

ADVERSE EFFECT OF LEAD ACETATE ON *DROSOPHILA MELANOGASTER*

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Abstract

As lead is an important environmental noxious waste which directly or indirectly contaminate the foods, soil water, and air thus, insects could be influenced easily by the lead. Therefore, lead was studied as lead acetate in different doses. viz 0.125 mg., 0.25 mg., 0.5 mg, 01 mg and 02 mg, on external morphology on *Drosophila melanogaster* at 48 hours post treatment. It was observed that under the effects of lead abnormalities and deformity were developed in the larvae of flies. Morphological changes were observed as elongated wings, de-shaped wings, elongated and folded legs, change in color of larvae, pupae and adults. Some other structural abnormalities of larvae and pupa shape were also observed. Thus the Dipterous 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. So, in this respect, *Drosophila melanogaster*, could be used as a test method for heavy metals effects.

Keywords: Heavy metal, lead acetate, *D. melanogaster*.

INTRODUCTION

Lead is generally used in industries. It is a significant environmental pollutant that contaminates food, water, urban soil and air. The exposure of this metal along with possible dangerous effect is an issue of urgent concern. Even though, many studies have been carried out in relation with the biological effects of lead, its toxic potential against insects remains to be explained well. Lead has been found to have a definite cytogenetic effect (Tachi & Nishime, 1975; Michailova, 1987a&b; Short, 1990; Wilson, 1995; Watson, 1999; Walter, 2000; Porter, 2002; Ramel, 2003; Talbot, 2004 and Margim 2005). In respect of effects of heavy metals, some studies have been carried out on *D. melanogaster*. It has been established that heavy metals (Zinc, Lead etc.) can induce the effects on feeding behavior of some diptera, and produce malformations, their structural and functional modifications (Michailova, 1987a and Timmermans 1988). Investigations on *Drosophila melanogaster* indicated developed abnormalities due to the effect of mutagenic factors on meiotic chromosomal non-disjunction (Ramel, 2003). However, sufficient data on the action of heavy metals and lead is limitedly available on the group of insects such as *Drosophila melanogaster*, which is widely distributed species of the family tephritidae.

Drosophila is belonging to the family Drosophilidae, also called "fruit flies" and less frequently called pomace flies, vinegar flies, or wine flies, *Drosophila* feed mostly on unripe or ripe fruit. As it breed rapid, and put down a lot of eggs. *D. melanogaster* is being used extensively in research as a model organisms (Eric 2001). The *D.*

melanogaster average life span is about 30 days at 29°C (84°F). Like other Diptrous insects The developmental process in *Drosophila melanogaster* varies with temperature. The developmental period from egg to adult take 7 days at 28°C (82°F). Under ideal conditions, the development time at 25°C (i.e., 77°F) is 8.5 days, the eggs, The eggs, which are about 0.5 mm long, hatch after 12-15 hours at 25°C (Ashburner 1978 and Ashburner 2005).

Among the heavy metals, lead, has been found to be widely distributed in the atmosphere, water, soils and food stuffs (Beliles 1975). It inhibits the activity of enzymes that are dependent on the presence of free sulphhydryl groups (SH). The clearest expression of these effects is the disturbance on the biosynthesis of heme, which in humans is accompanied by abnormalities in porphyrin metabolism (Valle and Ulmer 1972). Though, information about the mutagenic effects of lead salts in workers those are occupationally exposed the lead and information obtained from in vitro studies showed a non significant relationship (Maki-Paakkanen *et al.* 1981). Lead acetate is used as a topical astringent and is found to be a renal carcinogen in rats (Boyland *et al.*, 1962, Van Esch *et al.* 1962, Roe *et al.*, 1965, Mao and Molnar 1967, Choie and Richter 1974, Furst *et al.* 1976). In the Syrian hamster lead induces changes in the bronchio-alveolar area (Kobayashi and Okamoto 1974, ICPEMC 1984). It was found to produces infertility in mice (Varma *et al.*, 1974) and reduced the reproductive ability of rats (Stowe and Goyer 1971, Hackett *et al.* 1983, Hess and Sikov 1982). In *Drosophila melanogaster* lead was reported to induce enzymatic alterations in esterase and triose phosphate isomerase (Lower *et al.* 1976) and caused

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chromosomal non-disjunction effects (Ramel 1973).

