Adverse Effect of Lead Acetate on Light Weight Protein of *Bactrocera cucurbitae*

Rizwanul Haq[1,*], M. Farhan Ullah Khan[2] and Ehtesham Ul Haq[3]

[1]*Department of Botany, Federal Urdu University of Arts, Science & Technology, Gulshan-e-Iqbal Campus Karachi, Pakistan*

[2]*Department of Zoology, University of Karachi, Karachi, Pakistan*

[3]*Department of Zoology, Govt. National College, Karachi, Pakistan*

Abstract: Lot of insects are influenced easily by a number of pollutants; such as, the influence of lead (as lead acetate) on *Bactrocera cucurbitae. L*ead is considered to be an important toxic waste which could contaminate the environment, such as soil, air and water. Therefore, insects could be influenced by the lead. *Bactrocera cucurbitae,* was studied at 48 hours post treatment, under the effects of lead acetate, in different concentrations. Lead is found to exert a definite specific physiological and morphological effect on these flies.It was observed that under the effect of lead abnormalities and deformities were developed in the larvae of flies. Thus these flies could present a useful module for the quick transmission of the environmental hazards due to lead contamination, which exerts a specific physiological and morphological effect on these flies. The purpose of the present work was to determine the effects of lead on proteins as a major indicator of physiological features along with morphological features of larvae of *Bactrocera cucurbitae* flies.

Keywords: Effects, Lead acetate, Proteins *Bactrocera cucurbitae.*

INTRODUCTION

Lead is an important industrial heavy metal which contaminates environment and ultimately, food, water, urban soil and air. “As it is established that lead has been found to have a definite cytogenetic effect [1-10]. The detection of possible hazardous effects of this metal is, therefore; a matter of urgent concern. Although, many studies have been carried out to investigate the biological effects of lead, however, its potential toxicity against insects remained to be established. Some studies have been carried out on natural populations of *Bactrocera cucurbitae* in respect of effects of heavy metals. It has been found that contamination with heavy metals (Zinc, Lead etc.) can induce the effects on feeding behavior of Diptera, structural and functional modifications and malformations [11, 12]. Investigations on Diptera indicated abnormalities due to the effect on chromosomal meiotic nondisjunction [8]. However, sufficient data on the action of heavy metals and lead is inadequately available on the group of diptera insects, those are widely distributed species. *Bactrocera* has been reported on different varieties of mango, the adult flies has been found infesting fruit on maturation during the month of june [13]. *Bactrocera species* has been observed from the entire oriental region on a specific host plants [14]. The fruit fly *Bactrocera* complex have been reported in a vast field as pest of fruits in Asia [15]”. Presently, the species*, Bactrocera cucurbitae,* were used to observed the deleterious effects of lead metal.

The melon fruit fly, “*Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is distributed widely over the world. It has been reported to damage around 81 host plants and is a major pest of cucurbitaceous vegetables, particularly the bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*C. melo* var. *momordica*), and snake gourd (*Trichosanthes anguina*). *Bactrocera cucurbitae* has been reported on different varieties of mango, the adult flies has been found infesting fruit on maturation during the month of june [13]. The males pollinate the flowers and acquire the floral essence and store it in the pheromone glands to attract the females of their own species [16]”. Since immature, both the sexes male and female remains associated with the environment and therefore, it was found suitable to study the deleterious effects of the lead on it.

Among the heavy metals, lead, has been shown to be widely distributed in the atmosphere, water, soil and food [17]. Lead inhibits the activity of free sulphydryl groups (SH) dependant enzymes. The clearest manifestation of these effects is the disturbance on the biosynthesis of heme, which in humans is accompanied by abnormalities in porphyrin metabolism [18]. Lead acetate is used as a topical astringent and is

*Address correspondence to this author at the Department of Botany, Federal Urdu University of Arts, Science & Technology, Gulshan-e-Iqbal Campus Karachi, Pakistan; E-mail: rizwan-100@hotmail.com

