"ENVIRONMENTAL STUDIES OF DIFFERENT EFFECTS OF LEAD ON SOME PHYSIOLOGICAL AND MORPHOLOGICAL FEATURES OF DIPTERA FLIES"

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"ENVIRONMENTAL STUDIES OF DIFFERENT EFFECTS OF LEAD ON SOME PHYSIOLOGICAL AND MORPHOLOGICAL FEATURE OF DIPTERA FLIES"

Submitted

by

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under my supervision.
I permit it to submit for evaluation to the
Graduate Research Management Council,
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DEDICATION

The Dissertation is respectfully

dedicated to my parents

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ABSTRACT

ABSTRACT:

Lead is an important environmental toxic waste which almost contaminate the food, soil, water and air, hence, insects could be influenced easily by the lead. Therefore, lead was studied in the form of lead acetate using different doses. *viz* 0.125 mg., 0.25 mg., 0.5 mg., 1.0 mg and 2.0 mg, inrespect of its effects on external morphology of *Bactrocera dorsalis, Bactrocera zonata, Bactrocera cucurbitae, Drosophila melanogaster* and *Musca domestica* at 48 hours post treatment. It was observed that under the influenced of lead morphological abnormalities 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, several other structural abnormalities of larvae and pupae shape were also observed. It is shown that Dipterous flies could present a useful module for the assessment of lead contamination. The effect of lead acetate on proteins of five species of dipterious flies *Bactrocera dorsalis, Bactrocera zonata, Bactrocera cucurbitae, Drosophila melanogaster* and *Musca domestica* were also observed through electrophoresis, while Egg albumin 42.7 kDa was used as a reference protein and various proteins of different weights were found altered.

INTRODUCTION

INTRODUCTION:

Lead, a widely used 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 (Tachi and Nishime 1975; Michailova 1987b; Short 1990; Wilson 1995; Watson 1999; Walter 2000; Porter 2002; Ramel 2003; Talbot 2004 and Margim 2005). The detection of possible hazardous effects of this metal is, therefore; a matter of urgent concern. Though, many studies have been carried out to investigate the biological effects of lead, its toxic potential against insects remained to be established. Some studies have been carried out on natural populations of *Bactrocera dorsalis* and *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 Diptera, structural and functional modifications and malformations (Michailova 1987a and Timmermans 1988). Investigations on Diptera indicated abnormalities due to the effect on chromosomal meiotic nondisjunction (Ramel 2003). However, sufficient data on the action of heavy metals and lead is limited on the group of diptera insects, those are widely distributed species. *Bactrocera dorsalis and 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 (Qureshi *et al.*, 1991). *Bactrocera dorsalis and Bactrocera zonata* has been observed from the entire oriental region on a specific host plants (Kapoor 1970). The fruit fly *Bactrocera dorsaliss* complex have been reported in a vast field as pest of fruits in Asia (Drew and Hancock 1994)". Presently, the species *Bactrocera dorsalis, Bactrocera zonzta, Bactrocera cucurbitae, Drosophila melanogaster and Musca domestica* were used to determine the deleterious effects of lead metal.

The melon fruit fly, "*Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is widely distributed 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 (

Qureshi *et al.,* 1991). The males pollinate the flowers and acquire the floral essence and store it in the pheromone glands to attract con-specific females (Hong and Nishida 2000)". 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.

Musca domestica commonly known as "House fly is the most common insect found indoor, in out door in dairy farms poultry farms, horse stables and cattle farms also factories and mills etc. These flies are found associated with humans activities and animal feaces, feeding on garbages and are abundant in urban and rural areas where sanitary managements are badly enforced (Greenberg 1973; Graczyk *et al*., 2001., 2005). As these flies mostly found all over the world, therefore, housefly, *Musca domestica* L. (Diptera: Muscidae), is a true widely spread cosmopolitan insect of medical and veterinary importance (Greenburg 1971)".

Among the heavy metals, "lead, has been shown to be widely distributed in the atmosphere, water, soils and foods (Beliles 1975). 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 (Valle and Ulmer 1972). 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 harnster lead induces neoplastic changes in the bronchio-alveolar area (Kobayashi and Okamoto 1974, ICPEMC 1984). It also produces infertility in mice (Varma *et al.,* 1974) and reduces the reproductive ability of rats (Stowe and Goyer 1971; Hackett *et al.,* 1983; Hess and Sikov 1982). In *Drosophila melanogaster* lead induces enzymatic alterations in esterase and triose phosphate isomerase (Lower *et al***.,** 1976) and affects non disjunction (Ramel 1973). 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" (Maki-Paakkanen et *al.,* 1981).

Hypothesis:

Since lead is reported to cause adverse effects on various vital physiological ions and the DNA, therefore, it is expected that along with tetaromorphic effects the lead would cause some alteration on proteins on dipteral flies that could be a parametric indicator of food contamination due to environmental pollution with this heavy metal.

The purpose of the present work was to determin the effects of lead on proteins as a major indicator of physiological features alongwith morphology features of 3rd instar larvae of Diptera flies. In this connection phenotypic modifications in the different stages of metamorphosis along with its effects on proteins of these flies were studied under lead acetate exposure.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

Heavy metals and Lead:

"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). Lead acetate is found to be a renal carcinogen in rats (Mao and Molnar 1967 and Furst et al 1976). The doses of both salts lead acetate and nitrate were found to induce a significant number of these mutations at 5% level on the, lead acetate being less toxic and les> mutagenic than lead nitrate. Oregon R adult males were injected with lead acetate with almost 0.2 pl of the salts scllutions, stated by Sega and Lee (1970). In the Syrian harnster lead induces neoplastic changes and reduces the reproductive ability of rats (Stowe and Goyer, 1971). According to Alvares et al. (1972) lead inhibit the heme group synthesis".

Paton, (1973), reported that, "many studies have shown that lead ions have harmful and toxic effects on different organisms. Among the heavy metals, lead, has been shown to be widely distributed in the atmosphere, water, soils and foods, stated by Beliles (1975). Many studies have shown that lead ions have harmful and toxic effects on different organisms, reported by Tachi and Nashime (1975). Lead inhibit the heme group synthesis and produce cell death, reported by Goldberg *et al*. (1977). Heavy metal resistance is evidently a widespread phenomenon in invertebrates. Diptera and in particular *Drosophila* are the model organisms in these studies. Exposure to cadmium, reported by Chapco *et al*. (1978). Explain the lack of Pb ions accumulation in *C. piger* tissue (0,05 mg/g). The *Chironomids* are very tolerant to heavy metals and a mechanism for regulation and excretion of the metal from the body may exist", reported by Wetsel *et al,* (1978).

"Bengtsson & Rundgren (1982) observed a clear detrimental effect of copper, zink and lead contamination on enchytraeid species diversity, densities and occupation of the organic soil layer. In addition to the direct effect of heavy metals on the mortality and fecundity of invertibrates, changes in vegetation also have an indirect influence on the fauna, studied by Alstad *et al*. (1982)".

"The recent studies concerning the effect of heavy metals on forest ecosystems have generally demonstrated areduction in the species number and an impoverishment of the community structure of ground-living invertebrates, reported by Bengtsson *et al*. (1983). Lead administrated in excess it can compete with calcium turn into a poison, indicated by Pounds (1984). In the Syrian harnster lead induces neoplastic changes in the bronchio-alveolar area, stated by ICPEMC (1984). In the *I'radescantia* micronucleus test, the lead nitrate was found to be significant while lead acetate gave a borderline response", stated by Ma *et al.* (1984). "Shelton *et al*. (1986) indicated effects of lead are certain protein synthesis".

"The heavy metals and pesticides are two common and wide spread group of pollutant. Some studies have been carried out on natural populations of *Bactrocera cucurbitae* in respect of effects of heavy, metals, it has been established that contamination with heavy metals (Zinc, Lead etc.) can induce the effects on feeding behavior of some diptera, their structural and functional modifications and malformations (Michailova, 1987a and Timmermans 1988). However, sufficient data on the action of heavy metals and lead is limited available on the group of insects such as *Bactrocera dorsalis* and *Bactrocera zonata*, those are widely distributed species of the family tephritidae. Pollution effects on phytophagous insects whereas plant stress has negative effects on gall makers, observed by Preszler and Price, (1988). Insects living in polluted areas have been shown to accumulate heavy metals, in particular Ni and Cu, along with the obvious effects of pollution on growth rate and mortality and have harmful effects on immune defense, stated by Mitterböck and Fuhrer (1988). Experiment with *Chironomous reparius* and sediment spiked with cadium, zinc and copper, show high mortality rates, but no mentum deformities were induced, reported by Grootelaar *et al*. (1988). Heavy metal pollution has become one of the most important environmental problems in industrialized countries, reported by Nriagu and Pacyna (1988). Pollution effects on phytophagous insects vary often between insect guilds, reported by Larsson (1989). Many studies have shown that lead ions have harmful and toxic effects on different organisms. Pb ions damage chromosome structure and induce an increase in chromosome aberrations in mammalian species, (Dhir *et al*. 1990 and Hartwig *et al*. 1990). *Gl. barbipes* is phylogenetically younger and reacts to lead influence through different structural aberrations, reported by Michailova and Belcheva (1990). Pollution effects on

phytophagous insects and chewing insects, determined by Helio¨vaara *et al.* (1991). Mutagenesis and comutagenesis by lead compounds**,** stated by Roy (1992). Lead caused developmental abnormality (Kim *et al.,* 1997). Effect of heavy metals on *Aedes aegypti* (Diptera: Culicidae) larvae. reported by Rayms *et al.* (1998). Chronic exposure to lead acetate affects the development of protein kinase C activity and the distribution of the PKCgamma isozyme in the rat hippocampus. Reinholz *et al*. (1999). Effect of magnesium ions on pathogenicity of entomopathogenic micro-organisms applied into contaminated soil. Insect,reported by Ropek and Gorczyca. (2000). Lead effects on Drosophila is reported by Hirsch *et al.* (2003).

Biological effects of heavy metals on insects, reported by Sharma and Agrawal. (2005). The effect of lead nano particle present in the leaf of Calotrphis gigantea which result in the loss of the Painted Grass Hopper of the western ghats species in India", reported by Padmadhas and Ragunathan (2009).

Electrophoresis and Proteins:

Initially electrophoretic analysis apparatus was introduced by Tiselius (1937). "The techniques used in electrophoresis for separating proteins whether alone or in combination, have proved to be very useful in the calirification of structure function relationship for protein and peptides and the complex proteome analysis", (Andrews 1986).

"Electrophoresis is the process in which the relocation of ions under the pressure of an electric field. It is broadly used for categorization of proteins, nuclic acids,peptides etc. for the diagnostic and/ or preparative unification of organic macromolecules (Laemmli 1970). Disc electrophoresis and related techniques of polyacrylamide gel electrophoresis", reported by Maurer (1971). "Free amino acid composition of proteins in the hemolymph of rice stem impliment was found affected in the fat body", reported by Chang and Chang (1974). "Inhibition of phosphattases (proteins) by shikonin and analog has been reported by Gorgees *et al.* (1978) and Rashan *et al.* (1979) in histochemical sections".