The purpose of the present work was to find out the effects of lead ions in various doses on the physiological and morphological features of 3rd instar stage larvae and pupae of *Drosophila melanogaster*.

MATERIALS AND METHODS

The *Drosophila melanogaster* flies were reared in wide mouth bottles containing usual medium. The eggs of these flies were collected from fermenting fresh baker's yeast supplemented with sucrose. After removing the parental flies, the 72 hours old larvae were collected after three days and transferred to small bottles (10 larvae/bottle). Three bottles were prepared with 3 grams bananas, mixed with lead acetate in the doses of, 0.125 mg, 0.25 mg, 0.5 mg, 01 mg and 02 mg and the three bottles were left untreated as control. The 10 larvae were introduced in each bottle for 48 hours. Thereafter, alive larvae were transferred to the bottles containing pure bananas diet till pupation and adult emergence, after that, in different doses lead acetate effects was recorded on different stages of these flies.

RESULTS

Drosophila melanogaster larvae are normally, yellowish white, in color they are wide at anterior and taper at posterior end, with a pair of hooks in their mouth for digging and tearing food. They have spiracles at their anterior and posterior portions for respiration. They have body length of 5 mm., light brown at one end and dull white appearance at other end (Fig. 1).



Fig. 1. *Drosophila melanogaster* (larval stage), normal condition, yellowish white color wide at anterior and taper at posterior end with a pair of hooks in their mouth for digging and tearing food.

Morphological abnormalities in larvae

With 0.125 mg of lead acetate at 48 hrs. post-treatment, *Drosophila melanogaster* (larval stage), were appeared as slightly yellowish black color with broadened at one end and tapered at the other end. They had slightly curved and rough body, (Fig. 2). When larva treated with 0.25 mg of lead acetate, they appeared with yellowish dull white melanization, slightly curved body, with deep constrictions. Their physical appearance was slightly abnormal (Fig. 3). Larvae, treated with lead acetate in 0.5 mg. dose for 48 hours exposure, showed rod shape, yellowish dull white color body (Fig. 4). On the other hand treatment with slightly higher dose of lead acetate i.e. 1 mg (for 48 hrs) left the larvae with, elongated shape, dull white yellowish color and heavy melanized larvae were resulted with 2 mg of lead acetate treatment.

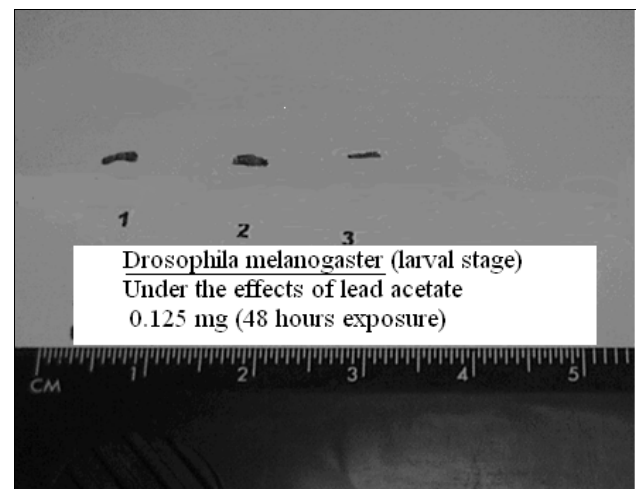


Fig. 2. *Drosophila melanogaster* (larval stage), under the effect of lead acetate, 0.125 mg. at 48 hours. Small toxic effect on larval structure.

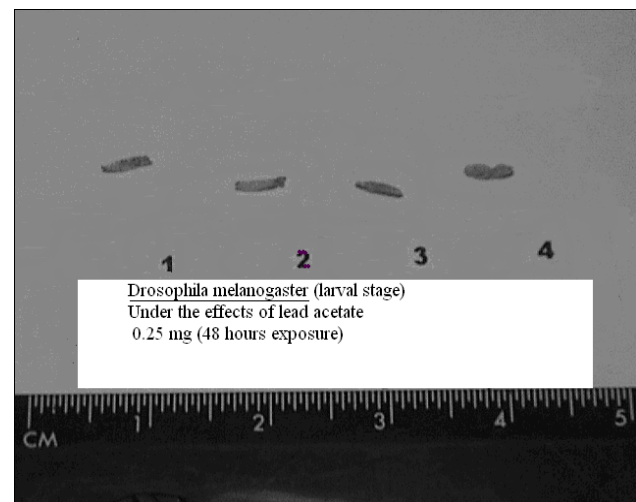


Fig. 3. *Drosophila melanogaster* (Larval stage). Effected with lead acetate, 0.25 mg. at 48 hours. Toxic effect of lead showing the deep constriction and curve on larvae.