ISSN: 1814-8085 / E-ISSN: 1927-5129/13

Solution	Prepration
i) Acrylamide-Bisacrylamide solution(30.0:0.8)	Dissolve 30 gm acrylamide and 0.8 gm bisacrylamide in deionized water.Make up the volume to 100 ml. Filter through Whatman no.1.
ii) 1.5 M Tris-HCl buffer:	Tris 18.2.0 gm,dissolve in 80 ml and adjust the pH of this solution to 8.8 using 0.1M HCl. Make up the volume to 100 ml with deionized water.
iii) 10% Sodium dodecyl sulfate:	Dissolve 1 gm SDS in 9 ml water and make the volume up to 10 ml with deionized water.
iv) 10% Ammonium per sulfate:	Dissolve 1 gm APS in 1ml water and make the volume up to 10 ml with deionized water.
v) Sample diluting buffer (SDB):	Dissolve 6.25 ml of 1M Tris-HCl pH 6.8 (Solution C), 2 gm SDS, 5 ml 2-mercaptoethonol and 10 ml glycerol together. Make volume up to 100 ml with deionized water.
vi) Reservoir Buffer:	Dissolve o.9 gm Tris, 3.6 gm Glycine and 1.0 gm SDS in 500 ml deionized water. Make up to 1liter.
vii) Staining solution:(Bromophenol Blue and 0.2% Comassic blue).	Dissolve 0.5 gm Coomassie blue in 18.75 ml acetic acid and 12 ml methanol, Make volume upto 2.50 ml with
viii) Destaining solution	Mix 10 ml Acetic acid and 30 ml methanol. Make up the volume to 100 ml.

Figure 1: Preparation of solutions.

found to be a renal carcinogen in rats [19-24]. In the Syrian harnster, lead induces neoplastic changes in the bronchio-alveolar area [25, 26]. It also produces infertility in mice [27] and reduces the reproductive ability of rats [28-30]. In *Drosophila melanogaster* lead induces enzymatic alterations in esterase enzyme and triose phosphate isomerase enzyme [31] and affects non disjunction [8]. However, information about the mutagenic effects of lead salts in humans who are occupationally exposed to them and information obtained from *in vitro* studies are contradictor [32].

Electrophoresis is being broadly used for categorization of proteins and peptides for the diagnostic and/ or preparative unification of organic macromolecules [33]. The process of electrophoresis first used by [34] for the separation of proteins has found many dimensions in analyzing and separating macromolecules. These techniques, whether alone or in combination, have proved to be very useful for proteins and peptides and the complex proteome analysis [35].

MATERIAL AND METHODS

The test materials, *Bactrocera cucurbitae* was also procured from the Diagnostic Laboratory, Department of Plant protection, Karachi. The cucurbits oviposited with *Bactrocera cucurbitae* were collected from the said laboratory for further rearing. Larvae were reared under aseptic conditions on a usual prescribed diet with a little amended procedure [36]. Insects were treated as batches of bottles with 3 grams bananas mixed with lead acetate in, 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg doses. A batch of three bottles were kept as control. 10 larvae were released in each bottle for 48 hours. After that mortality of larvae in each bottle was observed. Survivor larvae were kept in separate bottles on lead free bananas upto full formation. During that period of pupation and adult, effects of lead acetate in different concentration was observed.

The determination of lead acetate on protein of *Bactrocera cucurbitae* larvae were studied with lead acetate kept for 48 hours exposure. Thereafter, crushing and homogenizing of the treated and untreated larvae was made.

Reagents and Chemicals	Brand
N,N,Methylene bisacrylamide	Fluka
Acrylamide	Fluka
Tris (hydroxymethyl) aminomethane	Fluka
Ammonium persulfate	Merck
Sodium dodocylsulfate	Fluka
HCL	Merck
Glycine	Fluka
Bromophenol blue	Merck
TEMED	Merck

Figure 2: Reagent and chemicals are shown in Figures **1** and **2** respectively.