"The techniques used in electrophoresis for separating proteins whether alone or in combination, have proved to be very useful in the calirification of structure function relationship for protein and peptides and the complex proteome analysis", (Andrews 1986).Lead can induce change in some protein metabolism (Hilliard *et al.,* 1999). Influence of cadmium on growth, continued existence and grasp size of a common on Indian short horned grasshopper", reported by Chandrik *et al*. (2009).

Fruit flies:

Bactrocera dorsalis:

Herrera (1900), reported that "most of the baseline information on the biology of fruit flies comes from studies carried out at the beginning of the century. The period of relatively rapid advancement, reported by Herrera *et al.* (1910). The pupae of fruit-flies (*Ceratitis capitata)*, when exposed to 100-130 GY from a Cs-137 source, develops sterility in adult stages without showing any deleterious effect on longevity and mating behaviour of the flies (Kattyar 1962). The production of sperms during the life cycle of the three species of fruit flies *Bactrocera dorsalis*, *C.capitat*a and *Bactrocera cucurbitae*. reported by Steiner *et al.* (1962). Female attraction to methyl eugenol was first noted in field studies by Steiner *et al*. (1965). Bess *et al*. (1963), stated that, studies have indicated that 95% of the population of *Bactrocera dorsalis* develops in common guava, *Psidium guajava* L., and strawberry guava, *P. cattleianum* Sabine, and that population cycles are determined primarily by guava fruiting. Newell and Haramoto (1968), stated that, studies have indicated that 95% of the population of *Bactrocera.dorsalis* develops in common guava, *Psidium guajava* L., and strawberry guava, *P. cattleianum* Sabine, and that population cycles are determined primarily by guava fruiting. Oregon adult males were injected with lead acetate intraperitoneally", reported by Felix and Rodriguez- Arnaiz (1968).

Fitt (1981), stated that, "fruit flies Female attraction to methyl eugenol was extensively investigated. Most recent research has pointed to the importance of methyl eugenol as a pheromone precursor with proximate benefits and behaviors seen in males that acquire the compound. *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 (Kapoor and Agarwal., 1982). Bateman (1982), reported that the malathion was the usual choice of insecticide for fruit-fly control. Studies have indicated that 95% of the population of *Bactrocera dorsalis* develops in common guava, *Psidium guajava* L., and strawberry guava, *P. cattleianum* Sabine, and that population cycles are determined primarily by Vargas *et al*. (1983) in guava. *Bactrocera* are thought to have originated in the Indian block of Gondwana during the Cretaceous and Tertiary periods, where they were saprophagous, feeding on rotting fruit, reported by Munro (1984). Koyama *et al.* (1984), reported that, Methyl eugenol has subsequently demonstrated greatly useful in detection or control of the oriental fruit fly, for example, its use in several successful eradication programs. Beck and Turner (1985), Lysyk and Axtell (1985), stated that, surveillance programs for flies use a variety of sampling methods. Indoor populations can be monitored by spot cards, baited traps, or sticky tapes and cardsSix *Bactrocera* host fruits are now known to contain methyl eugenol, reported by Gaydou *et al.* (1986)."

"The melon fruit fly damage is the major limiting factor in obtaining good quality fruits and high yield (Srinivasan, 1959; Lall and Singh., 1969; Mote 1975; Rabindranath and Pillai 1986). Methyl eugenol has subsequently demonstrated great usefulness in detection and/or control of the oriental fruit fly, for example, its use in several successful eradication programs, but its attractiveness is limited to males, reported by Nakamori *et al.* (1988). In *Bactrocera dorsalis*, methyl eugenol is oxidized to 2-allyl-4,5-dimethoxyphenol and coniferyl alcohol, which are released during courtship, stated by Nishida *et al.* (1988). Lead is a pollutant heavy metal, which can be absorbed by the digestive system in a 10%, reported by Corey and Galvao (1989). Helio vaara *et al*. (1989), observed pollution induced effects include e.g reduction in pupal weight. The mechanism for the attractiveness of Alsynite to flies is uncertain, as is that for blue pigments, indicated by Zacks and Loew (1989)." "*Bactrocera* are widely distributed in tropical Asia, South pacific and Australia, with very few species in Africa and Europe, reported by Drew (1989). In fruit orchards, the use of synthetic chemicals have also caused out break of population of other insect pests like, scales on mango due to killing of their natural enemies, reported by Mohayuddin (1989). Plants rich in bioactive chemicals may provide potential alternative to currently used insect controlling

agents, plants including harmer, kuth, Balcher and neem, which carry repellent, anti-feedant qualities against stored grain insect pests, reported by Jilani *et al.* (1989). Host plant semi chemicals are particularly important in facilitating host plant location, which is the focal point for the ecology of the fly, stated by Drew (1989). *Bactrocera dorsalis* is the most abundant and widely distributed, reported by Vargas *et al*. (1990). The phytochemicals, biopesticide derived from the neem tree, *Azadirachta indica* A. Juss (Meliaceae) have been demonstrated to have insecticidal properties", determined by Schmutterer (1990).

Jilani *et al.* (1990) "have shown strong growth inhibiting effect of neem, turmeric and sweetflag oil against some major insect pests of stored grains. Fruit flies (Diptera: Tephritidae) in the subfamily Dacinae are a diverse, rapidly evolving group of more than 700 described species with a persistent effect on tropical agriculture, stated by Metcalf (1990). Para-coumaric acid from these rotting fruits is thought to have been a kairomone for ancestral dacines and also a precursor of both methyl eugenol and raspberry ketone, reported by Metcalf (1990). Lee *et al*. (1992), reported that oriental fruit fly, *Bactrocera dorsalis* (Hendel), is an important pest of fruit crops. There are no published descriptions of the morphology and ultrastructure of the alimentary canal in this fly, except for the rectal papillae. *Bactrocera* is a tephritid fly genus of 440 species distributed primarily in tropical Asia, the South Pasic, and Australia, stated by White and Elson-Harris (1992). *Bactrocera dorsalis* and *Bactrocera cucurbitae* are the major fruit fly pest species in Hawaii with 173 and 125 host plant species, respectively", reported by Metcalf and Metcalf (1992).

Fruit fly larvae in the pulp of fruit if eaten cause intestinal discomfort and diarrhea, reported by Hashim (1994). "In the case of *Anastrepha* (a Caribbean and Latin American pest), only seven (Aluja 1994) of the 197 described species are really economically important, reported by Aluja (1994) Methyl eugenol has subsequently demonstrated great usefulness in detection and/or control of the oriental fruit fly, stated by Jang and Light (1996).Yeast products are the main nutritional component in the diet used to mass-rear the adults and larvae of fruit flies in these programs, studied by Cangussu and Zucoloto (1997). Mature flies used were approximately 9-11 days old when tested and presumed to be mated (ca. > 95% of females from mixed cages are mated by day 7), stated by Jang *et al.* (1997). Data on color preference of house flies are confusing and at times contradictory, indicated by

Howard and Wall (1998). The phytochemicals, biopesticide derived from the neem tree, antifeedancy, reported by Su and Mulla (1998). A decrease survival of adult and negative effect on the development of eggs when fruit fly (*Rhagoletis indifferens*) ingested azadirachtin in food, observed by Van Randen *et al,* (1998b). Evaluated neem seed kernel extract and 7% azadirachtin against the developmental showed that high proportion of adults emerging from the treated pupae showed wing deformation preventing them from flying, reported by Hassan (1998). Incorporation of neem based insecticides in artificial larval diet resulted in a significant decrease in the formation of pupae and subsequent adult emergence of the Western fruit fly, reported by Van Randen and Roitberg (1998a). A decrease survival of adult and negative effect on the development of eggs when fruit fly (*Rhagoletis indifferens*) ingested azadirachtin in food. Longevity of *Bactrocera cucurbitae* fed continuously on sugar treated with 0.15% extract of *Acorus calamus* at 1ml/g sugar was 26.6 days than the 119.2 days for untreated flies", observed by Van Randen *et al.* (1998b).

"Female attraction to methyl eugenol was reported by Verghese (1998). Most recent research has pointed to the importance of methyl eugenol as a pheromone precursor with proximate benefits and behaviors seen in males that acquire the compound, reported by Tan and Nishida (1998). In agreement with this, six *Bactrocera* host fruits are now known to contain methyl eugenol, including the widely attacked common guava, *P. guajava*, and Strawberry guava, *P. cattleianum* Sabine, stated by Vernin *et al.* (1998). Other food-type attractants, such as hydrolyzed protein products (e.g. NuLure) and synthetic chemical blends (e.g. BioLure), are moderately attractive to both males and females fruit flies of many tephritid species, reported by Cornelius *et al.* (2000). *Bactrocera* are thought to have originated in the Indian block of Gondwana during the Cretaceous and Tertiary periods, where they were saprophagous, feeding on rotting fruit", reported by Drew and Hancock (2000).

Drew and Hancock (2000), reported that, "many *Bactrocera,* including *Bactrocera dorsalis*, are more closely associated with their hosts. The nickel-copper smelter at Monchegorsk, northwestern Russia, has been the focus of studies of pollution-induced changes in northern boreal ecosystems during the past decades, stated by Kozlov and Barcan (2000). *Bactrocera dorsalis* is generally considered polyphagous, with wide host range, they

nevertheless appear to display a strong preference for particular hosts, *T. catappa* along with *Psidium guajava* L. constituted the major hosts for *Bactrocera dorsalis* in a survey of Thailand and Malaysia, stated by Clarke *et al.* (2001). Influence of adult nutrition on male sexual performance in four neotropical fruit fly species of the genus *Anastrepha* (Diptera:*Tephritidae*), reported by Aluja *et al*. (2001). It was found a dose-dependent association between the incidence of active puffs and lead concentrations (Michailova *et al*., 2000b), along with a lot of somatic structural aberrations", reported by Michailova *et al*. (2002).

Verghese *et al.* (2002), stated that the "Oriental fruit fly (OFF), *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a direct pest on mango, Pest risk analysis suggests that *Bactrocera zonata* can establish in other countries of the Mediterranean region. The EPPO Workshop on *Bactrocera zonata* recommended that *Bactrocera zonata* should again be specified individually on the EPPO A1 list of pests recommended for regulation, which was done in 2002, in Paris".

Jiravanichpaisal *et al*. (2006), "determined the process, which is accompanied by blackening of the capsule due to melanization, leads ultimately to the death of a parasite within the capsule. Metals occur naturally in the environment in small quantities (volcanoes, erosion, spring water, bacterial activity), reported by Florea and Büsselberg (2006). The fly *Bactrocera dorsalis* is distributed in the Oriental regions of Bhutan, China, Myanmar, Thailand and India. Adults of the OFF are attracted to the para-pheromone, methyl eugenol, and several studies have shown the efficacy of methyl eugenol in monitoring and management, indicated by Verghese *et al*. (2006). Wheat germ oil (W) was used as a nutritional enhancer for fruit flies because our previous work showed that addition of wheat germ oil to a liquid diet would enhance performance, reported by (Chang and Vargas 2007). Oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) is a very destructive insidious pest of many tropical and subtropical fruits and vegetables. Predicted potential distribution of oriental fruit fly, *Bactrocera dorsalis* in Jiangxi Province, South China based on maximum entropy model" reported by Jian-Hong et al (2011).

