Hazardous Effects of Lead Acetate on Heavy Proteins of *Bactrocera zonata*

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Abstract: Lead is presume to be an important toxic waste which contaminate the environment, therefore, insects could be influenced easily by the lead., *Bactrocera zonata* was studied at 48 hours post treatment, under the effects of lead acetate, in different concentrations. It was observed that under the effects of lead abnormalities and malformation 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 zonata.*

INTRODUCTION

Lead is a heavy metal, widely use in industries and 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 zonata* 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 Chironomidae,structural and functional modifications and malformations [11,12]. Investigations on *Bactrocera zonata* indicated abnormalities due to the effect on meiotic nondisjunction [8]. However, sufficient data on the action of heavy metals and lead is limited available on the group of insects such as *Bactrocera zonata,* those are widely distributed species of the family tephritidae.

MATERIALS AND METHODS

The strains of the test materials, *Bactrocera zonata* were procured from the Plant protection and Diagnostic Laboratory, DPP, Karachi. The oviposited mangoes with *Bactrocera zonata* 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 [13]. The newly emerged 3rd instar larvae were collected in Petri dishes for the treatments. Batches of three bottles were prepared with 3 *Bactrocera. zonata* has been reported on different varieties of mango, the adult flies has been found infesting fruit on maturation during the month of June [14]. *Bactrocera spp.* is regular pest of fruits, which considered being responsible for causing up to 25-50% loss in fruit yield. It has now been considered to infesting heavily mango, peach, plum, citrus and many others [15]. *Bactrocera zonata* has been observed from the entire oriental region on a specific host plants [16]. Thus the use of Diptera flies could present a useful module for the rapid screening of the environmental hazards due to lead contamination, which exerts a definite physiological and morphological effect on Diptera. Therefore, presently, the species *Bactrocera zonzta,* were used to determine the deleterious effects of lead metal, to generate necessary data in respect of *Bactrocera zonzta* to use as a test system for heavy metals and to follow up the later teratomorphic effects on pure diet and kept till pupation and adult appearance. Next to each stage the effect of lead acetate of different concentration was recorded carefully.

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.

Preparation of Solutions

i) Acrylamide-Bisacrylamide solution (30.0:0.8)

ii) 1.5 M Tris-HCl buffer:

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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 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 zonata* (dipterious flies) is shown in Table **1**, in this respect *Bactrocera zonata,* protein were studied in comparision with Egg albumin as a reference protein. The relative flow (rf) of Egg albumin was found as 0.04. Protein rf. 0.04, 0.07, 0.16, 0.26, 0.33 and 0.43, have not been observed in treated *Bactrocera zonata*. While 0.05, 0.08, 0.15 and 0.32, have been dectected as altered in *Bactrocera zonata* untreated (Table **1**).

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

Protein (rf 0.05) is found in *Bactrocera zonata*. (treated) that is seemed to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera.zonata* is absent. This suggests that the protein I (rf. 0.05) is changed with some alteration in the treated insect. Protein V (rf 0.07) is found in *Bactrocera zonata* (untreated) that is seem to be lighter than the egg albumin,while corresponding protein, in the treated *Bactrocera zzonata* was absent at the same Rf. This suggests that the protein V was effected with some extend. Protein VI (rf 0.08) is found in *Bactrocera zonata* (treated) that is seem to be lighter than egg albumin, while corresponding protein, in the untreated *Bactrocera zonata* was absent. In the *Bactrocera zonata* protein VI (rf 0.08) was in the untreated while absent in treated ones. That suggest protein VI(rf 0.08) is affected at large extend. Protein X (rf 0.15) was found in *Bactrocera.zonata*. (treated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the untreated *Bactrocera.zonata*, is absent. This suggests that the protein X was changed with some alteration in the treated insect. Protein XI(rf 0.16) is found in *Bactrocera.zonata*. (untreated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Bactrocera.zonata*, is absent. This suggests that the protein XI was changed with some alteration in the untreated insect. Protein XVII (rf 0.26) wasd found in *Bactrocera zonata* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Bactrocera zonata*, was absent at the same Rf. That suggest that the protein XVII was affected at some extend. Protein XXII (rf 0.32) was found in *Bactrocera.zonata*. (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera. zonata*, was absent at the same Rf. This suggests that the protein XXII was affected at a small

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

Protein	Rf	Egg Albumin	*Bactrocera Zonata* Normal (untreated)	*Bactrocera zonata* treated
I.	0.04	+	-	-
II.	0.05		-	+
III.	0.07		+	-
IV.	0.08		-	+
V.	0.15		-	+
VI.	0.16		+	-
VII.	0.26		+	-
VIII.	0.32		-	+
IX.	0.33		+	-
X.	0.43		+	-

Protein: + = present - = absent.

extend. Protein XXIII (rf 0.33) was found in *Bactrocera zonata* (untreated) and was absent in treated at the same rf. That suggest that the protein XXIII (rf 0.33) was affected at some extend. Protein XXVIII (rf 0.43) is found in *Bactrocera zonata* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Bactrocera zonata*, was absent at the same rf. This suggests that the protein (rf 0. 43) was affected at a large extend.

DISCUSSION

Drosophila melanogaster 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 [17], 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 [18]. indicated that, lead is a pollutant heavy metal, which can be absorbed by the digestive system in a 10%, [19] indicated that when lead incorporated by cells, it produces free radicals, H2O2 and ·OH. [20] 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 [21] 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

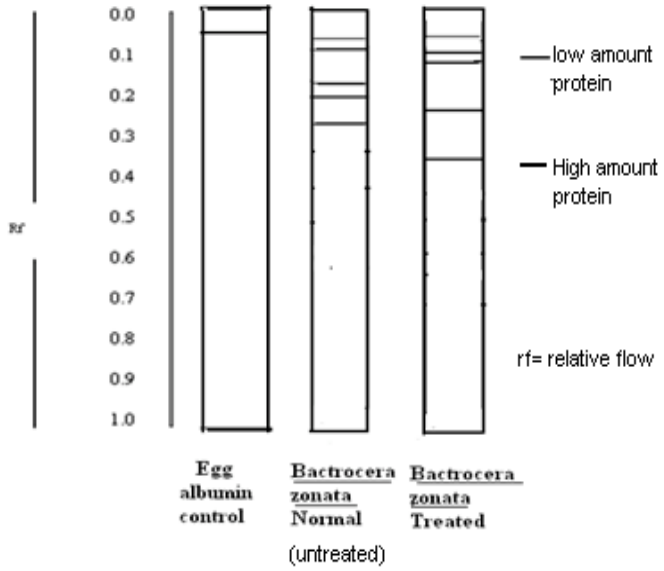

Figure 1: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera zonata.*

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.

[22] 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.

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