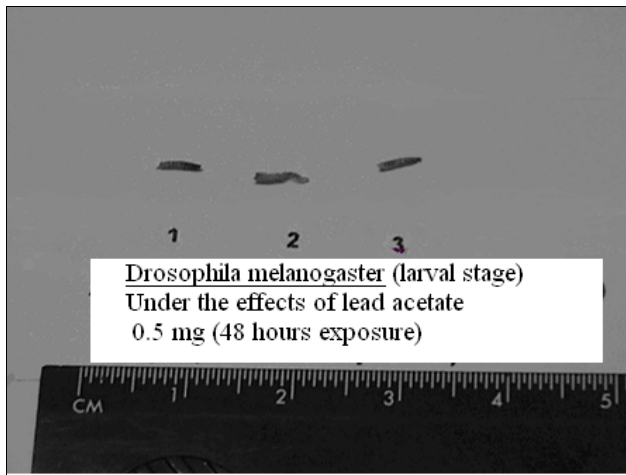


Fig. 4. *Drosophila melanogaster* (larval stage) effected with lead acetate 0.5 mg at 48 hours. Showing change in shape and structure of larvae.

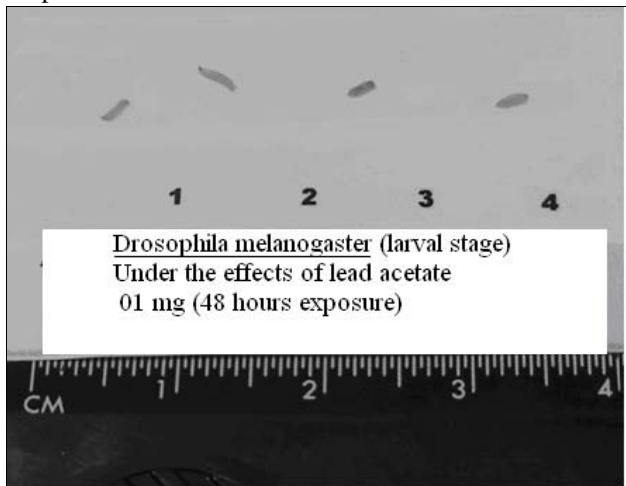


Fig. 5. *Drosophila melanogaster* (Larval stage) effected with lead acetate 01 mg. at 48 hours. Minute effect on melanization.

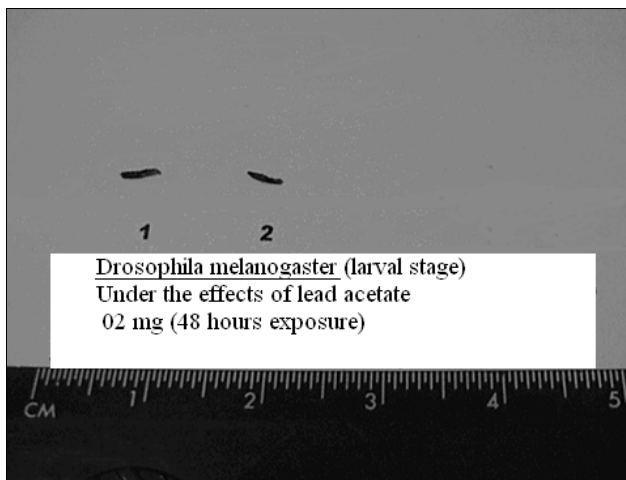


Fig. 6. *Drosophila melanogaster* (larval stage) effected with lead acetate 02 mg at 48 hours. Minute change in structure and melanization.

Morphological abnormalities in pupae

Drosophila melanogaster pupae, in normal condition, seemed to be tapered at the both the ends, while broaden at middle portion (Fig. 7). Cuticle of the pupae shows elasticity and their body length measures as 4 mm.