Bactrocera zonata:

"Efficacy of methyl eugenol as a male attractant for *Bactrocera zonata* Saunders (Diptera: Tephritidae), reported by Qureshi *et al*. (1976). Fletcher (1987), stated that for most *Bactrocera* spp., it is the adults that are best able to survive low temperatures, with a normal torpor threshold of 7 ° C, dropping as low as 2 ° C in winter. *Bactrocera. zonata* , however, overwinters in the larval or pupal stage. The morphology of larva described, reported by Jabbar Khan (1987). In North America, *Bactrocera zonata* trapped in USA (California), reported by Carey and Dowell (1989). A review of the biological aspects of male lures, determined by Cunningham (1989). Qureshi *et al.* (1991), reported that *Bactrocera zonata* is polyphagous, but is particularly a pest of peach, mango and guava. It is a significant pest in India and Pakistan. Publications from Pakistan show that it is possibly more important in that country than *Bactrocera dorsalis*".). "Pest risk analysis suggests that *Bactrocera. zonata* can establish in other countries of the Mediterranean region. The EPPO Workshop on *Bactrocera zonata* recommended that *Bactrocera zonata* should again be specified individually on the EPPO".

Qureshi *et al*. (1993), "reported the investigating development of *Bactrocera zonata* at different temperatures, showed that no stages developed at temperatures of 15 ° C or under, the optimum being at 25–30 ° C. The phytochemicals, biopesticide derived from the neem tree, *Azadirachta indica* A. Juss (Meliaceae) have been demonstrated to have insecticidal properties and repellency, reported by Sharma *et al*. (1993). The *Bactrocera zonata* geographical distribution were found in Bangladesh, India (Andhra Pradesh, Assam, Bihar, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal), Indonesia (Sumatra) (probable confusion with *Bactrocera maculigera*), Iran (southern), Laos, Myanmar, Nepal, pointed out by Kapoor (1993). Originally considered as an exclusively tropical fruit fly, *Bactrocera zonata* has established in Egypt. This raises questions about its possible survival during periods of cold weather, reported by White and Elson-Harris (1992) and Iwahashi and Routhier (2001). The baited with the male lure methyl eugenol (O-methyl eugenol), which attracts male flies at very low concentrations, studied by Qureshi *et al* . (1992). Yeast

products are the main nutritional component in the diet used to mass-rear the adults and larvae of fruit flies in these programs", studied by Cangussu and Zucoloto (1992). "*Bactrocera. zonata* since recorded for the first time in Egypt it caused a severe damage to a wide range of fruits including guava, peach, apricot and mango, reported by El-Minshawy *et al*. (1999). FAO/IAEA, 2000, reported the FAO/IAEA peach fruit fly action plan gives very detailed indications on trapping for *Bactrocera zonata*. Cadmium, copper, zinc and nickel are known to accumulate in insect herbivores as they consume metal exposed leaves (Lindqvist, 1992 and Kozlov *et al*., 2000b)". "Pest risk analysis suggests that *Bactrocera. zonata* can establish in other countries of the Mediterranean region. The EPPO Workshop on *Bactrocera zonata* recommended that *Bactrocera zonata* should again be specified individually on the EPPO".

Bactrocera cucurbitae:

" *Bactrocera cucurbitae* (Coquillett) is a major threat to cucurbits (Shah *et al*., 1948). Their Population buildups are associated primarily with fruiting of wild and cultivated, reported by Nishida (1953). The extent of damage lies between 30 to 100%, depending on the cucurbit species and the season. Its abundance increases when the temperatures fall below 32° C, and the relative humidity ranges between 60 to 70%. It prefers to infest young, green, soft-skinned fruits. It inserts the eggs 2 to 4 mm deep in the fruit tissues, and the maggots feed inside the fruit, eggs are also laid in the flower's corolla, and the larvae feed on the flowers. Some time larvae feed on the branches (Narayanan 1953). *Bactrocera cucurbitae* is the 2nd most abundant and widely distributed fruit fly species. Population buildups are associated primarily with fruiting of wild and cultivated cucurbits, reported by Harris *et al*. (1986)., reported that *Bactrocera cucurbitae* is the 2nd most abundant and widely distributed fruit fly species. Population buildups are associated primarily with fruiting of wild and cultivated cucurbits reported by Nishida (1953); Harris *et al*. (1986); Vargas *et al*. (1990), 1990; Uchida *et al*. (1990). *Bactrocera cucurbitae* is the 2nd most abundant and widely distributed fruit fly species. Population buildups are associated primarily with fruiting of wild and cultivated cucurbits, reported by Uchida *et al*. (1990). *Bactrocera cucurbitae* are the major fruit fly pest species in Hawaii with 173 and 125 host plant species, respectively,

reported by Metcalf and Metcalf (1992). Longevity of *Bactrocera cucurbitae* fed continuously on sugar treated with 0.15% extract of *Acorus calamus* at 1ml/g sugar was 26.6 days than the 119.2 days for untreated flies, observed by Van Randen *et al.* (1998b). Ovipositional deterrence of *Acorus calamus* L. on melon fly, *Bactrocera cucurbitae,* reported by Shakunthala and Thomas (2001).

Drosophila melanogaster:

Haramoto and Bess, (1970), studied "the mutagenic effects of two lead salts lead acetate and nitrate on sperm *of Drosophila melanogaste*". Lower *et al.***,** (1976), reported that *Drosophila melanogaster* lead induced enzymatic alterations in esterase and triose phosphate isomerase. Heavy metal resistance is evidently a widespread phenomenon in invertebrates (Diptera) and particularly in *Drosophila* are the model organisms exposure to cadmium, reported by Maroni *et al*. (1987). Heavy metal resistance is evidently a widespread phenomenon in invertebrates particularly in *Drosophila* are the model organisms exposure to cadmium", reported by Gill *et al.* (1989). "The *Drosophila melanogaster* lifespan is about 30 days at 29 °C (84 °F).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 (77 °F) is 8.5 days, the eggs, which are about 0.5 millimetres long, hatch after 12–15 hours (at 25 °C (77 °F). The eggs, which are about 0.5 millimetres long, hatch after 12–15 hours (at 25 °C (77 °F)". (Ashburner 1978 and Ashburner et al., 2005).

Musca domestica:

"House flies (*Musca domestica)* are cosmopolitan, ubiquitous, and are the vectors of more than 100 human and animal intestinal diseases" (Scott and Lettig, 1962, Greenberg, 1965). Thus, "he houseflies have been reported to disseminate numerous diseases such as typhoid and paratyphoid fevers, bacillary dysentery, cholera, hepatics carbuncles, bovine mastitis, conjunctivitis and poliomyelitis" (Greenberg, 1970, 1973; Gough and Jorgenson, 1983). Farnham *et al*., (1984), "stated that control of *Musca domestica* and *S. calcitrans* is mainly based on insecticides applied as baits, space or residual sprays, and larvicides".

"Extensive surveys of natural enemies attacking *Musca domestica* have been reported by Legner (1966). In Europe, only a few investigations have dealt with pupal parasitoids which attack *Musca domestica* and *S. calcitrans* populations, Three species of pupal parasitoid from *Musca domestica* puparia in outdoor manure heaps, reported by Mourier and Hannine (1969). Mourier (1971), reported that, in Europe, only a few investigations have dealt with pupal parasitoids which attack *Musca domestica* and *S. calcitrans* populations. Study has identified several new pupal parasitoids on *Musca domestica* and *S. calcitrans* compared with earlier studies in Denmark, Suppression of *Musca domestica* populations below annoyance levels failed in spite of late-season parasitism rates of 80% at the treated sites, reported by Mourier (1972)". "Widelyspread of natural enemies attacking *Musca domestica* and *S. calcitrans* have been reported by Rueda and Axtell (1985a). Keiding (1986), reported that House flies (*Musca domestica*) are international, everywhere, and are the vectors of more than 100 human and animal intestinal diseases. Flies *Musca domestica* transmit eye diseases such as trachoma and epidemic conjunctivitis, and infect wounds or skin with diseases such as cutaneous diphtheria, mycoses, yaws and leprosy". "House flies are vectors of a wide range of food-borne and other pathogens including *Escherichia coli* and *Shigella* spp, determined by Cohen *et al*. (1991). Extensive surveys of natural enemies attacking *Musca domestica* and *S. calcitrans* have been conducted, reported by Meyer *et al*. (1991). The housefly (*Musca domestica*) as a vector for emerging bacterial enteropathogens, reported by Nayduch and Stutzenberger (2000). Effect of age and sex on the sensitivity of antennal and palpal olfactory cells of houseflies, reported by Kelling *et al*. (2003). House flies pass with complete metamorphosis such as egg, larva or maggot, pupa and adult stages. Under suboptimal condition the life cycle require up to two months. The adults are 8- 12 mm long, the thorax is gray with four longitudinal dark lines on the back, the abdomen is slightly yellow, whole body cover with hair like projection. The female is larger than male and larger space between their compound eyes, flies have one pair of wings. The adult fly lives around two weeks to one month. Fly can lay approximately 500 eggs in several batches eggs are 1.5mm in length, with in a day larvae hatches from the eggs, feed on garbage and feaces and the adult as well. Nevertheless, the houseflies are the important mechanical and biological vectors for various pathogenic agents. These pathogens are transmitted on the fly's cuticle, proboscis, by regurgitation or through its faeces "(Fotedar *et al*., 1992b; Senna-Nunes *et al*.,

2002; Banjo *et al*., 2005).Toxicity, growth regulatory and repellent activities of medicinal plant extracts on *Musca domestica* L. (Diptera: Muscidea)", reported by Herve *et al*. (2008).

MATERIALS AND METHODS

MATERIALS AND METHODS:

Initial strains of the test materials, *Bactrocera dorsalis* and *Bactrocera zonata* were procured from the Plant protection and Diagnostic Laboratory, DPP, Karachi. The oviposited mangoes with *Bactrocera dorsali* and *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 (Huiye and Jian-Hong liu 2005). The newly emerged 3rd instar larvae were collected in Petri dishes for the treatments. three batches of bottles were prepared with 3 gram feed of mangoes and bananas pulp mixed with lead acetate in the desired concentration, of 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 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 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 (Huiye and Jian-Hong liu 2005). 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.

A colony of *Musca domestica* L. was started from the pupae obtained from Pakistan Council of Science and Industrial Research (PCSIR) laboratories at Karachi. The insect was reared, following the method of (Ashrafi *et al*., 1966), in cages (42x 32x 32 cms.) having 20- mesh screens on the sides and top. The bottom of the cages were made of hard boards. A lateral opening, at one side of cage, was provided with long sleeves of muslin cloth.The

temperature and relative humidity was maintained at 29 ± 2 ^{o}C and 60 ± 10% R.H. respectively respectively.

Larvae were grown on these diets for 8 days, then removed from the medium and allowed to pupate in covered glass bottles at 29-30 °C. The newly emerged 3rd instar larvae were collected in Petri dishes for the treatments. The newly emerged 3rd instar larvae were collected in Petri dishes for the treatments. Insects were treated as batches of bottles with 3 grams bananas mixed with lead acetate were used as diet, doses of lead acetate were used as, 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg. 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. Surviving larvae were kept in separate bottles on lead free bananas upto pupal formation. During that period pupation and adults effects of lead acetate of different concentration was observed on different flies.