Preparation of Gel

In the process of electrophoresis, the capillary tubes of electrophoresis were cleaned by water and ethanol then dried it by air. The lower mouth of capillaries were covered by rubber stopper. 10 ml resolving gel was prepared with the above mentioned ingredients. The

Table 1: Values of Various Proteins Observed in Lead Acetate Treated and Untreated *Bactrocera cucurbitae* Larvae

Protein	Rf	Egg Albumin (control)	Bactrocera Cucurbitae normal (untreated)	Bactrocea cucurbitae (treated)
	0.04	+	-	-
	0.47		+	-
	0.70		-	+
	0.72		+	-
	0.79		-	+
	0.84		+	-
	0.89		-	+
	0.93		+	-
	0.98		-	+

mix solution was filled in the each electrophoresis tube, then added 0.1 ml ammonium sulphate and 0.008 ml TEMED in capillaries, then left for 3-4 hours for polymerization. After that 200µl. (micro litre) sample was added and then Bromophenol solution was added. After 30-40 min. the mouth of above and lower part of capillaries were exposed with Reservior Buffer solution in the electrophoresis tank for one day under 110 volt. After that gel were exposed to coomassi blue solution for 2 hours, after colorization of Gel, It was kept in the destaining solution for removing the excess color on the Gel then the bands of proteins were observed. After this process the length and bands on Gel was measured for Rf determination. Egg albumin was also run simultaneously, for the comparison.

RESULTS

The effect of lead acetate on proteins of *Bactrocera cucurbitae* (dipterous flies) is shown in Table **1**, in this respect *Bactrocera cucurbitae,* protein were studied in comparision with Egg albumin as a reference protein. The rf. of Egg albumin was found as 0.04. Protein rf. 0.04, 0.47, 0.72, 0.84, 0.93, have not been observed in treated *Bactrocera cucurbitae*. While protein rf. 0.70, 0.79, 0.79, 0.89 and 0.98 have been observed as altered in *Bactrocera cucurbitae.*

Electrophoratic expression of various protein flow as compared to egg albumin in treated and untreated *Bactrocera cucurbitae* shown in (Figure **3**).

DISCUSSION

Protein (rf 0.47), found in *Bactrocera cucurbitae* (untreated) lighter than the egg albumin, while corresponding protein in treated *Bactrocera cucurbitae*, was not found at the same rf. This suggests that the protein (rf 0.47) was effected under the lead treatment. Protein (rf 0.70) was found in *Bactrocera cucurbitae* (treated), it was be lighter than the egg albumin, while corresponding protein in the lead treated *B.cucurbitae*, was absent at the same rf. That suggest that the protein (rf 0.70) was affected. Protein (rf 0.72) was found in *Bactrocera cucurbitae* (untreated) that seems to be lighter than the egg albumin, while corresponding protein in the lead treated *Bactrocera cucurbitae* was absent at the same rf. This suggests that protein (rf 0.72) was also affected. Protein (rf 0.79) is found in *Bactrocera cucurbitae* (treated) that seems to be lighter than the egg albumin, while corresponding protein in the untreated *Bactrocera cucurbitae*, was absent at the same rf. This suggests that the protein (rf 0.79) was affected as well. Protein (rf 0.84) was found in *Bactrocera cucurbitae* (untreated) that is lighter than the egg albumin, while corresponding protein in the treated *Bactrocera cucurbitae*, was absent at the same rf. This suggests that the protein (rf 0.84) was affected with some extension in the untreated insect. Protein (rf

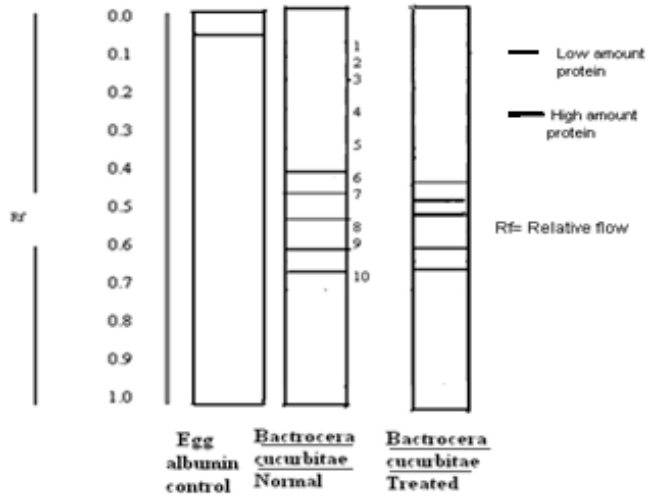

Figure 3: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera cucurbitae*.

