicity and induce liver damage. This also suggests a potential risk to humans who may come in contact with this supplement since liver and kidney are the major sites of chemical and drug metabolism.

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Toxicity of triethyllead chloride (TriEL) on cytoplasmic shuttle streaming, structure, growth and migration of the plasmodial slime mold *Physarum polycephalum*.

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Abstract

Lead is one of the poisonous heavy metals that affects the biotic system. In this research, the toxic effects of different concentrations of triethyllead chloride(TriEL) was studied on the acellular slime mold *Physarum polycephalum* cytoplasmic shuttle streaming, growth, structure and migration of *Physarum* plasmodium. The plasmodium was treated with different concentrations of TriEL (10, 20, 30, 40, 50 and 60 µM). The results showed that concentrations of TriEL<10 µM had no obvious effect on the studied parameters. Initial or slight signs of toxicity appeared at Concentrations between 20 - 50 µM. However, 50 µM TriELwas sublethal. They caused mostly irreversible condensation of the plasmodial strands, blebbing of the plasma membrane, vacuolization of the cytoplasm and elongation of the cytoplasmic shuttle streaming period. 60 µM TriEL was lethal and caused irreversible high blebbing of the plasma membrane, direct and rapid stop of cytoplasmic streaming followed by contraction of the whole plasmodium, strong blebbing of the plasma memmbrane, depigmentation, vacuolization and complete fixation of the cytoplasm (neither growth nor migration occurred on nutrient agar plates).

الملخص

يعتبر الرصاص من العناصر الثقيلة السامة التي تؤثر على النظام الحيوي. تم في هذا البحث دراسة التأثير السام لتراكيز مختلفة من ثلاثي إثيل الرصاص الكلوريدي (TriEl) على الفطر الهلامي اللاخلوي فيزاروم بوليسيفالوم. وشملت الدراسة التأثير على الانسياب السيتوبلازمي الرتيب، النمو، التركيب والزحف للفطر المدروس. تم معاملة طور البلازموديوم بتراكيز من TriEl (60,50,40,30,20,10 ميكرومول). أظهرت النتائج أن تركيز 10 ميكرومول أو أقل ليس له تأثير واضح على الظواهر المدروسة. أما التراكيز بين 20-50 ميكرومول فكانت غير قاتلة حيث أنها سببت إنقباضاً غير منعكس للعروق البروتوبلازمية، تكون الفقاعات في الغشاء البلازمي، زيادة الحويصلات والفراغات في السيتوبلازم والزيادة في مدة دورة الانسياب السيتوبلازمي الرتيب. أما التراكيز 60 ميكرومول فكان التركيز القاتل للفطر حيث تسبب بتلف وتفقع غير منعكس للغشاء الخلوي، توقف مباشر للانسياب السيتوبلازمي، تبعه إنقباض كامل للبلازموديوم وانتشار للصبغة إلى الخارج وزيادة عدد الفقاعات في السيتوبلازم. ولم يظهر البلازموديوم أي شكل من أشكال النمو أو الحركة على أطباق بتري الغذائية.

Keywords: *Physarum polycephalum* ,triethyllead chloride, cytoplasmic shuttle streaming, lethal dose, plasmodium .

1. Introduction

The last few years gave direct attention to studies that concern with the effect of toxic heavy metals found in the ecosystem. The toxic metals come to ecosystem from various pollution resources such as smoking, cars smoke, sewage, fires and variable resources that use those toxic metals in manufacturing processes(Gloag, 1981; WHO, 1977). TriEL at substantially lower concentrations is able to bring about drastic changes in the organization of intermediate filaments system of mammalian cells (Zimmerman *et al*., 1986). TriEL interacts and disrupts microtubules isolated from mammalian cells(Roderer, 1984). Moreover, TriEL inhibits cell proliferation of normal human lymphocytes (Stiakaki *et al*. 1997), and was found to have erythrocyte haemolytic activity(Kleszcynska *et al*. 1997). The random motility of cells was not markedly inhibited by TriEL, whereas chemotaxis directed cellular movements was strongly inhibited (Zimmerman *et al*., 1986).