Drosophila melanogaster flies were reared in culture bottles containing standard medium. Eggs were collected from these flies on fermenting fresh baker's yeast supplemented with sucrose (Graf *et al*., 1991). After removing the parental flies, the egg collection bottles were kept for development. Three days later, the 72 hour larvae were collected. Immediatly after that, the larvae were transfered to small bottles (10 larvae/bottle). Three bottles were prepared with 3 grams bananas, mixing with lead acetate as diet purpose for each concentration, as, 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg and three bottles as control, 10 larvae were released in each bottle for 48 hours. After that noted the mortality of larvae in each bottle. The live larvae were transfered in seperate bottles on pure feed of bananas for pupation and adults emergence, after that, the effect of lead acetate on different concentration has been noted on different flies. For the study of effects of lead acetate on protein, the following process was followed.

A disc electrophoreses apparatus was employed for the purpose. Tube of electrophoresis apparatus were made clean and rinsed with water followed by ethonal and then air dried, after that, the bottom mouth of the tubes were closed with rubber stopper. Then, 10 ml resolving gel solution was prepared with the described ingredient solution components (Table B). There after, the capillaries tubes were filled by the mix solution and

then added the 0.1ml. ammonium persulfate and 0.008 ml. TEMED in capillaries tube and then left the apparatus for 4-5 hours, for polymerization.

After polymerization of the Gel, 200µl. sample was poured in the tube with Bromophenol solution 100 µl. The above and lower mouth of capillaries tube were dip with the Reservoir Buffer solution, for one day, and kept under 110 voltage current for one day On the second day Buffer solution were removed and then the Gel was extracted from the capillaries with the help of water pressure through syring, in separate petri dishes, after that the Gel was treated eith coomassic blue solution, for 2 hours, then after colorization of Gel. Destaining solution was used for decolourization of the Gel and then observed the bands of proteins were observed. (Reagents, chemicals,prepration of solutions and Gel are shown in Table A,B,C). The observed rf of various protein bands are presented in (Table 6—11).

RESULTS

RESULTS:

Mortality effect of lead acetate on *Bactrocera dorsalis, Bactrocera zonata, Bactrocera cucurbitae,Musca domestica and Drosophila melanogaster* (3^{rd} instar larvae) at post 48 hours exposure period, indicated in (Table: I,2,3,4,and Table5 respectively).

The mortality curve of *Bactrocera dorsalis* (3^{rd} instar larvae) at post 48 hours exposure (Curve 1) was plotted at probit log mortality graph paper. The obtained curve indicated that the LD_{50} as 0.19 mg. At the doses less than 0. 19 mg of lead acetate developed abnormalities on physiological and morphological characters in adult stages, such as, folded legs, curved spotted wings, and more melanization. Whereas in *Bactrocera zonata* the mortality curve at probit log mortality graph paper (Curve 2), showed LD_{50} as 0.150 mg of lead acetate. The dose less than 0.150 mg. caused abnormal effect on physiological and morphological characters of adults, as well.

In normal condition, *Bactrocera dorsalis* larvae were found possessing elongated cylindrical body, fusiform in shape and creamy white in color. A band of spinules encircled the body on the first body segment is found. Respiratory organs, the spiracles were found located one on each side of the head of the larvae. The caudal segment was smooth, the posterior spiracles were found located on third segment. It has a body length of 10 mm (FigI).

Larvae treated,with lead acetate, 0.125 mg at 48 hours exposure,were elongated cylindrical shape, anterior end narrow and curved ventrally, with anterior mouth hooks, ventral fusiform in shape, and flattened caudal end, 9 mm. in length, segmented marks on whole body morphologically changed structure and more melanization was observed (Fig II).

Larvae treated with lead acetate, 0.25 mg at 48 hours exposure, were found same dull white, slightly segmented, curve at middle portion of the body, deep contraction at middle portion of the body, 7 to 9 mm. in length. Toxic effect of lead acetate as morphological changed in shape, structure and melanization is shown in (Fig III).

Larvae, under the effects of lead acetate 0.5 mg at 48 hours exposure were found with dull white elongated cylindrical body, pointed at anterior end and flattened at posterior end, curved at anterior portion of the body. The body length was 10 mm. No prominent change in larvae body was observed.(Fig IV).

Larvae treated with lead acetate 1.0 mg and 2.0 mg respectively, at 48 hours exposure were dull white elongated cylindrical body, pointed at anterior end and flattened at posterior end, slightly segmented body, 7 to 10 mm. in length, No morphology change was recorded on larvae body in this case as well. (Fig V, VI).

Normal *Bactrocera dorsalis* pupae were observed as a capsule like structure, elastic cover, body length 4mm. yellowish brown pigmentation, slightly segmented body (Fig VII).

Larvae, treated with lead acetate, 0.125 mg and at 48 hours exposure, formed pupae yellowish brown, slightly elastic, 3 to 4 mm in length. Morphological change appeared in the shape of a pointed projection at one end. (Fig VIII).

Pupae obtained from treated larvae with lead acetate 0.25 mg at 48 hours exposure formed pupae were, redish yellowish dark brown, capsule shape, slightly elastic, 4 to 5 mm. in length, showing toxic effect on the structure of pupa body and changed melanization. (Fig IX).

Pupae obtained from the larvae effected with, lead acetate 0.5 mg at 48 hours exposure yellowish brown, abnormally, rough surface, pointed at one end, contracted at middle portion, 4 to 5 mm. in length, seemed abnormal in shape, with slightly abnormalities on structure, and melanization (Fig X).

Pupae treated with, lead acetate 1.0 mg at 48 hours exposure were with same yellowish brown pigmentation. Slight abnormalities appeared on shape, rough surface, pointed projection at one end. Body length 4 mm (Fig XI).

Pupae effected with lead acetate 2.0 mg at 48 hours exposure were found same pigmentation as above yellowish, slightly segmented capsule shape, 4 to 5 mm. in length. Morphologically slightly changed structure and melanization was observed. (Fig XII).

Adult normal fly *Bactrocera dorsalis* was observed as in a triangular shaped body, 8 mm in length. The wing were about 7.3 mm. in length, yellow, greenish brown pigmentation, black marking on the thorax, the abdomen has two horizontal black stripes and a longitudinal median stripe extending from the base of the third segment to the apex of the abdomen with two scape and pedicel red-brown, antennae on head. (Fig XIII).

Adult developed from the treated larvae with lead acetate, 0.125 mg, at 48 hours exposure were found with elongated body, about 8 mm. in length, expanded transparent wings with a slight cutting at the edges, yellowish dark brown pigmentation, folded legs, dark brown eyes, physical changes on curved wings and folded leg were prominent. (Fig XIV).

Adult developed from effected larvae with lead acetate, 0.25 mg, at 48 hours exposure were with 8 mm. in body length, expanded transparent wing, cutting at the edges, black strips on abdomen, yellowish green brown body pigmentation, folded legs, dark brown eyes (Fig XV).

Adult developed from the treated larvae with lead acetate, 0.5 mg, at 48 hours exposure were found with abnormal body in shape, about 8 mm. in length, expanded transparent wing, cutting at the edges, folded legs, dark brown eyes, specimen with lengthy wing was also obtained. (FigXVI).

Adult developed from the treated larvae with lead acetate, 1.0 mg, at 48 hours exposure were found with a body length of 8 mm., transparent wing, dark body pigmentation and black marking on thorax as above, folded legs, eyes dark brown melanized, Changed morphological appearance on body structure and wings. (Fig XVII).

Bactrocera dorsalis (Adult stage) effected with lead acetate, 1.0 mg at 48 hours. Showed toxic effect as enlarged wings and folded legs. Adult through the larvae effected with lead acetate, 2.0 mg, at 48 hours exposure were slightly abnormal body shape, about 7 mm. in length, veinleted transparent wing, black strips on thorax, folded legs, with dark brown malanized eyes, in most of the cases abnormal physical appearance was observed, absence of one wing and elongated legs were the marked abnormality in the most cases. (FigXVIII).

Bactrocera zonata, normal Larva was observed as elongated body, fusiform in shape, pointed at anterior end, broad at posterior end, slightly segmented body. creamy white color, anterior spiracle, mouth hook present, creeping welt present for movement. 7 to 10 mm in length (FigXIX).

Larvae treated with lead acetate, 0.125 mg, at 48 hours exposure were observed as dull white creamy boby, abnormal in shape, curved at anterior and posterior end, rough surface, 5 to 6 mm. in length, showing hazardrous effects on shape and structure of larvae. (Fig XX).

Larvae treated with lead acetate, 0.25 mg at 48 hours exposure, elongated smooth shiny body, slightly segmented, pointed at anterior end, 5 to 7 mm. in length. Toxic effect showing structural abnormalities and melanization (Fig XXI).

Larvae treated with lead acetate 0.5 mg, at 48 hours exposure were found as elongated straight, slightly shiny body, pointed at anterior end and flattened at posterior end, 7 mm. in length. Slightly abnormalities on body structure. (Fig XXII).

Larvae effected with lead acetate 1.0 mg, at 48 hours exposure were, 8 to 10 mm. in length, cylindrical body, broader at one end and tapering at other end. Segmented body, dull white colour were also observed. (Fig XXIII).

Larvae treated with lead acetate 2.0 mg., at 48 hours exposure were 7 to 8 mm. in length. Abnormalities appeared on curved larvae, rough surface and melanization. (Fig XXIV).

Pupa in normal condition was observed as yellowish brown in colour, 4 to 5 mm. in length, elastic body cover rounded and slopy at both the end, slightly segmented body, small capsule shape. body length 5 mm. (Fig XXV).

Larvae effected with lead acetate, 0.125 mg at 48 hours exposure were dark melanization, dumbly shape, slightly elastic cover, 5 mm. in length, with a pointed projection at one end, toxic effect of lead acetate showed abnormalities in structure, with head projection at one end. (Fig XXVI).

Pupae obtained from the treated larvae with lead acetate 0.25 mg at 48 hours exposure were dumbly shaped, yellowish brown in color, slightly elastic body, 5 mm in length. An abnormal structure with spotted body was also observed. (Fig XXVII).

pupae from larvae effected with lead acetate 0.5 mg at 48 hours exposure were with a slightly effected body shape, 5 mm. in length, morphologically changed body structure. (Fig XXVIII).

Pupae, formed from effected larvae with lead acetate 1.0 mg at 48 hours exposure, were abnormal in shape, spotted body with a body length 4 to 5 mm. Dark brown malanization and abnormal shape with spotted body. (Fig XXIX).

Larvae treated with lead acetate 2.0 mg at 48 hours exposure, pupae were slightly in abnormal shape, with changed melanization as brown in colour and spotted body, 4 to 5 mm in length. (Fig XXX).

Bactrocera zonata adult in normal condition were observed as redish brown in colour, marking on abdomen and thorax, transparent expanded wings, with a small brown spot on the tip of each wing a pair of dark marks on tergite III. Body length 8 mm. and wings are 7 mm. in length. Two spots were found present as one in each furrow, just above the

mouth. The antennae were scape and pedicel red-brown. They were found shorter than the vertical length of the head. (Fig XXXI).

Larvae effected with lead acetate, 0.125 mg at 48 hours exposure formed adult with slightly segmented marking on abdomen, dark body colour, blakish brown marking, 8 mm. in length, cut edges of wing showed the abnormal effect of lead acetate, as developed abnormalities on wing edges, and reduced abdomen. (Fig XXXII).

