Heavy Weight Protein Affected by Lead Acetate in *Bactrocera dorsalis*

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Abstract: The studies were carried out on toxic effects of lead acetate, which could contaminate the environment, such as food, water, air and soil, therefore insects could be influenced easily by the lead., *Bactrocera dorsalis* 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 deformity were developed in the larvae of flies. Thus these flies could present a useful module for the quick transmission of the environmental hazards of lead, which exerts a specific physiological and morphological effect on their bodies.

Keywords: Effects, lead acetate, *Bactrocera dorsalis.*

INTRODUCTION

Lead is toxic heavy metal, widely use in industries, 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 issue 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 dorsalis* 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 dorsalis* 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 dorsalis,* which are widely distributed species of the family tephritidae.

Bactrocera dorsalis 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 spp.* is a 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 [14]. *Bactrocera dorsalis* has been observed from the entire oriental region on a specific host plants [15]. Thus, the fruit fly *Bactrocera dorsalis* complex have been reported in a vast field as pest of fruits in Asia [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 flies. Therefore, presently, the species *Bactrocera dorsalis,* were used to determine the deleterious effects of lead metal, to generate necessary data in respect of *Bactrocera dorsalis,* to use as a test system for heavy metals and to follow up the later teratomorphic effects.

Electrophoresis is being broadly used for categorization of proteins and peptides for the diagnostic and/ or preparative unification of organic macromolecules [18]. The process of electrophoresis first used by [19] 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, [20].

MATERIAL AND METHODS

Initial strains of the test materials, *Bactrocera dorsalis* were procured from the Plant protection and

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Solution	Prepration
i) Acrylamide-Bisacrylamide solution (30.0:0.8)	Dissolve 30 gm acrylamideand0.8 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 18.02 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.

Diagnostic Laboratory, DPP, Karachi. The oviposited mangoes with *Bactrocera dorsali* 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 [17]. The newly emerged 3rd instar larvae were collected in Petri dishes for the treatments. Batches of three bottles were prepared with 3 gram feed of mangoes and bananas pulp mixed with lead acetate in the desired concentration, as, 0.125 mg, 0.25 mg, 0.5 mg, 01 mg and 02 mg and a batch of three bottles was kept as control. Thereafter, 10 larvae were released in each bottle for 48 hours exposure. At the post 48 hours exposure mortality count was made then the surviving larvae were transferred in separate bottles on pure diet and kept till pupation and adult emergence. At each stage the effect of lead acetate of different concentration was recorded.

The determination of lead acetate on protein of *Bactrocera dorsalis* 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 solution, 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 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.

Reagents and Chemicals	Brand
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

Figure 2: Reagents and Chemicals.

RESULTS

The effect of lead acetate on proteins of *Bactrocera dorsalis* (dipterious flies) is shown in Table **1**, in this respect *Bactrocera dorsalis,* protein were studied in comparision with Egg albumin as a reference protein. The rf. of Egg albumin was found as 0.04 as indicated in Table **1**. Protein rf. 0.03, 0.05, 0.21. 0.34 have not

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

Rf	Egg Albumin (control)	*Bactroca dorsalis* normal (untreated)	*Bactrocera dorsalis* (treated)
0.03		+	-
0.04	+		+
0.05		+	-
0.06		-	+
0.09		-	+
0.21		+	-
0.22		-	+
0.33		-	+
0.34		+	-

Protein: + = present - = absent Rf= Relative flow.

been observed in treated *Bactrocera dorsalis*. While protein rf. 0.04, 0.06, 0.22, 0.33, have been dectected as altered in *Bactrocera dorsalis.* Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera dorsalis* shown in Figure **3**.

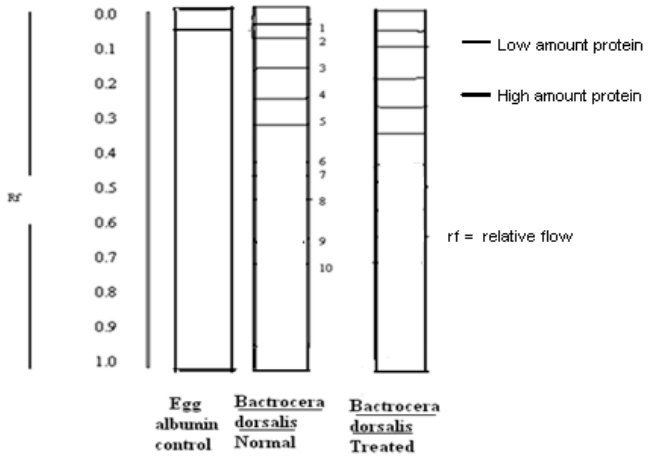

Figure 3: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera dorsalis.*

DISCUSSION

Protein relative flow (rf 0.03) is found in *Bactrocera.dorsalis*. (untreated) that is seemed to be lighter than the egg albumin, while corresponding protein, in the treated *Bactrocera.dorsalis*, is found at the same rf (0.04) that of egg albumin. This suggests that the protein (rf 0.03) is changed with some alteration in the treated insect. Protein (rf 0.05) is found in *Bactrocera dorsalis* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Bactrocera dorsalis*, was found at the rf (0.06). This suggest that the protein (rf. 0.05) is changed with some deletion or alteration in treated insect.

Protein (rf 0.09) was found in *Bactrocera dorsalis* (treated) that is seem to be lighter than the egg albumin. While nothing is present in *Bactrocera. dorsalis* untreated. This suggest that the protein (rf 0.09) was affected with some extend in the treated insect. Protein (rf 0.21) was found in *Bactrocera dorsalis* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Bactrocera dorsalis*, was absent at the same rf. That suggest that the protein (rf 0.21) was affected at a small extend. Protein (rf 0.22) was found in *Bactrocera dorsalis* (treated) that is seemed to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera dorsalis* was absent. This suggests that the protein rf 0.22 was changed with some alteration in the untreated insect. Protein (rf 0.33) was found in *Bactrocera dorsalis* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera dorsalis*, was absent found at the same rf. This suggests that the protein (rf 0.33) was changed with some alteration in the treated insect.

Protein (rf 0.34) was found in *Bactrocera dorsalis* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Bactrocera dorsalis*, was absent at the same rf. This suggests that the protein (rf 0.34) was affected with some alteration in the treated insect.

Bactrocera dorsalis 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. [21], 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. [22]. indicated that, lead is a pollutant heavy metal, which can be absorbed by the digestive system in a 10%, [23] indicated that when lead incorporated by cells, it produces free radicals, H2O2 and ·OH. [24] 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. [25] 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.

[26] 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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