Few studies were done concerning the effect of pollution with heavy metals on soil microorganisms.Terayama *et. al*.(1978) studied the toxicity of heavy metals and insecticides on slime mold *Physarum polycephlum*. Wang *et. al*.(2006), and Zeng *et. al*.(2006) discussed the influence soil heavy metals pollution on soil microbial biomass and enzyme activities. Also, Skerving (1993), Bressler and Goldstein (1991) showed that lead is able to inhibit or mimic the actions of calcium, and to interact with proteins (including those with sulfhydryl, amine, phosphate, and carboxyl groups). Shraideh (1999) showed that TriEL induced dramatic changes in the ileum contractile activity in mice.

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There is still a need for detailed studies concerning the physiological and ultrastructural effects of lead on significant microorganisms inhabiting our environment.

The acellular slime mold, *Physarum polycephalum*, represents a suitable model for studying the effect of environmental pollutants,i.e,lead compounds on motility, behavior and ultrastructure of living organisms. Acellular slime molds or myxomycetes represents a strange group of microorganisms. They show plant and animal like characteristics.

Physarum polycephalum is characterized by having a phagotrophic somatic phase (phaneroplasmodium), which is a yellowish, creeping, multinucleated mass of protoplasm enveloped by a slimy sheath and differentiated into two main regions known as massive front and a posterior network of connected veins (fig.1) (Wohlfarth-Bottermann, 1979). These veins which are seen under the light microscope consist of ectoplasm (gel) and a flowing endoplasm (sol), which exhibits a rhythmic shuttle streaming. In addition, the motive force generation in *Physarum* is based on cytoplasmic actin-myosin interaction similar to that of smooth muscles of higher organisms (Kessler, 1982).

The present research concentrated on the following objectives:

A-Studying the effect of different concentrations of TriEL on *Physarum polycephalum*.

B-Investigation of structural and morphological effects of TriEL on phaneroplasmodia of *Physarum polycephalum*.

In studying the effect of TriEL on *Physarum polycephalum* plasmodia, four criteria were taken into consideration:

1. Microscopic responses of whole plasmodium (*In vivo*).
2. Effect on plasmodial migration.
3. Effect on plasmodial structure and shuttle streaming periodicity.
4. Effect on plasmodial growth and viability.

2. Materials and Methods

2.1. Study object

Phaneroplasmodium of *Physarum polycephalum*.(Jordanian isolate similar to ATCC 44912) was used in this study(Shraideh, 1988).

2.2. Effect of test solutions on cytoplasmic shuttle streaming

The shuttle streaming (protoplasmic streaming) is an energy-requiring process. The rate of protoplasmic streaming can be extremely fast (over 1 mm per second) and the duration of an entire cycle of the shuttle streaming is 1.5-3.0 min .Its rhythmicity is a result of its reverse direction every few minutes.

Protoplasmic streaming of phaneroplasmodium of *Physarum polycephalum* (starved on 1.5 % non-nutrient agar media for 24 h) was carried out after submersion of plasmodia in a physiological salt solution (PSS) as control for about 10 min. PSS conisted of: 6.0 mM NaCl, 3.0 mM KCl, 1.0 mM $CaCl_2$, 0.1 mM $NaHCO_3$, 0.5 mM $MgCl_2$. Submerged plasmodium in PSS was observed under a phase contrast microscope (at 100X and 400X magnifications). After 5-10 min. adaptation (when streaming period was nearly stable), shuttle streaming periods were recorded and followed for about 2 hs (Shraideh, 2006).

The PSS solution was removed and replaced by different concentrations (10, 20, 30, 40, 50,& 60 µM) of TriEL (TriEL was obtained from Ventron, Karlsruhe, Germany). After 5-10 min. submersion, shuttle streaming periods were recorded and followed. For every tested solution, 30 periods were measured, the mean and standard deviation were calculated (Table 2) . Recorded values were compared with that of a control plasmodium (streaming and morphology under PSS). Also morphological changes were observed at least for 2 h. Depigmentation, the release of pigments and decoloration of plasmodia were observed by the yellowish coloration of submersion solution.