Bactrocera zonata Adult effected with lead acetate developed from 0.25 mg. at 48 hours exposure, marking bands on abdomen, blakish brown marking lines on thorax, transparent veinleted wings, antenna on head, red brown body colour, redish black eyes, 8 mm. in length,abnormalities appeared on wings.Notoxication recorded. (Fig XXXIII).

Adult, formed from effected larvae with lead acetate, 0.5 mg at 48 hours exposure, werered brown body color, body length 7 mm., abnormal wing structure and, folded legs. (Fig XXXIV).

Bactrocera cucurbitae normal larva were found as cylindrical maggot shape, anterior portion narrow, with pointed mouth hook, slightly curved at anterior end, elongated fusiform in shape and flattened caudal end,7 to 10 mm. , in length, anterior spiracles slightly convex, dull white colour, segmented creeping welts supporting movement. (Fig. XXXV).

Bactrocera cucurbitae larvae treated with lead acetate 0.125 mg., at 48 hours exposure were found as cylindrical rough shape, dull white brown in colour, anterior portion narrow, with pointed mouth hook, slightly curved at anterior end, slightly rough surface, elongated fusiform in shape ,7 to 10 mm. in length, slightly abnormal structure with pointed projection, curved, swollen and shrinkake body (Fig. XXXVI).

Bactrocera cucurbitae larval effected with lead acetate 0.25 mg., at 48 hours exposure were formed as were formed as elongated cylindrical fusiform shape, dull white brown melanization, anteriorly narrow, slightly curved at anterior end, rough surface, posteriorly broad end, elongated, body length 7 mm.. Abnormally curved body and rough shrinkage structure (Fig. XXXVII).

Bactrocera cucurbitae larvae effected with lead acetate 0.5 mg., at 48 hours exposure, were found elongated cylindrical rough shape, dull white brown in colour, anteriorly narrow and posteriorly broad, curve at middle portion, rough surface, fusiform in shape, 5 to 9 mm. in length. abnormal structure with rough and body (Fig. XXXVIII).

Bactrocera cucurbitae larvae treated with lead acetate 1.0 mg., at 48 hours exposure, were found as elongated cylindrical body, maggot shape, dull white brown in colour, rough surface, fusiform in shape, 8 to 10 mm. in length. Abnormal curve structure (Fig. XXXIX).

Bactrocera cucurbitae larvae were treated with lead acetate 2.0 mg., at 48 hours exposure were found as elongated cylindrical body, dull white brownish in colour, slightly rough surface, fusiform in shape, 8 to 9 mm. in length. abnormal structure, rough surface, shrinkage body and changed colour. (Fig. XL).

Bactrocera cucurbitae normal pupa were found as 6 mm. in length, ringed by narrow yellow bands, around each segments, elliptical and dull white, yellowish brown in colour, slightly shining (Fig. XLI).

Bactrocera cucurbitae pupae effected with lead acetate, 0.125 mg. at 48 hours exposure were found as ringed by narrow yellow bands, around each segments, elliptical and dull white, yellowish brown in colour, slightly shining, 6 mm. in length, Physical appearance shows abnormalities in shape and structure (Fig. XLII).

Bactrocera cucurbitae pupae treated with lead acetate, 0.25 mg. at 48 hours exposure, were found as ringed by narrow yellow bands, around each segments, elongated, yellowish dark brown melanization, body length 5 mm., physical appearence changed melanization (Fig. XLIII).

Bactrocera cucurbitae pupae effected with lead acetate, 0.5 mg. at 48 hours exposure were ringed by narrow yellow brown bands, around each segments, capsule shape yellowish dark brown melanization, slightly abnormal shape, 5 mm. in length, second and forth indicated abnormalities in structure and shape and changed melanization (Fig. XLIV).

Bactrocera cucurbitae pupae treated with lead acetate 1.0 mg. at 48 hours exposure, were found as ringed by narrow yellow brown bands, around each segments, body swollen at middle and anterior end, dull white yellowish brown colour, 4 to 6 mm. in length, physiological abnormalities appeared on pupa no. first and fourth in shape and melanization. (Fig. XLV).

Bactrocera cucurbitae pupae treated with lead acetate, 2.0 mg. at 48 hours exposure, were ringed by narrow yellow brown bands, around each segments, dull white yellowish brown colour, narrow at one end and slightly broad at other end, 5 mm. in length., no toxicities appeared. (Fig. XLVI).

Bactrocera cucurbitae Adult in normal condition were body length 8 mm., long third antennal segment, the dorsum of the thorax reddish yellow with light yellow markings, and the head yellowish with black spots, head and eyes are dark brown, yellowish brown body with a yellow spot above the base of the first pair of legs, yellow stripe, with curved lines on either side, transparent wings and thick brown band extending along the leading edge, brown spot at the tip. brown spoted at wing margin. Abdomen reddish yellow with bands on the second and third abdominal segments, moveable head, yellowish legs. Body length 8 mm. (Fig. XLVII).

Bactrocera cucurbitae Adult developed from effected larvae with lead acetate 0.125 mg. at 48 hours exposure were thorax redish yellow, with light yellow markings, abdomens are reddish yellow with light black bands on the second and third abdominal segments, the pointed projection at abdominal end, wings are transparent,veinleted, about 6 mm. in length, elongated folded legs, head and eyes are dark brown melanized, 8 mm. in length. Abnormalities appeared on spoted wing and elongated legs. (Fig. XLVIII).

Bactrocera cucurbitae Adult emerged from effected larvae with lead acetate 0. 25 mg. at 48 hours exposure, were found as wings are transparent,veinleted and curved, head and eyes are dark brown melanized thorax light redish yellow, abdomens are brown yellow with light black bands on the second and third abdominal segments, small antena on head

about 6 mm. in length, elongated folded legs, 8 mm. in length, abnormalities appearedon curved wings and on abdomen. (Fig. XLIX).

Bactrocera cucurbitae Adult effected with lead acetate 0.5 mg. at 48 hours exposure, were found as thorax brown yellow, with light yellow markings, abdomens are reddish brown yellow with light black bands on the second and third abdominal segments, wings were transparent, spoted and veinleted, about 6 mm. in length, elongated folded legs, head and eyes are reddish dark brown melanized, 8 mm. in length. no toxicities appeared. (Fig. L).

Bactrocera cucurbitae Adult developed from effected larvae with lead acetate 1.0 mg. at 48 hours exposure, were found as thorax brown yellow, with light yellow markings, abdomens were found as reddish brown yellow with black bands wings were elongated, 7 mm. in length transparent, spoted and veinleted, elongated folded reduce legs, head and eyes are reddish brown in colour, 8 mm. in length. Abnormalities appeared on elongated expanded wings. (Fig. LI).

Bactrocera cucurbitae Adult effected with lead acetate 2.0 mg. at 48 hours exposure, wings were found as elongated transparent,veinleted and curved, head and eyes were reddish brown melanized thorax light brown yellow, abdomen is light brown yellow with light black bands on the second and third abdominal segments, small antena on head about 6 mm. in length, folded legs, 8 mm. in length. no toxicities appeared. (Fig. LII).

Musca domestica larvae in normal condition. Were observed up to 3 to 9 mm long, typical creamy whitish in color, cylindrical but tapering toward the head. The head contains one pair of dark hooks. The posterior spiracles are slightly raised and the spiracular openings are sinuous slits which are completely surrounded by an oval black border. (Fig. LIII).

Musca domestica effected larvae with lead acetate 0.125 mg. at 48 exposure hours were found that the abnormal larva was thick on at one end and tapper from other end. The middle portion of larvae was dull white dark brown and malanized at proximal end, elongated cylindrical body with fusiform in shape slightly curved in middle portion, 7 to 9 mm. in length (Fig. LIV).

Musca domestica treated with lead acetate 0.25 mg. at 48 hours. the larvae were thick in at posterior end and tapper from anterior other end, curved at middle portion dull white at anterior end, elongated fusiform in shape, 8 to 9 mm. in length (Fig. LV).

Musca domestica treated with lead acetate 0.5 mg.at 48 hours, were found as in elongated body, thick at one end and thin at other end, fusiform in shape, dark brown melanized, were abnormal in shape, curved body and rough surface, 6 to 7 mm. in length, seem morphologically abnormal shape, colour and condition (Fig. LVI).

Musca domestica treated with lead acetate 1.0 mg. at 48 hours,treated larvae were dark brown at the tip of the tapering end, middle portion is yellowish brown, anterior end dark brown about, 6 to 10mm. in length, stuff and curved cylendrical body seemed abnormal condition in shape, size and colour (Fig. LVII).

Musca domestica (larval stage), treated with lead acetate, 2.0 mg. at 48 hours, were rough cylindrical body, anterior and posterior end are drak brown, middle portion of the body was dark brown malanized, slightly curved body, 8 to 12 mm., in length, physiologically and morphologically was found abnormal condition in shape, structure and colour (FigLVIII).

Musca domestica in normal condition. The pupal was found as 8 mm. in length varies in color from dark to yellow, red brown, up to black as per pupa ages. The shape of the pupa was bluntly round at both ends (Fig. LIX).

Musca domestica effected pupae with lead acetate 0.125 mg., at 48 hours exposure were found as blakish drak brown,narrow at anterior end, some time immature dead fly was found inside the puparium, usually reduced 5 to 6 mm in length, pupae were found (Fig LX).

Musca domestica effected pupae with lead acetate 0.25 mg. at 48 hours exposure larvae, were dark brown malanization, dumble shape, segmented body, 6 to 8 mm in length (Fig. LXI).

Musca domestica pupae formed from the treated larvae with lead acetate 0.5 mg., at 48 hours,found abnormal in shape, elongated pointed projection at both the end, melanization dark brown, rough surface, 4 to 5 mm in length, morphologically seemed abnormal structure in shape, size and melanization (Fig. LXII).

Musca domestica (Pupal stage) effected with Lead acetate 1.0 mg. at 48 hours, dark brown malanization elongated cylindrical and spoted body, rounded at one end and tapper at other end, about 7 mm in length, seemed projection of inborn fly at one end, prohibited with the cover of pupal capsule, showing morphologically abnormal structure and melanization (Fig. LXIII).

Musca domestica (Pupal stage), were treated with Lead acetate 2.0 mg.at 48 hours. Larvae gave pupa those seem to be segmented brownish capsule,dumble shape, seemed inborn fly inside the pupal cover, leg and head projection could be seen, over the surface of pupa. 5 to 8 mm in length, slightly pointed at one end over all showing the morphological change in structure and melanization (Fig. LXIV).

Normal adult *Musca domestica* were found as with brown reddish eyes, sponging mouth part, thorax bears four narrow black strips, the abdomen was with yellow to gray dark lines and dark marking on the sides, transparant veinlated wings,7 mm. in length (Fig.LXV).

Musca domestica Adult emerged from the treated larvae with lead acetate 0.125 mg at 48 hours exposure were morphologically were abnormal in shape and structure, wing with cut at proximal end, abnormal legs, red compound eyes, light black thorax, dull white abdomen was found, they were,7 mm. in length (Fig. LXVI).

Musca domestica Adult developed from lead acetate 0.25 mg. treated larvae at 48 hours exposure were found with folded legs, transparent wing with less venation , back of thorax was found with three longitudinal lines, abdomen dull white and 7 mm. in length (Fig. LXVII).