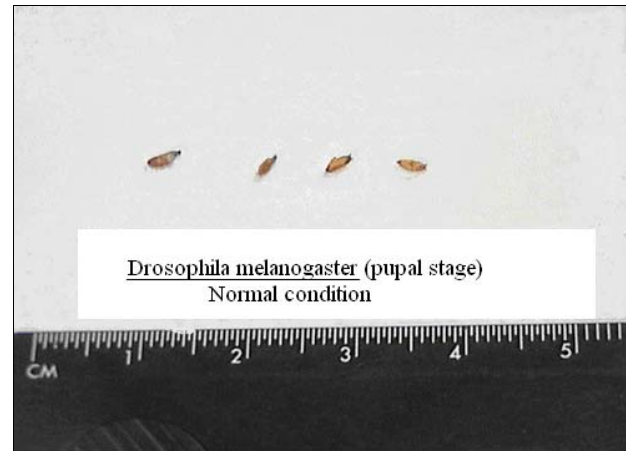


Fig. 7. *Drosophila melanogaster* (papal stage) in normal condition. seem taper at both the end, broader at middle portion, cuticle (skin) slightly elastic, slightly segmented.

Treatment of larvae with 0.125 mg of lead acetate for 48 hrs, produced pupae with minimum abnormality on body structure (Fig. 8). With 0.25 mg. of dose for 48 hours exposure, brown yellowish and dark melanized pupae were observed. (Fig. 9).

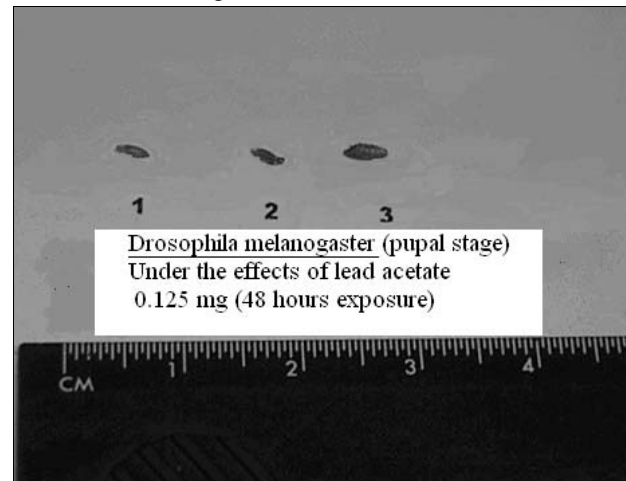


Fig. 8. *Drosophila melanogaster*, pupal stage, effected with lead acetate, 0.125 mg. at 48 hours.

Lead acetate affected pupae with slightly higher dose i.e., 0.5 mg showed hardened cuticle with slight pigmentations (Fig. 10). Exposure with .01 mg brought a slight change in morphological structure and melanization (Fig. 11). On the other hand with 2.0 mg lead acetate, pupae showed abnormal projection at one end, showed dark brown melanization and physically slightly abnormal structure with rough surface (Fig. 12).

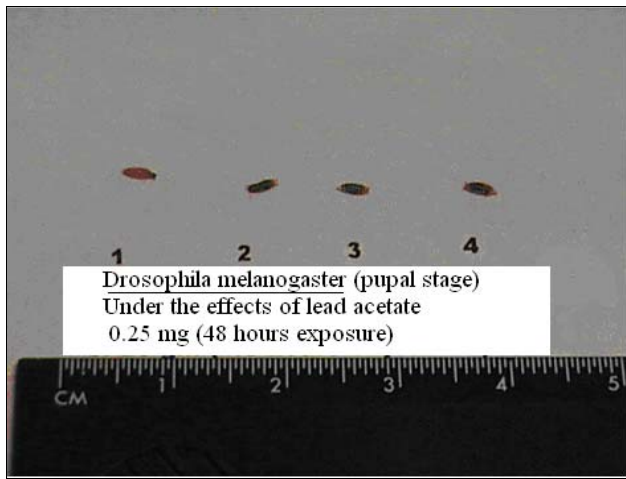


Fig. 9. *Drosophila melanogaster* (pupal stage) effected with lead acetate 0.25 mg at 48 hours. Slightly effect of lead acetate indicates the structural change on pupae