2.3. Effect of test solutions on growth of Physarum plasmodium:

Corn- agar plates were used for growth test. Corn-agar medium consisted of: 6.0 gram rolled oats+1.0 gram D-glucose+7.5 gram agar- agar +500 ml distilled water. Contents were mixed, sterilized in autoclave at 121^{o}C for 20 min and poured in sterile plates. Petri dishes were prepared including growth media (control) and successive concentrations of TriEL (10, 20, 30, 40, 50 and 60 µM), which were added individually to the growth medium. Small pieces of *Physarum* plasmodia were allowed to grow on medium surface and growth was observed and sketched at intervals of 2h (Shraideh, 2006).

2.4. Viability test:

Viability of phaneroplasmodium of *Physarum polycephalum* (starved on 1.5% non–nutrient agar medium for 24 hours) was studied after treating with two different concentrations (50 and 60µM) of TriEL for 4 h. Treated plasmodia were transferred to 1.5 % agar-agar plates and kept overnight. Their ability to make phaneroplasmodia was compared to that of control (PSS treated plasmodia)(Korohoda *et. al*., 1983).

2.5. Effect of test solutions on migration of Physarum plasmodium

1.5% non-nutrient agar medium was used for migration test. Petri dishes were prepared as follows:

- A-1.5% non-nutrient agar media (control).
- B-Successive concentrations of TriEL were added to 1.5% non-nutrient agar medium (migration test).

Small pieces of *Physarum* plasmodia were allowed to migrate on the surface of the agar plates. Migrating plasmodia were sketched at intervals of 2h.

2.6. Time lapse phase contrast photomicrography:

The effect of two concentrations (50, 60µM) of TriEL on phaneroplasmodia of *Physarum polycephalum* (starved on 1.5 % non-nutrient agar media for 24 h) was followed using Zeiss stereophotomicroscope. Photography was started under PSS at 0- time (few min. after submersion of the plasmodium with PSS), then with the test solutions(50, 60µM of TriEL) at 10 min. intervals.

All experiments of this research were done at a temperature of 24-26^{o}C.

3. Results

3.1. Effect of TriEL on Physarum polycephalum phaneroplasmodia

The effects of different concentrations of TriEL on whole phaneroplasmodia migrating on agar surface are summarized in table (1), which shows the sequence of events following the treatment of the plasmodia with different cocentrations of TriEL.

3.2. Effect of TriEL on plasmodial migration:

The effect of different concentrations of TriEL on migration ability of *Physarum* plasmodia on non-nutrient agar was investigated. Migration of the plasmodia was followed overnight and sketched at intervals of 2 h. The results are shown in Figure 2. Plasmodia transferred into TriEL media were compared to those on control media. The results showed that:

1. In B1(Plasmodium transferred into 10 µM TriEL media) there were no obvious changes in migration.
2. In B2, B3, B4(Plasmodium transferred into [20-40 µM TriEL media) showed gradient decrease in migration ability.
3. In B5(Plasmodium transferred into sublethal dose [50 µM TriEL] media) showed a very short distance migration ability.
4. In B6(Plasmodium transferred into lethal dose [60 µM TriEL] media) showed no migration ability at all.

3.3. Effect of TriEL on the viability of Physarum plasmodia:

Treatment of plasmodia with (50 and 60 µM) of TriEL affected their viability. The results are shown in Figure 3. Plasmodia treated with 50 µM of TriEL showed very weak spreading ability compared with control. While treatment with 60 µM TriEL resulted in complete inhibition of phaneroplasmodia

3.4. Effect of TriEL on plasmodial structure& shuttle streaming periodicity:

The protoplasmic strand of *Physarum* in a cross section is composed of 2 distnct regions; ectoplasm and endoplasm (figure 1). The more viscous or gel ectoplasm represents about 80% of volume of the protoplasm. It is rich in labyrinth of invaginations from which vesicles are pinched off to the inside. Contractile vacuoles and other kinds of vacuoles are present. The endoplasm, which is the central part is less viscous and includes streaming nuclei, mitochondria, ribosomes, pigment granules, vacuoles, vesicles and other organelles. The protoplasm in *Physarum* plsmodial strands(veins)exhibits reversible or regular shuttle streaming with period duration of approximately 1.3-3.0 min.(Shraideh, 1988).