Musca domestica Adult emerged from the treated larvae with lead acetate 0.5 mg. at 48 hours exposure. A pair of wide transparent curved wing with venations, legs were folded

and thick, reduce dull white yellowish abdomen, thorax is blakish gray with five longitudinal lines, dark red brown eyes, 9 mm. in length. toxic effect were appeared as curved wing and legs were formed as well (Fig.LXVIII).

Musca domestica Adult developed from effected larvae with lead acetate 1.0 mg., at 48 hours exposure . A pair of veinleted transparent wings, slightly pointed end posteriorly rounded abdomen with dull white appearence, blakish gray thorax, with 4 longitudinal lines, dark red brown compound eyes, black thick folded legs, 8 mm. body length, morphologically abnormal wing structure (Fig. LXIX).

Musca domestica Adult formed from lead acetate 2.0 mg, at 48 hours exposure larvae were physical appeared abnormal, with one wing projection, and reduced oval abdomen, yellowish dull white malanization, back of the thorax is dark gray, folded and curved thick legs, dark red brown compound eyes, body length 7 mm. abnormal structure of one wing (Fig. LXX).

Drosophila melanogaster larvae in normal condition, were yellowish white cooler, wide at anterior and tapper at posterior end, with a pair of hooks in their mouth for digging and tearing food, anterior and posterior (rear) spiracles, which are openings for their breathing tubes, body length 5 mm., light brown at one end and dull white appearance at other end (Fig. LXXI).

Drosophila melanogaster larval treated with lead acetate, 0.125 mg at 48 hours were found as slightly yellowish black colour, broad at one end and tapper at other end, slightly curved and rough body, small toxic effect on larval structure. (Fig. LXXII). Larvae treated with lead acetate, 0.25 mg. at 48 hours exposure were yellowish dull white malanization, slightly curve shape, deep constriction on larval body, physical appearance slightly abnormal.(Fig. LXXIII). Larvae effected with lead acetate 0.5 mg. at 48 hours exposure were rod shape, yellowish dull white colour, slightly abnormal structure. (Fig. LXXIV). Larvae treated with lead acetate 1.0 mg. at 48 hoursexposure, elongated sloping shape, dull white yellowish color, Pigmented slightly curved body. (Fig. LXXV). Larvae

effected with lead acetate 2.0 mg. at 48 hours exposure were found as slightly curved body, slightly toxic effect on body structure and dark melanization (Fig. LXXVI).

Drosophila melanogaster pupae, in normal condition, pupa found to be tapper at the both, broader at middle portion, cuticle (skin) slightly elastic, body length 4 mm (Fig. LXXVII).

Drosophila melanogaster pupae formed from treated larvae with lead acetate, 0.125 mg. at 48 hours. Showed minute toxic effect on body structure.(Fig. LXXVIII). Pupae formed from treated larvae with lead acetate 0.25 mg. at 48 hours exposure were brown yellowish or dark melanization, slightly abnormal physical appearance on structure and melanization (Fig. LXXIX).

Pupae effected with lead acetate 0.5 mg. at 48 hours exposure larvae were, slightly hard cuticle, they were in slightly changed structure and pigmentation. (Fig. LXXX). Pupae formed from treated larvae with lead acetate 1.0 mg. at 48 hours exposure were slightly change in morphological structure and melanixation. (Fig.LXXXI). Pupae effected with lead acetate 2.0 mg. at 48 hours exposure, abnormal projection at one end, malanization dark brown, physically slightly abnormal structure and rough surface (Fig. LXXXII).

Drosophila melanogaster, Normal adult, brick red eyes, yellow brown colour, transverse black ring, across their abdomed. Veinleted expanded transparent wings (Fig. LXXXIII).

Drosohpila melanogaster, adult formed from the larvae effected with lead acetate, 0.125 mg. at 48 hours exposure. Change in physical appearance on wing and legs. (Fig. LXXXIV). Adult formed treated larvae with lead acetate, 0.25 mg. at 48 hours exposure were elongated legs, transparent curved wings and yellow green body, slightly effected. wings and swollen abdomen. (Fig. LXXXV). Adult formed from effected larvae with lead acetate 0.5 mg. at 48 hours exposure were, slightly abnormal wing and curved abdomen (Fig. LXXXVI).

Adult emerged by effected larvae with lead acetate, 1.0 mg. at 48 hours exposure, curve down abdomen, red brown eyes, folded curved legs, slightly changed abdomen and folded legs. (Fig. LXXXVII). Adult formed from effected larvae with lead acetate, 2.0 mg. at 48 hours exposure were with upside curved wing, abdomen with abnormal projection. folded legs.Changed physical appearence on curved wing and abdomen (Fig. LXXXVIII).

The effect of lead acetate on proteins of five species of dipterious flies is shown in Table: 6, in this respect five species. *Bactrocera dorsalis, Bactrocera zonata, Bactrocera cucurbitae, Drosophila melanogaster* and *Musca domestica* protein were studied in comparision with Egg albumin as a reference protein. The rf. of Egg albumin was found as 0.04, the rf. of various proteins obtained from *Bactrocera dorsalis* (untreated control) were found to be 0.03, 0.05, 0.21 ,0.34,0.43,0.58,0.63, 0.72, 0.87 and 0.97. As compared the rf. with *Bactrocera dorsalis* (treated), It was found to be 0.04,0.06, 0.09, 0.22,0.33,0.44, 0.56, 0.75, 0.87, 0.95. Compared with *Bactrocera zonata* (untreated control), the values were found to be 0.07,0.16, 0.26,0.33,0.43, 0.56, 0.66, 0,78,0.87, 0.93 respectively. The rf. of protein *Bactrocera zonata* (treated) were found to be 0.05, 0.08, 0.15, 0.32,0.46, 0.56, 0.65, 0.72, 0.81, 0.92. in Comparison the rf. Of various proteins of *Bactrocera cucurbitae* (untreated control), were found to be 0.08, 0.15, 0.24, 0.34,0.47, 0.56, 0.65, 0.72, 0.84, 0.93, however; the rf. protein of *Bactrocera cucurbitae* (treated) reflected the values as 0.03, 0.12, 0.31, 0.36, 0.56, 0.65, 0.70, 0.79, 0.89, 0.98. As compared the rf. with *Drosophila melanogaster* (untreated), the values were found to be 0.09, 0.23, 0.32, 0.43, 0.50, 0.64, 0.73, 0.78, 0.87, 0.93. The rf proteins of *Drosophila melanogaster* (treated) showed the values as 0.05, 0.14, 0.22, 0.30, 0.42, 0.50, 0.61,0.76,0.86,0.94. The rf. of the protein of *Musca domestica* (untreated) were found to be 0.03, 0.08, 0.18, 0.27, 0.35, 0.46, 0.53, 0.72, 0.81, 0.93. On the other hand the rf. of Protein of *Musca domestica* (treated) indicated the values as 0.08, 0.18, 0.28, 0.35, 0.45, 0.53, 0.61, 0.71, 0.85, and 0.96, respectively.

As indicated in Table 7. Protein rf. 0.03, 0.05, 0.21. 0.34, 0.043, 0.58, 0.63, 0.72 and 0.97 have not been observed in treated *Bactrocera dorsalis*. While protein rf. 0.04, 0.06, 0.22, 0.33, 0.44, 0.56 0.75 and 0.95 have been dectected as altered in *Bactrocera dorsalis.*

As shown in Table 8. Protein rf. 0.04, 0.07, 0.16, 0.26, 0.33, 0.34, 0.66, 0.78,0.87 and 0.93 have not been observed in treated *Bactrocera zonata*. While 0.05,0.08,0.15, 0.32, 0.46, 0.65, 0.72, 0.81 and 0.92 have been dectected as altered in *Bactrocera zonata*.

Protein rf. 0.04, 0.08, 0.15, 0.24, 0.34, 0.47, 0.72,0.84 and 0.93 have not been observed in treated *Bactrocera cucurbitae*. While protein rf. 0.03, 0.12, 0.31, 0.36, 0. 70, 0.79, 0.89 and 0.98 have been dectected as altered in *Bactrocera cucurbitae* (Table 9).

Protein rf. 0.04, 0.09, 0.23, 0.32, 0.43, 0.64, 0.73, 0. 78, 0.87 and 0.93 have not been observed in treated *Drosophila melanogaster*. While protein rf. 0.05, 0.14, 0.22, 0.30, 0.42, 0.61, 0.76, 0.86 and 0.94 have been dectected as altered in *Drosophila melanogast* (Shown in Table 10).

Protein rf. 0.03, 0.04, 0.45, 0.72, 0.81 and0.93 have not been observed in treated *Musca domestica*. While protein rf. 0.28, 0.45, 0.61, 0.71, 0.85 and 0.96 have been dectected as altered in *Musca domestica*, indicated in (Table 11).

DISCUSSION

DISCUSSION:

The results of different flies *Bactrocera cucurbitae,* treated with lead acetate 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg of different concentrations, revealed an increase in mortality directly proportional to all different concentrations with a sigmoid- curve (Curve3). Parke *et al*. (1991) have shown that "after the exposure of certain compound the mortality rate was increased with the increase in the concentration of the chemical compound, these results are in line with the present findings".

The present studies showed that lead acetate caused influence mostly on the morphology and development of the under test insects. However, the differences due to increase in concentration of lead acetate effected the level of these changes as compared to control.

Heavy metal in Diptera and specially *Drosophila* were used as model organisms in exposure to cadmium stated by Magnusson and Ramel (1986). "Insects living in polluted areas have been shown to accumulate heavy metals, in particular Ni and Cu, along with the obvious effects of pollution on growth rate and mortality, reported by Warrington (1987). Positive relationship was found in *Chironomus* between the copper concentration and the incidence of deformation of the pectin epipharyngis, as observed by Kosalwat and Knight, (1987). *Chironomous reparius* was studied with sediment spiked with cadium, zinc and copper,high mortality rates were observed, but no deformities were found induced, as observed by Grootelaar *et al*. (1988). The concentration of lead ,cooper, cadmium and zinc in *Chironomus* larvae from several location in the polluted Dyle Basin and Dommel River in Belgium, were studied and compared with normal larvae, (Janssens de Bisthoven *et al.*, 1995)". The present studies showed that lead acetate caused influence mostly on the morphology and development of the under test insects. However, the differences due to increase in concentration of lead acetate effected the level of these changes as compared to control. There results are almost in line with the previous workers in toxicity development.

Musca domestica treated with lead acetate 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg of different concentrations, revealed an increase in mortality directly proportional to

all diverse concentrations with a sigmoid- curve (Curve 4). As already indicated that "after the exposure of definite chemical compounds, the mortality rate increased with the increase in the concentration of the chemical constituent to provoke these results are in line with the present results. Ahmed and Naqvi (1985), reported that heavy metal compounds divergence is evidently a extensive observable fact in invertebrates". "Heavy metal resistance in Diptera are the model organisms in exposure to cadmium indicated by Magnusson and Ramel (1986). Positive affiliation was found in *Chironomus* between the copper concentration and occurrence of deformation of the pectin epipharyngis, reported by Kosalwat and Knight (1987). Insects living in contaminated areas have been revealed to accumulate heavy metals compounds, as Ni and Cu, along with the observable effects of contamination on growth rate and mortality, indicated by Warrington (1987). *Chironomous reparius* treated with sediment spiked with cadium, zinc and copper,high mortality rates were observed, but no malformation were, detected by Grootelaar *et al*. (1988). The concentration of lead, cooper, cadmium and zinc in Diptera larvae from several location in the polluted Dyle Basin and Dommel River in Belgium, and compared levels in normal larvae, stated by Janssens de Bisthoven *et al*. (1995)". The present observation showed that lead acetate induced effects mostly on the morphology and development of the under test insects. the differences due to increase in concentration of lead acetate influenced various changes as compared to control is in line with the previous works.

