Heavy Protein Alteration under the Effects of Lead Acetate in *Bactrocera cucurbitae*

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Abstract: Lead is supposed to be an important poisonous waste which could contaminate the environment, therefore, insects could be influenced easily by the lead., *Bactrocera cucurbitae* was studied at 48 hours post treatment, under the effects of lead acetate, in different concentrations of 0.125 mg., 0.25 mg., 0.5 mg, 1.0 mg and 2.0 mg. It was observed that under the effects of lead abnormalities and deformity 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.

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

INTRODUCTION

Lead, a widely in use industrial heavy metal, is a significant environmental pollutant that contaminates 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 toxic potential 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 limited 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 determine 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 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 con-specific females [16]". Since immature, both the sexes male and female remains associated with the environment 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, soils and foods [17]. lead inhibits the activity of enzymes that are dependant on the presence of free sulphydryl groups (SH). 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 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 and triose phosphate isomerase [31] and affects non disjunction [8]. However, information about the mutagenic effects of lead salts in humans who are

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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, wheather 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 Plant protection and Diagnostic Laboratory, 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 was 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 formation. During that period pupation and adults 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.

1. Preparation of solutions:

i) Acrylamide-Bisacrylamide solution(30.0:0.8)

ii) 1.5 M Tris-HCl buffer:

iii) 10% Sodium dodecyl sulfate:

iv) 10% Ammonium per sulfate:

v) Sample diluting buffer (SDB):

vi) Reservoir Buffer:

vii) Staining solution: (Bromophenol blue and 0.2% Comassic blue).

viii) Destaining solution

Reagent and Chemicals

Acrylamide (Fluka)

N,N,Methylene bisacrylamide (Fluka)

Tris (hydroxymethyl) aminomethane (Fluka

HCL (Merck)

Sodium dodocylsulfate (Fluka)

Ammonium persulfate (Merck)

Glycine (Fluka)

TEMED (Merck)

Bromophenol blue (Merck)

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 above ingredient. The mix solution was filled in capillaries tube, then added the 0.1 ml ammonium sulphate and 0.008 ml TEMED in capillaries, then left it 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 de-staining 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 (dipterious 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, 0.08, 0.15, 0.24, 0.34, have not been observed in treated *Bactrocera cucurbitae*. While protein rf. 0.03, 0.12, 0.31, 0.36, have been dectected

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

Rf	Egg Albun	*Bactrocera cucurbitae* normal	*Bactrocea cucurbitae* treated
0.03		-	+
0.04	+	-	-
0.08		+	-
0.12		-	+
0.15		+	-
0.24		+	-
0.31		-	+
0.34		+	-
0.36		-	+

as altered in *Bactrocera cucurbitae* (Table **1**).

Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera cucurbitae* shown in (Figure **1**).

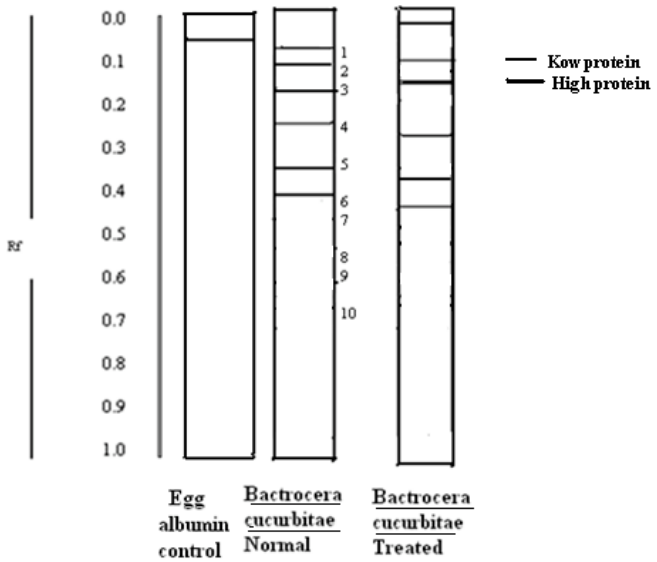

Figure 1: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera cucurbitae*.

DISCUSSION

Protein I (rf 0.03) is found in *Bactrocera. cucurbitae*. (treated) that is seemed to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera.cucurbitae*, is absent This suggests that the protein I (rf. 03) is changed with some alteration in the treated insect.

Protein VI (rf 0.08) is found in *Bactrocera cucurbitae* (untreated) that is seem to be lighter than egg albumin, while corresponding protein, in the treated *Bactrocera cucurbitae* was absent. This suggests that the protein V was effected with some extend.

Protein VIII (rf 0.12) is found in *Bactrocera cucurbitae* (treated) that is seemed to be lighter than the egg albumin, while it is absent in the untreated ones. That suggests the protein VIII is affected on small extend.

Protein X (rf 0.15) was found in *Bactrocera cucurbitae* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Bactrocera cucurbitae* is absent. This suggest that the protein X is affected at a large extend.

Protein XVI (rf 0.24) was found in *Bactrocera cucurbitae* (untreated) that was seems to be lighter than the egg albumin, while corresponding protein, in the treated *Bactrocera cucurbitae* was absent. This suggests that the protein XVI was affected at alow extend.

Protein XXI (rf 0.31) was found in *Bactrocera cucurbitae* (treated) that is 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 XXI was affected at a small extend.

In the *Bactrocera cucurbitae* protein XXIV (rf.0.34) was found in the untreated treated ones that is seems to be lighter than the egg albumin, while it is absent in the treated insect. That suggest that the protein XXIV (rf.0.34) was also affected at some extent

Protein XXVI (rf 0.36) was found in *Bactrocera cucurbitae* (treated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the untreated *B.cucurbitae*, was absent at the same rf. This suggests that the protein XXVI (rf. 0.36) was changed with some alteration 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 the 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: 10 ml

6%

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

Gel Casting:

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