The endoplasm(inside stationary ectoplasm) streams in one direction for a short while, stops and then turns back for a while and so on.

Table 2 summarizes the effect of TriEL on shuttle streaming periodicity.

The effects of a sublethal concentration (50 µM) and the lethal concentration (60 µM) of TriEL were investigated on the structure and behaviour of treated phaneroplasmodia (fig.4). After half an hour of addition of the lethal concentration of TriEL only vacuolization of endoplasm occurred. After 1 hour darkening and more vacuolization were observed. After 1.25 hour addition of the lethal concentration, high vacuolization, blebbing of plasma membrane and stop of shuttle streaming occurred.

By comparing results with control it was found that the lethal concentration of TriEL caused complete fixation and disruption of the plasmodium after 1.5 hours of treatment.

3.5. Effect of TriEL on growth of Physarum polycephalum:

Mixing the corn agar media with the different concentrations (10-60 µM)TriEL affected the growth of *Physarum* plasmodia. The effect of TriEL was investigated and reported as sketches.

Figure 5 shows the results obtained. Plasmodia transferred into TriEL-media were compared to those transferred to control media. The results showed that in B1-B5 (Plasmodium transferred to 10,20,30,40 or 50 µM TriEL media) there was a gradient decrease in migration ability. 50 µM TriEL medium resulted in a very short distance migration, that was highly inhibited after 3 h. While in B6 (plasmodium transferred into the lethal concentration (60 µM)TriEL), the results showed no migration ability at all, with extreme depigmentation.

Table 1.Effect of gradient concentrations of TriEL on *Physarum* whole plasmodia.

Concentrations [µM TriEL / ml]	Effect
PSS(control)	Plasmodium showed normal migration, small vacuoles in streaming endoplasm. Stationary ectoplasm.
10.0	Plasmodium has good migration ability but darker in color. The plasmodium slowed down streaming velocity, with a low degree of vacuolization.
20.0-30.0	Slow migration ability of plasmodium was noticed.. Vacuolization, depigmentation and contraction of the frontal region was noticed after 2h of treatment.
40.0-50.0	Condensation of the whole plasmodium. Decolorization and release of pigments. Very little migration ability was observed.
60.0	Direct stop of cytoplasmic streaming. No migration ability.Condensation, depigmentation of plasmodium was observed after 2 h of treatment.

Table 2 .Effect of gradient concentrations of TriEL on plasmodial shuttle streaming periodicity and plasmodial structure.

Treatment	Streaming Period				Observations
	Mean ± SEM	Percentage	n	N	
PSS	2.07±0.03 min	100.0%	30	3	Regular streaming, no large vacuoles.
10µM	2.23±0.04 min	107.7%	30	3	Regular streaming. Little effect on period duration.
20µM	2.91±0.04 min	140.6%	30	3	Elongation in streaming period and little vacuolization.
30µM	2.95±0.03 min	142.5%	30	3	Elongation in streaming period, obvious vacuolization.
40µM	3.02±0.08 min	145.9%	30	3	Blebbing and disturbance occurred in large veins. Difficulty to monitor the shuttle-streaming period.
50µM Sublethal concentration	----------	----------	---	4	Very long shuttle streaming periods (up to 5 min) or sometimes very short periods (up to59-sec.). Blebbing was obvious.
60µM Lethal concentration	----------	----------	---	4	Immediate stop of cytoplasmic streaming followed by decolorization. Viability test showed no migration ability.(irreversible effect)

*n: number of periods analyzed.
*N: number of plasmodia used.
*Percentage: Mean of streaming periods of treated plasmodia/ Mean of streaming periods of the control (100%)
*SEM: Standard error of mean