Drosophila melanogaster, were treated with lead acetate 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg of different concentrations, the results revealed an increase in mortality rates directly proportional to all different concentrations with a sigmoid- curve (Curve 5) likewise Parke *et al*. (1991). "Certain heavy metal resistance in Diptera and specially *Drosophila* are the model organisms in exposure to cadmium reported by Magnusson and Ramel (1986). Insects living in contaminated areas have been revealed to accumulate heavy metals, particularly Ni and Cu, along with the obvious effects of toxic waste on growth rate and mortality, reported by Warrington (1987). Positive affiliation was obtained in *Diptera* between the copper concentration and the incidence of deformation of the pectin epipharyngis, elaborated by Kosalwat and Knight (1987). *Chironomous reparius* and residue spiked with copper, zinc and cadium, indicated the high mortality rates, reported by

Grootelaar *et al*. (1988). The concentration of lead and other heavy metals in *Dipterian* larvae from numerous site in the contaminated area Dyle Basin and Dommel River in Belgium, were compared with normal larvae reported by Janssens de Bisthoven *et al.* (1995)". The present findings showed that lead acetate developed abnormality on the morphology and development of the under the observed insects. The differences due to increase in concentration of lead acetate effected the various changes as compared to control. These results are expected from the previous findings.

Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera dorsalis, Bactrocera zonata, Bactrocera cucurbitae, Drosophila melanogaster* and *Musca domestica* shown in (Fig. 1,2,3,4 and 5 respectively) and (Table 6—11).

Protein I (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 I is changed with some alteration in the treated insect. In the *Bactrocera. Cucurbitae*. Protein I is found in the untreated treated ones while it is absent in the treated insect and not any immediate protein is noted in *Bactrocera. dorsalis*. That suggest that the protein I is affected at a large extend. The similar case is that of *Musca domistica*. Protein III (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 III is changed with some deletion or alteration in treated insect. In the *Bactrocera zonata* protein III is found in the treated ones while it is absent in the untreated insect and not any immediate protein was found as noted in *Bactrocera dorsalis* case. That suggests that the protein III is effected at a large extend. The similler case is that of *Drosophila melanogaster*.

Protein V (rf 0.07) is found in *Bactrocera zonata* (untreated) that is seemed to be lighter than the egg albumin,while corresponding protein, in the treated *Bactrocera* 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 cucurbitae* protein VI was in the untreated while absent in treated ones.In the

Musca domestica protein VI was present in the untreated and untreated ones. That suggest protein VI is affected at large extend. Protein VII (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 VII was affected with some extend in the treated insect. In the *Drosophila melanogaster* protein VII is found in the untreated ones, while it is absent in treated insect and not any immediate protein is noted.

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 IX (rf 0.14) is found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumim, while corresponding protein in *Drosophila melanogaster* untreated is absent, that suggest that protein IX is affected at some 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. In the *Bactrocera cucurbitae.* Protein X is found in the untreated ones. That suggest that the protein X is affected at a large extend.

Protein XI (rf 0.16) is found in *Bactrocera zonata*. (untreated) that is seemed 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. That suggest that the protein XI is affected at some extend. Protein XII (rf 0.18) remained unaltered as it found in *Musca domestica* (untreated) as well as in the treated *Musca domestica* it was present at the same rf (0.18). That suggest that the protein XII was affected at a large extend. Protein XIII (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 XIII was affected at a small extend.

Protein XIV (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 XIV was changed with some alteration in the untreated insect. In the *Drosophila melanpogaster* Protein XIV was found in the treated ones. That suggest that the protein XIV was affected at a low extend. Protein XV (rf 0.23) was found in *Drosophila melanogaster* (untreated) that is seemed to be lighter than the egg albumin, while corresponding protein, in the treated *Drosophila melanogaster*, was absent. That suggest that the protein XV was affected at some 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 XVII (rf 0.26) was found in *Bactrocera zonata* (untreated) that is seemed 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 XVIII (Rf 0.27) was found in *Musca domestica* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Musca domestica*, was absent. This suggests that the protein XVIII was affected with some extend. Protein XIX (rf 0.28) was found in *Musca domestica* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera dorsalis*, was absent at the same rf .This suggests that the protein XIX was affected with some extend.

Protein XX (rf. 0.30) was found in the *Drosophila melanogaster* (treated) ones while it is absent in the untreated insect at the same rf. That suggest that the protein XX was affected at a low 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.

Protein XXII (rf 0.32) was found in *Bactrocera zonata*. (treated) that is seem 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 extend. Protein XXIII (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 XXIII was changed

with some alteration in the treated insect. In the *Bactrocera zonata* protein XXIII was found in the untreated and it was absent at the same rf in the treated That suggest that the protein XXIII was affected. Protein XXIV (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 XXIV was affected with some alteration in the treated insect. In the *Bactrocera cucurbitae* protein XXIV was found in the untreated treated ones while it is absent in the treated insect . That suggest that the protein XXIV was also affected. Protein XXV (rf 0.35) was found in *Musca domestica* (untreated) it is lighter than the egg albumin, and it remained unaltered, in the treated *Musca domestica*, while it was absent in the rest of four under consider species. 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 was changed with some alteration in the treated insect. Protein XXVII (rf 0.42) is found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the untreated *Drosophila melanogaster*, was absent at the same rf. This suggests that the proteinXXVII was changed with some alteration in the treated insect.

Protein XXVIII (rf 0.43) 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 absent at the same rf. This suggests that the protein XXVIII is changed with some alteration in the untreated insect. The similar case is that of *Bactrocera zonata* and *Drosophila melanogaster* at the same rf. That suggest that the protein XXVIII was affected at a large extend.Protein XXIX(rf 0.44) is 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 at the same rf. This suggests that the protein XXIX was changed with some alteration in the treated insect. Protein XXX (rf 0.45) is found in *Musca domestica* (treated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the untreated *Musca domestica*, was absent at the same rf. This suggests that the protein XXX is changed with some alteration in the treated insect. Protein XXXI (rf 0.46) 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 XXXI was affected and formed with some alteration in the treated insect. In the *Musca domestica* protein XXXI was found in the untreated treated ones while it is absent in the treated insect .That suggest that the protein XXXI was affected at a low extend. Protein XXXII (rf 0.47) was found in *Bactrocera cucurbitae* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Bactrocera cucurbitae*, was found at the same rf . This suggests that the protein XXXII was changed with some alteration in the untreated insect. Protein XXXIII(rf. 0.50) was found in the *Drosophila melanogaster* (untreated) that was seems to be lighter than egg albumin, while corresponding protein, in the treated *Drosophila melanogaster* was at the same rf. That suggests that the protein XXXIII is affected with some extend.Protein XXXIV (rf 0.53) was found in *Musca domestica* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Musca domestica*, was found at the same rf. This suggests that the protein XXXIV was affected with some alteration in the insect. Protein XXXV (rf 0.56) 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 at the same rf. This suggests that the protein XXXV was affected with some alteration in the treated insect. In the *Bactrocera zonata* and *Bactrocera cucurbitae* protein XXXV was found in the untreated treated and treated ones. That suggest that the protein XXXV was affected with some extend. Protein XXXVI (rf 0.58) 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 XXXVI was affected with some alteration in the insect.

Protein XXXVII (rf 0.61) was found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Drosophila melanogaster*, was absent at the same rf. and in *Musca domestica* protein XXXVII was found in treated ones while untreated was absent at the same rf. That suggest that the protein XXXVII was affected at a low extend. Protein XXXVIII (rf 0.63) 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 XXXVIII was affected with some extend. Protein XXXIX (rf 0.64) was found in *Drosophila melanogaster* (untreated) that is seems to be lighter than the

egg albumin, while corresponding protein ,in the treated *Drosophila. melanogaster*, was absent at the same rf. This suggests that the protein XXXIX was affected with some alteration in the untreated insect. Protein XL (rf 0.65) was found in *Bactrocera zonata* (treated) 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 XL affected with some alteration in the treated insect. In the *Bactrocera cucurbitae* Protein XL was found in the untreated treated and treated ones at the same rf. That suggest that the protein XL was affected at some extend. Protein XLI (rf 0.66) was 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 XLI was affected with some alteration in the untreated insect.

Protein XLII (rf 0.70) was found in *Bactrocera cucurbitae* (treated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Bactrocera cucurbitae* was absent at the same rf. That suggest that the protein XLII was affected at low extend. Protein XLIII (rf 0.71) was found in *Musca domestica* (treated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the untreated *Musca domestica*, was absent at the same rf. This suggests that the protein XLIII was affected with some alteration insect.

Protein XLIV (rf 0.72) 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 protein XLIV was affected with some alteration in the untreated insect. In the *Bactrocera zonata* Protein XLIV was found in the treated ones while it is absent in the untreated insect and in the *Bactrocera cucurbitae* and in the *Musca domestica* untreated protein XLIV was found at the same rf. That suggest that the protein XLIV was affected at some extend. Protein XLV (rf 0.73) was found in *Drosophila melanogaster* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Drosophila melanogaster*, was absent at the same rf. This suggests that the protein XLV was affected with some alteration in the untreated insect.Protein XLVI (rf 0.75) was found in *Bactrocera dorsalis* (treated) 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 XLVI was affected with some alteration in the treated insect.

Protein XLVII (rf 0.76) was found in *Drosophila melanogaster* (treated) that it seems to be lighter than the egg albumin, while corresponding protein, in treated *Drosophila melanogaster,* was absent. This suggests that the protein XLVII was affected with some alteration in the treated insect.Protein XLVIII (rf 0.78) was found in *Bactrocera zonata* (untreated) that seems to be lighter than the egg albumin, while corresponding protein, in the treated *Bactrocera zonata*, was absent at the same rf. In the *Drosophila melanogaster* Protein XLVIII is found in the untreated treated ones while it is absent in the treated insect That suggest that the protein XLVIII was affected at some extend. Protein XLVIX (rf 0.79) is found in *Bactrocera cucurbitae* (treated) that seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Bactrocera cucurbitae,* was absent at the same rf. This suggests that the protein XLVIX was affected with some extension.

Protein L (rf 0.81) was found in *Bactrocera zonata* (treated) that 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 L was affected with some extension in the treated insect. In the *Musca domestica* Protein L was found in the untreated treated ones while it is absent in the treated . That suggest that the protein L was affected at a low extend.