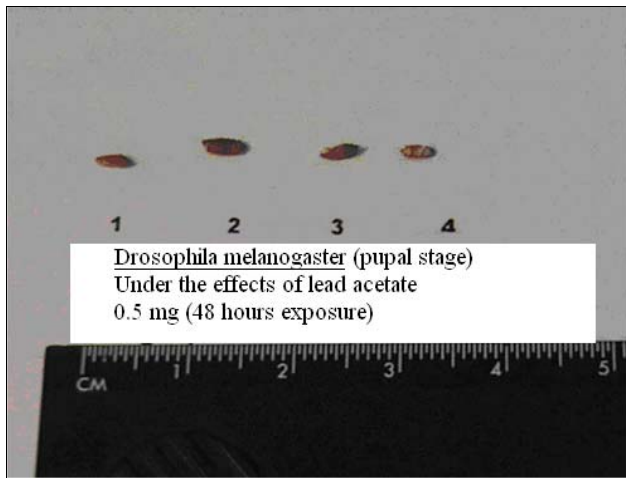


Fig. 10. *Drosophila melanogaster*, effected with lead acetate 0.5 mg for 48 hours. Showing toxic effect of lead acetate on structure and size of pupae

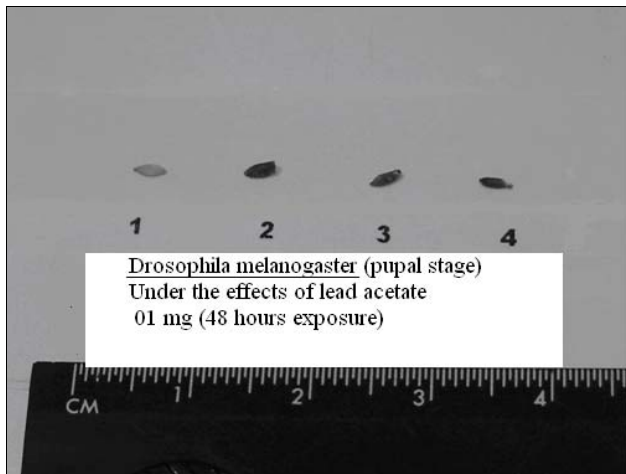


Fig. 11. *Drosophila melanogaster*, effected with lead acetate 0.1 mg at 48 hours. Showing toxication on morphological structural abnormalities and pigmentation.

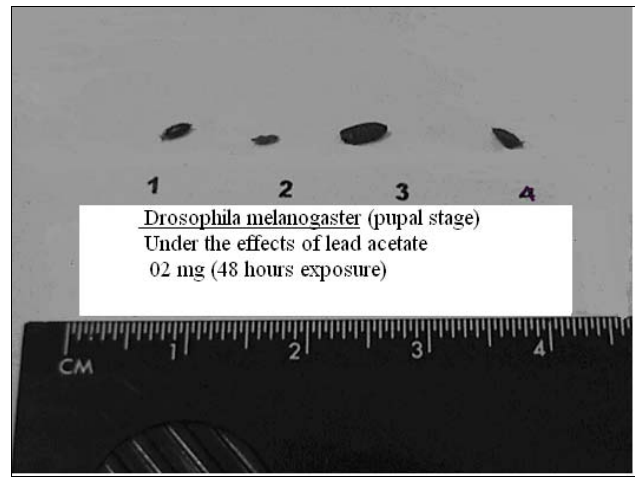


Fig. 12. *Drosophila melanogaster* (pupal stage) effected with lead acetate 0.2 mg at 48 hours. Showing toxic effect on structure and size of pupae

Morphological abnormalities in adult

Drosophila melanogaster, Normal adult *Drosophila* have red eyes, yellow brown body with a patch of black color at the end of abdomen in males. In females this patch pattern is found broken into black striation across their abdomen. Veinleted expanded transparent wings are present (Fig. 13).