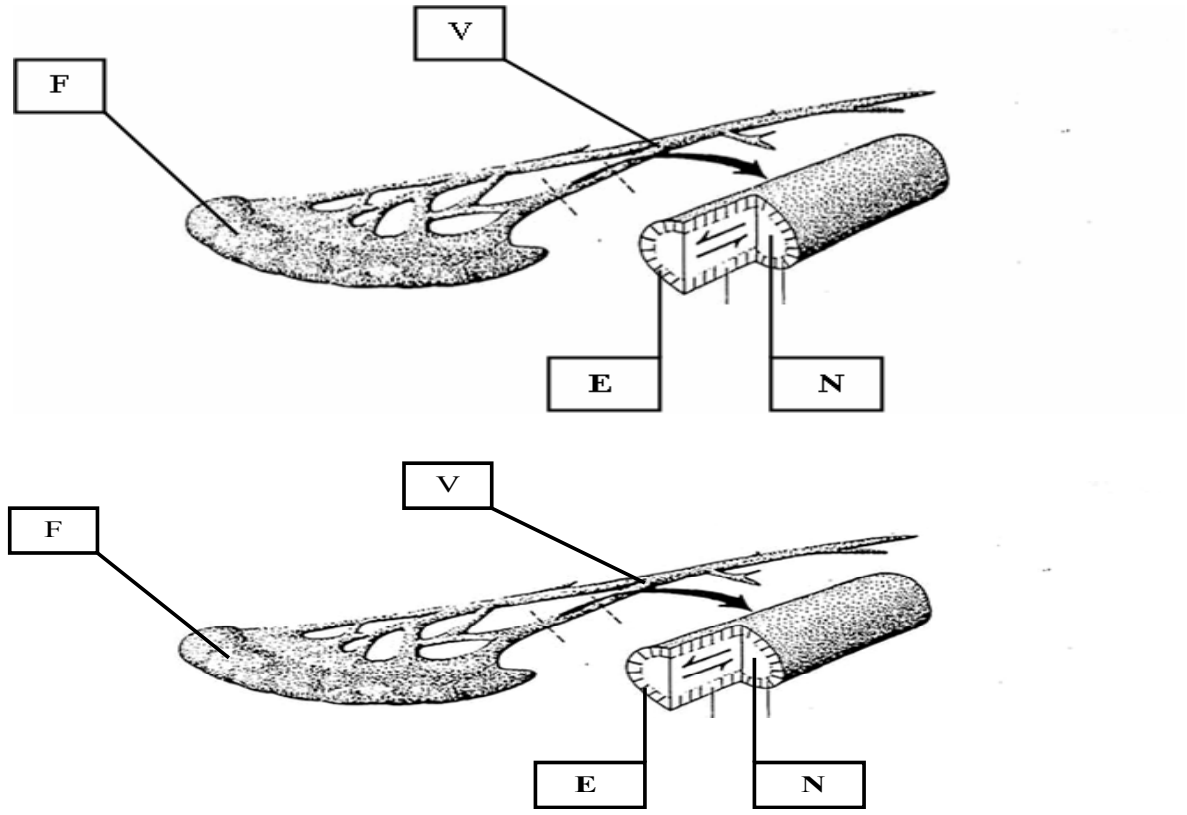

Figure 1: Plasmodium of *Physarum polycephalum*

F: Front , V: Veins, E: Ectoplasm and N: Endoplasm. (Adopted from Wohlfarth-Bottermann, 1979)