0.89) was found in *Bactrocera cucurbitae* (treated) that seems to be lighter than the egg albumin, while corresponding protein in the untreated *Bactrocera cucurbitae*, was absent at the same rf. This suggests that the protein (rf 0.89) was affected with some extension in the treated insect.Lighter than the egg albumin. Protein (rf 0.93) was found in *Bactrocera cucurbitae* (untreated), while corresponding protein in the treated ones, was absent at the same rf. This suggests that the protein (0. 93) was affected in the untreated insect lighter than the egg albumin. Protein (rf 0.98) was found in *Bactrocera cucurbitae* (treated). while corresponding protein in the untreated Bactrocera cucurbitae, was absent at the same rf. This suggests that the protein (rf 0.98) was affected with some extension in the treated insect.

Bactrocera cucurbitae treated with different doses of lead acetate viz. 0.125 mg., 0.25 mg., 0.5 mg., 1.0 mg and 2.0 mg resulted deformities. [37], indicated cellular damage in processes of lead exposed to PC-12 cells.After lead exposure the N-acetyleysteine (NAC), glutathione (GSH), glutathione disulfide (GSSG) and malondialdehyde (MDA), were found effected after treated to various doses of lead acetate, these results could be correlated with the present findings with the presence of affected proteins in the lead treated insects. {38]. indicated that, lead is a pollutant heavy metal, which can be absorbed by the digestive system in a 10%, [39] indicated that when lead incorporated by cells, it produces free radicals, H2O2 and ·OH. [40] found free radicals can also produce simple breaks in the DNA chains these results resembled with present finding. that exposure of lead produced the abnormal morphological effects in the larvae and the adults emerged therefrom. [41] reported newly hatched nymphs of an Indian short horned grasshopper Oxya fuscovittata(Marschall) Orthoptera: Acrididae were fed on foods treated with three sub lethal concentrations of CdCl i.e. 2 25 ppm in oat or dose 1 (d1), 50 ppm in oat or dose2 (d2) and 100 ppm in oat or dose3 (d3) until they reached on adult stage for a complete generation. Growth was measured in terms of specific growth rate (SGR), average daily growth (ADG), percent weight gain (PWG) and Growth rate (GR). They observed that growth retardation occurred significantly with the increase of doses in both sexes. Adult life period found reduced in both sexes however, in females a significant difference was found only with higher doses (d2 and d3). Lower survival was in d3 was observed. These adverse effect of heavy metals on diptera are in line with the present findings [42], found morphological changes in wild Drosophila species that found over almost all of Europe, under the effects of lead. The effects of lead on inversion polymorphism were studied by cytological analysis of gene arrangements on all of the five acrocentric chromosomes, as well as by cytological analysis of karyotypes on all of the four autosomes. The frequencies of particular gene arrangements on the four autosomes changed significantly in the samples maintained on medium not supplemented with lead. The frequencies of some gene arrangements on all of the five acrocentric chromosomes changed significantly in the flies maintained on media supplemented with lead. The length of exposure to different lead concentrations results in a significant change in the frequency of a few gene arrangements on two autosomes. Their results showed that different concentrations of lead, and exposure period caused affects on chromosome. the effects on the DNA configuration and chromosome cause effects on morphology and the physiology of the affected organism, in this way presently the obtaining of altered protein bands ,deform larvae, pupae and deform adults are in the line with the previous findings.

Appendix 1: Component Volumes (ml) Per Mold Volume of Solution Components

Solution components: 6%	10 ml
H_2O (Deionized water)	5.3
30% acrylamide mix	2.0
1.5 M Tris (pH. 8.8)	2.5
10% SDS	0.1
10% ammonium persulfate	0.1
TEMED	0.008

REFERENCES

[1] Tachi K, Nishime S. Cytogenetic effects of lead acetate on rat bone marrow cells. Arch Environ Heal 1975; 403: 144-47.