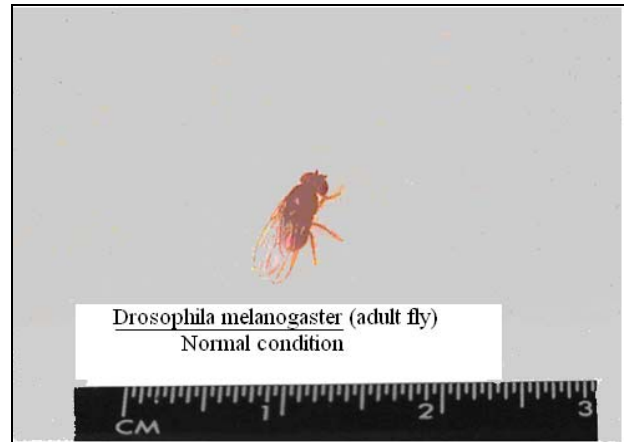


Fig.13. *Drosophila melanogaster* (Adult stage) in normal condition. brick red eyes, yellow brown color, transverse black ring, across their abdomed. Veinleted expanded transparent wings.

When *Drosophila melanogaster*, adult obtained from the treated larvae with 0.125 mg of lead acetate their leg and wings become abnormal. When a slightly higher dose i.e. 0.25mg of lead acetate were applied the legs of adult become elongated and twisted wing were observed, while the abdomen become swollen (Fig. 14 and Fig. 15). Adult, those were produced from with lead acetate 0.5 mg affected larval treatment, showed slightly abnormal wings and curved abdomen (Fig. 16). Adult emerged after the larvae treated with lead acetate, 1 mg. appeared in curve down abdomen, folded and curved legs, and folded wings

as well (Fig. 17). Adult effected with lead acetate(2.0 mg) at larval stage resulted in upside curved wings, abdomen with abnormal projection and folded legs(Fig. 8).

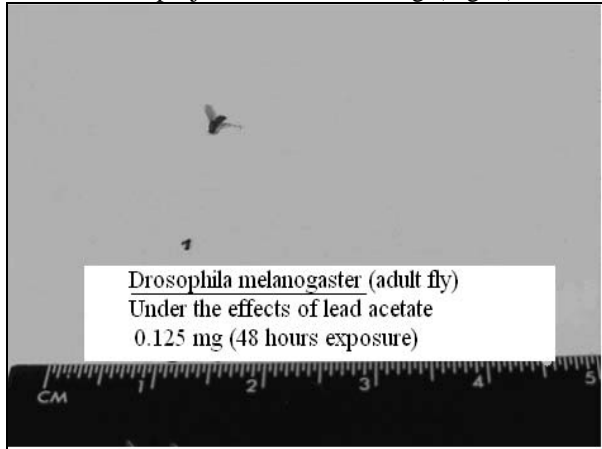


Fig. 14. *Drosophila melanogaster*, adult stage, effected with lead acetate, 0.125 mg at 48 hours. Showing slightly toxic effect on curved wing and legs.

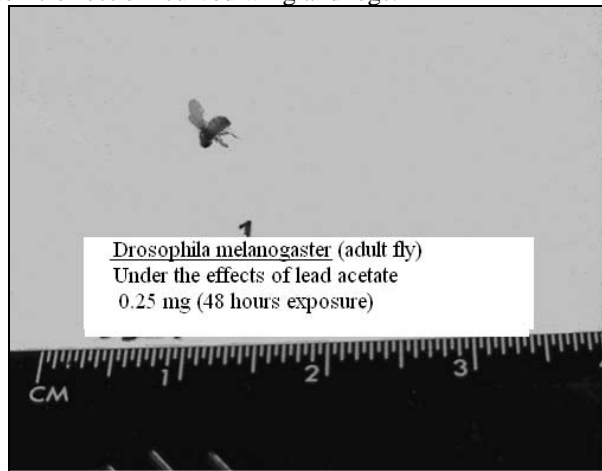


Fig. 15. *Drosophila melanogaster* (Adult stage) effected with lead acetate, 0.25 mg at 48 hours. Showing toxic effect on expanded wings and abdomen.

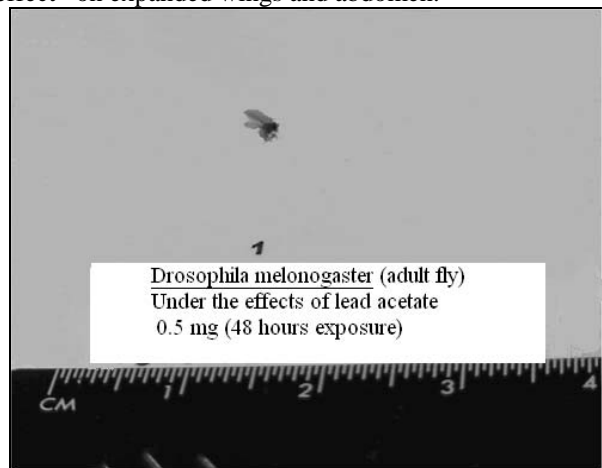


Fig. 16. *Drosophila melanogaster* (Adult stage) effected with lead acetate 0.5 mg at 48 hours. Slightly toxic effect on wings and curved abdomen.