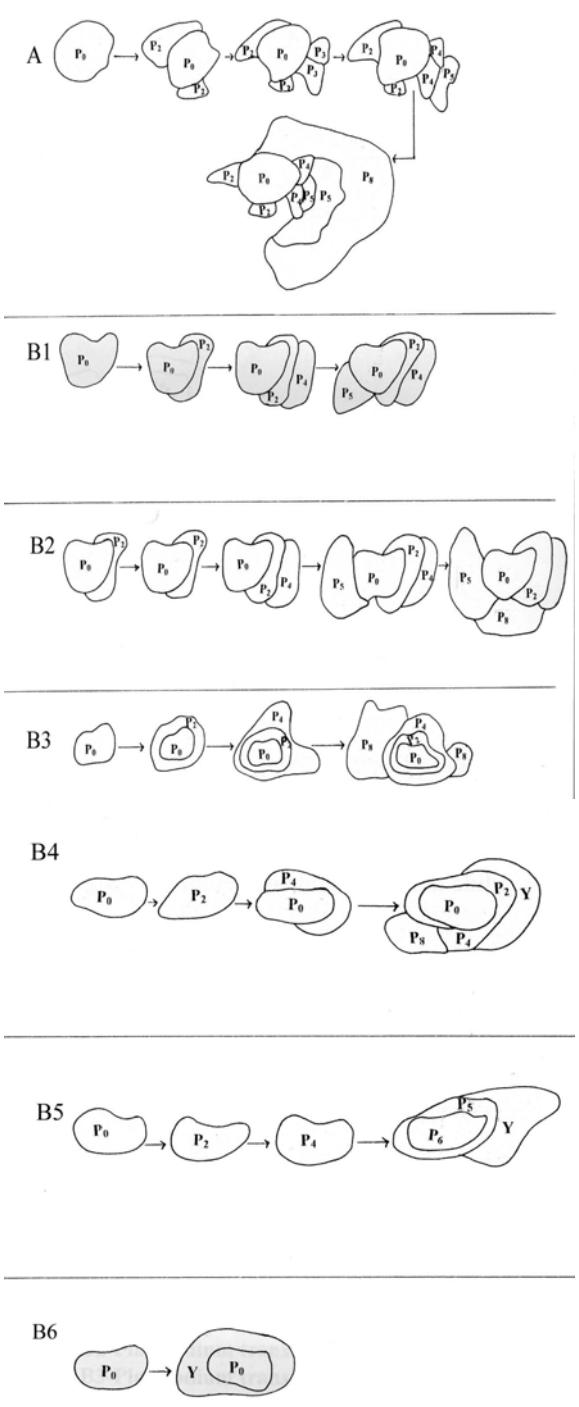

Figure 2. Sketches showing migration ability of plasmodia treated with TriEL.
P_0:Codes for plasmodium at zero time.
P_2:Codes for plasmodium after 2 h.
P_3:Codes for plasmodium after 4 h.
P_5:Codes for plasmodium after 6 h.
P_8:Codes for plasmodium after 8 h.
Y:Yellow color caused by depigmentation.
A-Plasmodium transferred to 1.5% agar medium (control).
B-Plasmodium transferred into 1.5%agar medium containing gradient concentrations of TriEL:
B1-Plasmodium transferred into 10 µM TriEL medium.
B2-Plasmodium transferred into 20 µM TriEL medium.
B3-Plasmodium transferred into 30 µM TriEL medium.
B4-Plasmodium transferred into 40 µM TriEL medium.
B5-Plasmodium transferred into 50 µM TriEL medium (Sublethal concentration).
B6-Plasmodium transferred into 60 µM TriEL medium (Lethal concentration).

4. Discussion

This study is one of few studies concerning the effect of environmental contamination with heavy metals on slime molds inhabiting our environment. In this study we investigated the effect of different concentrations of TriEL on different parameters of life of the acellular slime mold *Physarum polycephalum* isolated from a north forest in Jordan (Shraideh, 1988).

The results showed that concentrations of TriEL (10, 20, 30, 40 and 50 µM) represent the sublethal concentrations while the concentration (60 µM) represents the lethal concentration, which affected the activity, structure and life of the acellular slime mold *Physarum polycephalum.*

TriEL concentration of 10 µM of TriEL caused a little effect on structure or streaming of the cytoplasm. TriEL concentrations of 20 and 30 µM caused vacuolization of the cytoplasm, increasing the streaming period about 40%, and very low ability of migration.While 40 and 50 µM induced high vacuolization and condensation of the cytoplasm, blebbing of plasmalemma and very long streaming periods.On the other hand, the lethal concentration (60 µM) caused an immediate stop of cytoplasmic streaming, followed by depigmentation of plasmodium. Similar effects were observed from treatment of *Physarum* plasmodia with CdCl2 and PbCl2 (Shraideh 2006, 2007 respectively).

From these structural observations we can conclude that TriEL affected the integrity of the biological membranes. The weakening of the membrane led to the observed effects i.e. vacuolization of the cytoplasm, blebbing of plasmalemma and its disruption. Terayama *et. al*.(1978) suggested that the toxicity of heavy metals in the slime mold *Physarum polycephalum* is accompanied by some changes in the cell membrane. could be also explained by the ability of Lead can bind membrane proteins (Skerving, 1993) and is able to substitute Ca^{++}(Bressler and Goldstein, 1991) which causes disturbance in permeability of plasma membrane and normal function of membrane proteins. This may explain the observed weakening of membrane, blebbing and loss of pigments in the TriEL-treated plasmodia.