[2] Michailova P. Comparative karyological studies of three species of the genus *Glyptotendipis* Kieff. (Diptera, *Chironomidae*) from Hungary and Bulgaria and *Glyptendipis salinus* sp. n. from Bulgaria. Folia Biol (Krakwo) 1987b; 35: 43-56.

[3] Short C. Varion in sister-chromatid exchange among 100 species of the general insect population. Proc Environ 1990; 29: 140-49.

[4] Wilson BS. Sister chromatid exchange in larvae of insects. Lab 1995; 62: 135-44.

[5] Watson N. Chromosome aberrations and sister chromatid exchanges in insects of lead polluted. Exp Sci 1999; 90: 64-69.

[6] Walter R. Cyntheses prozesse an den Riesenchromosomen Von 2000.

[7] Porter DM. Mutagenicity new horizons in genetic toxicology. Proc Tox 2002; 55: 32-37.

[8] Ramel C. Effects of metal compounds on chromosome aegregation. Mutat Res 1973; 21: 45-46. http://dx.doi.org/10.1016/0165-7992(73)90062-6

[9] Talbot PS. Sister-chromated exchange frequency correlated with age, sex and lead poisoning. Environ Tox 2004; 25: 27-33.

[10] Margim A. Chromosome affected in experimental lead poisoning. Tox 2005; 41: 6-14.

[11] Michailova P. The effect of metal compounds on chromosomsegregation. Ist Nat Conf Plovidiv 1987a; pp. 168-173.

[12] Timmermans KP. Heavy metal body burden in insects larvae as related to their feeding behaviour. Symp Abst 76, 1988.

[13] Qureshi ZA, Hussain T, Siddiqui QH. Relative preference of mango varities by *Dacus. Zonata* (Saunders) and *D. dorsalis* Hendel. Pakistan J Zool 1991; 23(1): 85-87.

[14] Kapoor UC. Indian *Tephritidae* with their recorded hosts. Orient Insects 1970; 4: 207-51. http://dx.doi.org/10.1080/00305316.1970.10433957

[15] Drew RAI, Hancock D. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae*: Dacinae*) in asia. Bull Entomol Res 1994; (Suppl 2).

[16] Hong KT, Nishida R. Mutual reproductive benefits between a wild orchid, Bulbophyllum patens and Bactrocera fruit flies *via* a floral synomone. J Chem Ecol 2000; 26: 533-46. http://dx.doi.org/10.1023/A:1005477926244

[17] Beliles RP. Metais. In *Toxicology.* Casarett LJ, Doull J. Eds. Macmillan Pub. New York 1975; pp. 477-482.

[18] Valle BL, Ulmer DD. Biochemical effects of mercury, cadmium and lead. Ann Rev Biochem 1972; 41: 91-128. http://dx.doi.org/10.1146/annurev.bi.41.070172.000515

[19] Boyland E, Dukes CE, Grover PL, Mitchlcy BCV. Thc induction of renal tumoun by feeding lead acetate to rats. Br J Cancer 1962; 16: 283-88. http://dx.doi.org/10.1038/bjc.1962.33

[20] Van Esch GJ, Gendersen H, Vink HH. The induction of renal tumors by f e e d i i of basic lead acetate to rats. Br J Cancer 1962; 16: 289-97. http://dx.doi.org/10.1038/bjc.1962.34

[21] Roe FJC, Boyland E, Dukes CE, Mitchley BCV. Failure of testosterone or xanthopterin to influence the induction of renal neoplasms'by lead in rats. Br J Cancer 1965; 19, 860-66. http://dx.doi.org/10.1038/bjc.1965.99

[22] Mao P, Molnar JJ. The finc structure and histochcmistry of lead induced renal tumors in rats. Am J Pathol 1967; 50: 571-80.

[23] Choie DD, Richter GW. Cell proliferation in mouse kidney induced by lead. 1 Synthesis of DNA. Lab Invest 1974; 30: 647-51.