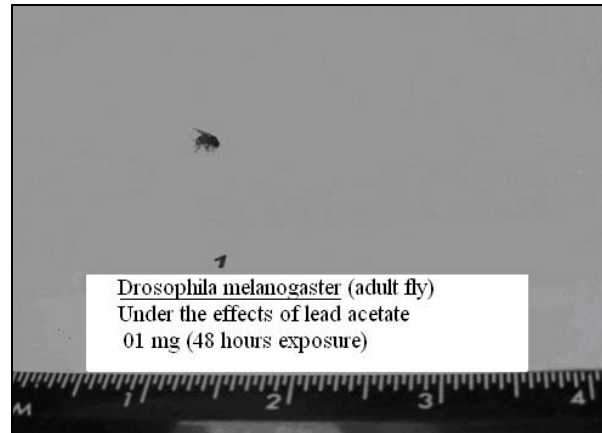


Fig. 17. *Drosophila melanogaster* (Adult stage) effected with lead acetate, 01 mg at 48 hours. Minute effect on abdomen and folded legs.

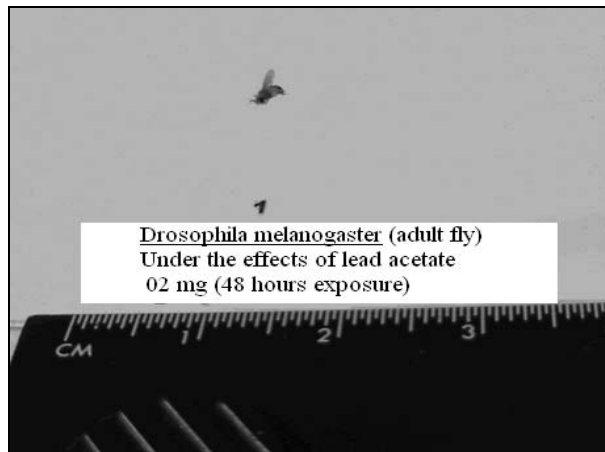


Fig. 18. *Drosophila melanogaster* (Adult stage) effected with lead acetate, 02 mg at 48 hours. Showing toxic effect on wings and abdomen

DISCUSSION

The results of *Drosophila melanogaster*, treated with various doses of lead acetate viz., 0.125 mg, 0.25 mg, 0.5 mg, 01 mg and 02 mg resulted an increase in mortality rates to the all different doses with a sigmoid curve (Curve 1). Parke *et al.* (1991) have shown that after the exposure of lead acetate the death rate increased with the increase in the dose of the chemical compound. These results are in line with the present findings. Ahmed and Naqvi (1985) reported that heavy metal resistance is obviously an extensive observable fact in invertebrates. Heavy metal resistance in Diptera and specially *Drosophila* are the model organisms in exposure to cadmium stated by Magnusson and Ramel (1986). Insects living in contaminated areas have been shown have accumulated heavy metals, in particular Ni and Cu, along with the clear effects of pollution on growth rate and mortality, reported by Warrington (1987). Positive relationship was found in *Chironomus* between the copper

dose and the occurrence of deformation of the pectin epipharyngis, indicated by Kosalwat and Knight (1987). Presently, lead was found causing deleterious effects on insects at various stages those were exposed at larval stage; these effects could be related with either extracellular changes or intracellular changes due to the lead ions. Lead has been reported to have a definite cytogenetic effect by various author e.g. Tachi & Nishime (1975), Michailova (1987b), Short (1990), Wilson (1995), Watson (1999), Walter (2000), Porter (2002), Ramel (2003) Talbot (2004) and Margim (2005) these reports indicate the explanations for the expected results similar to that obtained in the present studied.

The work on present studies also demonstrated that lead acetate caused influence generally on the morphology and development of the under test insects. The differences due to increase in dose of lead acetate effected the level of these changes as compared to control.

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