The internal organization of protoplasmic strands (veins) and the presence of actomyosin fibrils in the ectoplasm of the strand are important for shuttle streaming, migration and growth of the plasmodium (Wohlfarth-Bottermann, 1979). Disruption of internal

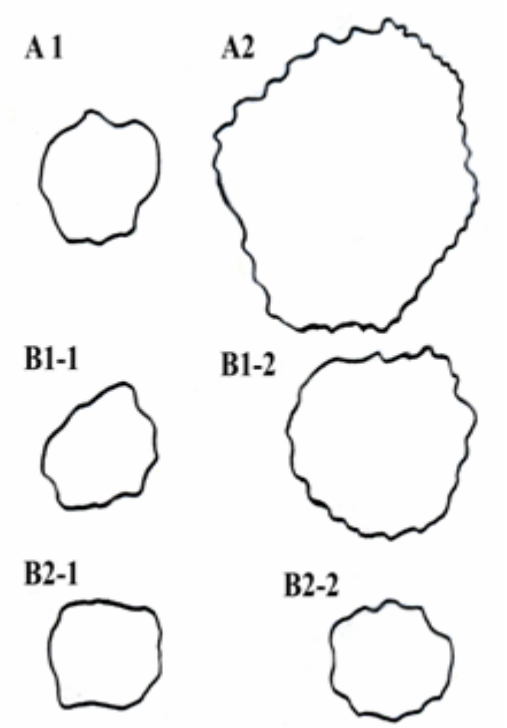

Figure 3. Sketches showing effect of TriEl on viability of *Physarum* plasmodia

A-Control plasmodium at:
A_1: Zero time. A_2: After 4 h.
B-Plasmodium treated with TriEL concentrations of:
B_1: Sublethal concentration (50 μM):
B_{1-1}: Sublethal concentration at zero time.
B_{1-2}: After 4 h treatment with the sublethal concentration.
B_2: Lethal concentration (60 μM):
B_{2-1}: Lethal concentration at zero time.
B_{2-2}: After 4 h treatment with the lethal concentration.
Magnification = 6 X.

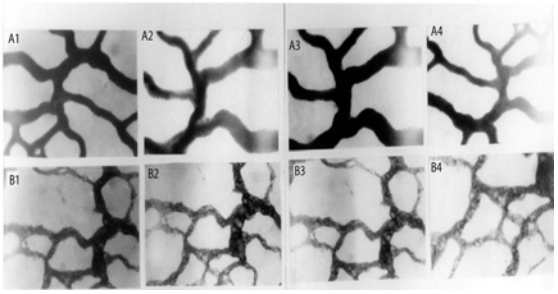

Figure 4. Time laps photomicrographs showing effect of TriEL on the shuttle streaming and structure of *Physarum* phaneroplasmodium.

A-Control plasmodium (PSS solution). Note normal structure of protoplasmic strand.
A_1: After 30 min.
A_2: After 60 min.
A_3: After 75 min.
A_4: After 90 min.
B-Plasmodium treated with TriEL lethal concentration (60 μM)
B_1: After 30 min. There was a slight vacuolization of cytoplasm.
B_2: After 60 min. Condensation and more vacuolization of cytoplasm was observed.
B_3: After 75 min. High condensation, extensive vacuolization of cytoplasm and
blebbing of plasmalemma was observed.
B_4: After 90 min. Condensation and extensive vacuolization of cytoplasm, blebbing of plasmalemma were observed. Also disruption of structural organization of plasmodium was obvious.
Magnification = 5X