[24] Furst A, Schlauder M, Sasmore DP. Tumongenic activity of lead chromate. Cancer Res 1976; 36: 1779-3.

[25] Kobayashi N, Okamoto T. Effects of lead oxides on the induction of lung tumours in Syrian Hamster. J Natl Cancer Inst 1974; 52: 1605-7.

[26] ICPEMC. Report of. ICPEMC task group 5 on the differentiation between genotoxic and non-genotoxic carcinogens. Mutat Res 1984; 133: 1-49. http://dx.doi.org/10.1016/0165-1110(84)90002-2

[27] Varma MM, Joshi SR, Adeyami AO. Mutagenicity and infertility follow- ing administration to lead sub-acetate to swiss male mice. Experientia 1974; 30: 486-87. http://dx.doi.org/10.1007/BF01926307

[28] Stowe HD, Goyer RA. The reproductive ability and progeny of F, lead toxic rats. Fertility Sterility 1971; 22: 755-60.

[29] Hackett PL, Hess JO, Sikov MR. Effect of dose level and pregnancy on the distribution and toxicity of intravenous lead in rats. J Ton Environ Health 1983; 9: 1007-20. http://dx.doi.org/10.1080/15287398209530221

[30] Hess JO, Sikov MR. Distribution and effects of intravenous lead in the fetoplacental unit of the rat. J Tox Environ Health 1982; 9: 1021-32. http://dx.doi.org/10.1080/15287398209530222

[31] Lower WF, Drobney VK, Rose PS, Putnam CW. Environmental and laboratory monitoring of biotic indicators of heavy metals. Mutat Res 1976; 38: 386. http://dx.doi.org/10.1016/0165-1161(76)90109-6

[32] Maki-Paakkanen JM, Sorsa M, Vainio H. Chromosome aberrations and sister chromatid exchanges in lead exposed worken. Hereditas 1981; 94: 269-75. http://dx.doi.org/10.1111/j.1601-5223.1981.tb01764.x

[33] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bactriophage T4. Nature 1970; 227: 680. http://dx.doi.org/10.1038/227680a0

[34] Tiselius A. A new apparatus for electrophoretic analysis of colloidal mixtures. Transactions of the Faraday Society 33: 524. segregation. Ist Nat Conf Plovidiv 1937; pp. 168-173.

[35] Andrew AT. Electrophoresis: Theory, techniques and biochemical and clinical applications. Clarendon Press, Oxford 1986.

[36] Huiye and Jian-Hong Liu. Population dynamics of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) in the Kunming area, Southwestern China. Insect Sci 2005; 12: 387-92. http://dx.doi.org/10.1111/j.1005-295X.2005.00048.x

[37] Nukhet AB, Elizabeth A. Franklin NE. Effects of Nacetylcysteine on lead-exposed PC-12 cells. Arch Environ Contam Toxicol USA 2005; 49: 119-23. http://dx.doi.org/10.1007/s00244-004-0025-0

[38] Corey OG, Galvao CL. Plomo. Serie Vigilancia 8. Metepec, Edo. De México. Centro Panamericano de Ecología Humanay Salud Org Panam Salud O. M. S. 1989; pp. 103.

[39] Roy NK. Mutagenesis and comutagenesis by lead compounds. Res 1992; 298: 97-103.

[40] Friedberg EC, Walker G, Siede W. DNA Repair and Mutagenesis. ASM Press, Washington 1995; pp. 16-17.

[41] Chandrik M, Arijit G, Parimalendu H. Influence of Cadmium on Growth, Survival and Clutch Size of A Common Indian Short Horned Grasshopper, Oxya fuscovittata. Am-Eurasian J Toxicol Sci 2009; 1(1): 32-36.

[42] Kalajdzic P, Stamenkovic-Radak M, Andjelkovic M. The effect of different concentrations of lead on inversion polymorphism in Drosophila subobscura. Hereditas 2006; 143: 41-46. http://dx.doi.org/10.1111/j.2006.0018-0661.01939.x

Received on 24-01-2013 Accepted on 26-03-2013 Published on 10-04-2013

http://dx.doi.org/10.6000/1927-5129.2013.09.38