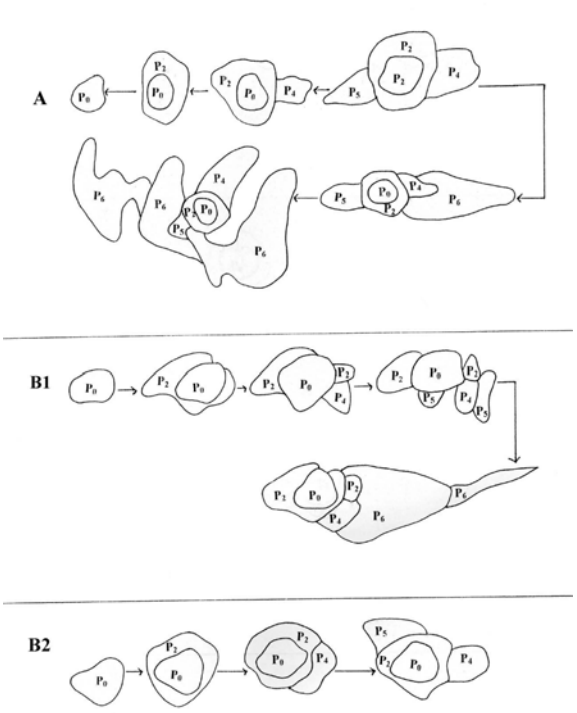

Fig 5. Sketches showing growth ability of plasmodia treated with TriEL.

P_0: Codes for plasmodium at zero time.
P_2: Codes for plasmodium after 2 h.
P_3: Codes for plasmodium after 3 h.
P_4: Codes for plasmodium after 4 h.
P_6: Codes for plasmodium after 6 h.
P_8: Codes for plasmodium after 8 h.
Y: Yellow color caused by depigmentation.
A-Plasmodium transferred into corn agar medium (Control).
B-Plasmodium transferred into growth media containing gradient concentrations of TriEL:
B1-Plasmodium transferred into 10 μM TriEL growth medium.
B2-Plasmodium transferred into 20 μM TriEL growth medium.
B3-Plasmodium transferred into 30 μM TriEL growth medium.
B4-Plasmodium transferred into 40 μM TriEL growth medium.
B5-Plasmodium transferred into 50 μM TriEL growth medium (Sublethal concentration).
B6-Plasmodium transferred into 60 μM TriEL growth medium (Lethal concentration).

organization, vacuolization and disturbance of actomyosin fibrils could explain the drastic effects of TriEL treatment These effects range from slow of shuttle streaming, vacuolization and at end by complete irreversible stop of cytoplasmic streaming, loss of pigments and blebbing of plasma membrane.

TriEL has been found to disassemble microtubules in cultured mammalian cells(Zimmermann, et al, 1988) and is able to disrupt microtubules and inhibit motility of *Dictyostelium discoideum*(Sroka et al, 2002). *Physarum* contains a microtubule assembly that supports actin-myosin organization, which is responsible for motility, streaming, and support of plasma membrane(Diggens & Williams, 1987). Treatment of *Physarum* plasmodia with

different concentrations of TriEL may disrupt and disassemble microtubules and cause disorganization of actin-myosin organization. This may explain the results of plasmodial treatment with TriEL, including membrane blebbing, stop of motility and irregular or complete stop of cytoplasmic streaming.

These results are in agreement with those of other studies which showed that lead is able to replace and fuction as Ca^{++} (Bressler and Goldstein, 1991), to inhibit ion channels and to increase membrane permeability (Skerving, 1993).

The inhibitory effect of TriEL on plasmodial growth and migration can also be explained by the findings of Wang, *et. al*. (2006) who showed that heavy metals pollution affected negatively microbial biomass, activity and community composition in soil. Also Malecka, *et. al.* (2001) discussed the ability of lead to bind nucleic acids causing condensation of chromatin, stabilization of the DNA double helix and thus inhibiting the process of replication and transcription. They also showed that lead can exert a negative effect on mitochondria by decreasing the number of mitochondrial cristae, which in turn lowers the capacity of ATP production. This may explain the slow migration and weak growth of lead treated *Physarum* plasmodia. TriEl has been found to affect dramatically the ileum contractile activity in TriEL treated mice (Shraideh, 1999).

Finally we can say that pollution of the environment with heavy metals like lead will have a bad effect on the environmental structure and equilibrium.

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المجلة الأردنية للعلوم الحياتية
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هيئة التحرير

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