Protein LI (rf 0.84) was found in *Bactrocera cucurbitae* (untreated) seems to be lighter than the egg albumin, while corresponding protein ,in the treated *Bactrocera cucurbitae*, was absent at the same rf. This suggests that the protein LI was affected with some extension in the untreated insect. Protein LII (rf 0.85) was found in *Musca domestica* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Musca domestica,* was absent at the same rf. This suggests that the protein LII was affected with some extension in the treated insect. Protein LIII (rf 0.86) was found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Drosophil melanogaster* was absent at the same rf. This suggests that the protein LIII was affected with some extension in the treated insect. Protein LIV (rf 0.87) was found in *Bactrocera dorsalis* untreated and treated on the same rf that seems to be lighter than the egg albumin, This suggests that the protein LIV was affected with some extension in the untreated and treated insect. In the *Bactrocera zonata* Protein

LIV was found in the untreated treated ones while it is absent in the treated. That suggest that the protein LIV was affected at a low extend, while same case was reported in *Drosophila melanogaster*.

Protein LV (rf 0.89) was found in *Bactrocera cucurbitae* (treated) it 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 LV was affected with some extension in the treated insect. Protein LVI (rf 0.92) 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 LVI was affected with some extension in the treated insect. Protein LVII (rf 0.93) were found in *Bactrocera zonata*, *Bactrocera cucurbitae*, *Drosophila melanogaster*, *Musca domestica* (untreated) that seems to be lighter than the egg albumin, while corresponding protein, in the treated ones, were absent at the same rf. This suggests that the protein LVII were affected with some extension in the untreated insect. Protein LVIII (rf 0.94) was found in *Drosophila melanogaster* (treated) that seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Drosophila melanogaster*, was absent at the same rf. This suggests that the protein LVIII was affected with some extension in the treated insect. Protein LIX (rf 0.95) was found in *Bactrocera dorsalis* (treated) that seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera dorsalis*, was absent at the same rf. This suggests that the protein LIX was affected with some extension in the treated insect. Protein LX (rf 0.96) was found in *Musca domestica* (treated) that seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Musca domestica*, was absent at the same rf. This suggests that the protein LX was affected with some extension in the treated insect. Protein LXI (rf 0.97) was found in *Bactrocera dorsalis* (untreated) that it 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 LXI was affected with some extension in the untreated insect. Protein LXII (rf 0.98) was found in *Bactrocera cucurbitae* (treated) that 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 LXII was affected with some extension in the treated insect.

Dipterous flies 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 and change in various proteins. Nukhet et al.(2005), "indicated cellular damage in processes of lead exposed to PC-12 cells.After lead exposure various enzymes 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 as presently various morphological deformalities and altered proteins were absorved under the effects of lead in the lead treated insects. Corey and Galvao, (1989). indicated that, "lead can be absorbed by the digestive system in a 10%,presently various proteins were found altered as (Roy 1992) indicated that when lead incorporated by cells, it produces free radicals H_2O_2 and OH".probably these radicals brought about protenious changes and some changes appeared as morphologically as found presently in the *Bactrocera dorsalis Bactrocera zonata, Bactrocera cucurbitae, Drosophila melanogaster* and *Musca domestica*. Since Friedberg *et al*. (1995) found free radicals can also produce simple breaks in the DNA chains these results are in agreement with present finding in view of altered observed proteins in the treated Diptera. That the exposure of lead produced the abnormal morphological effects in the larvae and the adults emerged there from. If lead administrated in excess "it can compete with calcium (Pounds 1984), turn into a poison (Foulkes 1993), inhibit the protein group synthesis (Alvares *et al*., 1972; Goldberg *et al*., 1977), and produce cell death", these results are in agreement with present finding of altered proteins and deformaties due to lead administration. Chandrik *et al.* (2009) reported that "newly hatched nymphs of an Indian short horned grasshopper *Oxya fuscovittata* 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. Lower survival was observed". These adverse effect of heavy metals on insects are in line with the present findings.

Kalajdzic *et al.* (2006), found morphological changes in wild Drosophila species, 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, deformed larvae, pupae and deformed adults are in the line with the previous findings.(Kalajdzic *et al.,* 2006).

Lead caused developmental abnormality (Kim *et al.,* 1997), presently, lead is found causing alteration in various proteins of *Bactrocera dorsalis, Bactrocera zonzta, Bactrocera cucurbitae, Drosophila melanogaster and Musca domestica* (Table 6,--11) and morphological abnormalities, Present findings on lead effect on proteins of Dipterous flies is in the line with the previous workers.

Shelton *et al.* (1986) indicated effects of lead in certain protein synthesis, present results showed that lead causing morphological abnormalities on it alteration in different proteins of *Bactrocera dorsalis, Bactrocera zonzta, Bactrocera cucurbitae, Drosophila melanogaster and Musca domestica* (Table 6,- 11), present findings indicated that lead caused its effect on proteins of Dipterion flies in the same line with the above scientists.

Lead can induce change in some protein metabolism (Hilliard *et al.,* 1999), current results also indicated that lead causing alteration in various proteins of *Bactrocera dorsalis, Bactrocera zonzta, Bactrocera cucurbitae, Drosophila melanogaster and Musca domestica* (Table 6,- 11), Present findings on lead effect on proteins of Dipterous flies is in the similar line with the above researchers,

Lead effects on Drosophila is reported by Hirsch *et al.* (2003), however present results showed alteration in different proteins of *Bactrocera dorsalis, Bactrocera zonzta, Bactrocera cucurbitae, Drosophila melanogaster and Musca domestica* due to the lead effects (Table 6- 11), present findings indicated that lead make prominent effects on proteins of Dipterous flies it is in the same line with the above workers.

SUMMARY

SUMMARY

Lead is a heavy metal and it is considered as a potent environment pollutant. It causes soil contamination and the, food, water and air as well. Lead causes adverse hazardous effects on life. Due to this act its of pollution it is regarded as a matter of urgent concerned, many studies have been carried out in this respect. However, there is still some thirst remained on insect part.

Hence, lead is a toxic matter therefore, it is expected to develop it adverse effects on dipterous flies as well, such as *Bactrocera dorsalis, Bactrocera zonata, Bactrocera cucurbitae, Drosophila melanogaster and Musca domestica* etc Lead acetate in concentrations of 0.125, 0.25, 0.5, 1.0 and2.0 mg were studied in this relation

As stated above, different concentration on larvae of Dipterous flies, developed abnormalities and malformation, respectively. Adverse effects on morphological and physiological characters were observed as a change in larval, pupal morphology and folded, elongated legs, curved wings of adults etc. Moreover, colour change of larvae, pupae and adult was also observed as a prominent feature.

Proteins are essential bio-compound in nature. All enzymes are protein in nature. Lead acetate on protein exerted some electrophoratic changes. Presently, under the effect of lead acetate various protein have been found altered.

TABLES

Table A: Reagents and Chemicals:

Reagents and Chemicals	Company/Manufacturer
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

Table B: composition of Electrophoresis solutions:

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 18.2.0 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.

Preparation of Gel:

Table C : Component volumes (ml) per mold volume of

Solution components:

Solution component	Total volume 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

Table: I Mortality effect of lead acetate on *Bactrocera dorsalis* (3rd instar larvae) at post 48 hours exposure period.

S.No	Lead acetate in mg	Mortality/replicate	average % Mortality
1	0.125	04	43.33
2		04	
3		05	
4	0.25	05	53.33
5		05	
6		06	
7	0.50	06	60.00
8		06	
9		06	
10	1. 00	07	70.33
11		07	
12		07	
13	2.00	07	80.00
14		08	
15		08	
16	Control	1.0	13.33
17		1.0	
18		2.0	

Number of larvae exposed in each replicate = 10

Table: 2 Mortality effect of lead acetate on *Bactrocera zonata* (3^{rd} instar larvae) at post 48 hours exposure period.

S.No.	Lead acetate in mg	Mortality/replicate	% Mortality
1	0.125	05	46.66
2		04	
3		05	
4	0.25	05	53.33
5		05	
6		06	
7	0.50	07	63.33
8		06	
9		06	
10	1.00	08	73.33
11		07	
12		07	
13	2.00	08	80.00
14		08	
15		08	
16	Control	1.0	10.00
17		1.0	
18		1.0	

Number of larvae exposed in each replicate = 10

Table 3: Mortality effect of lead acetate on *Bactrocera cucurbitae* (3^{rd} instar larvae) at post 48 hours exposure period.

S.No.	Lead acetate in mg	Mortality/replicate	% Mortality
1	0.125	04	46.66
2		05	
3		05	
4	0.25	05	53.33
5		05	
6		06	
7	0.50	05	63.33
8		07	
9		07	
10	1.00	07	73.33
11		07	
12		08	
13	2. 00	08	80.00
14		08	
15		08	
16	Control	01	10.00
17		02	
18		00	

Number of larvae exposed in each replicate = 10

Table 4: Mortility effect of lead acetate on *Drosophila melanogaster* (3^{rd} instar larvae) at post 48 hours exposure period.

S.No.	Lead acetate in mg	Mortality/replicate	% Mortality
1	0.125	05	46.66
2		04	
3		05	
4	0.25	06	56.66
5		05	
6		06	
7	0.5	06	63.33
8		07	
9		06	
10	1.0	07	73.33
11		08	
12		07	
13	2.0	08	83.33
14		08	
15		09	
16	Control	2.0	10.00
17		1.0	
18		0.0	

Number of larvae exposed in each replicate = 10

Table 5: Mortality effect of lead acetate on *Musca domestica* (3^{rd} instar larvae) at post 48 hours exposure period.

S.No.	Lead acetate in mg	Mortality/ replicate	% Mortality
1	0.125	06	42.22
2		06	
3		07	
4	0.25	07	48.88
5		07	
6		08	
7	0.50	08	55.55
8		08	
9		09	
10	1. 00	09	64.44
11		10	
12		10	
13	2. 00	10	71.11
14		11	
15		11	
16	Control	2.0	10.00
17		0.0	
18		1.0	

Number of larvae exposed in each replicate = 10

Table 6: Rf values of various proteins observed in lead acetate treated and untreated Dipterous larvae.

Protein	Rf	Egg Albun	Bactroca dorsalis normal	Bactrocera dorsalis treated	Bactrocera Zonata normal	Bactrocera Zonata treated	Bactrocera cucurbitae normal	Bactrocea cucurbitae treated	Drosophila melanogasr normal	Drosophila melanogastr treated	Musca domestica normal	Musca Domestica treated
I.	0.03		+	-	-	-	-	+	-	-	+	
II.	0.04	+		+	-	-	-	-	-	-	-	
III.	0.05		+	-		+	-	-	-	+	-	
IV.	0.06		-	+	-	-	-	-	-	-	-	
V.	0.07				+	-	-	-	-	-	-	
VI.	0.08		-			+	+	-	-	-	+	+
VII.	0.09		-	+	-	-	-	-	+	-	-	
VIII.	0.12							+				
IX.	0.14		-	-	-	-	-		-	+	-	
X.	0.15		-	-	-	+	+	-	-	-	-	
XI.	0.16		-	-	+	-	-	-	-	-	-	
XII.	0.18			-	-	-	-	-	-	-	+	+
XIII.	0.21		+	-	-	-	-	-	-	-	-	
XIV.	0.22		-	+	-	-	-	-	-	+	-	
XV.	0.23		-	-	-	-	-	-	+	-	-	
XVI.	0.24		-	-	-	-	+	-	-	-	-	
XVII.	0.26		-	-	+	-	-	-	-	-	-	
XVIII.	0.27		-	-	-	-	-	-	-	-	+	
XIX.	0.28		-	-	-	-	-	-	-	-	-	+
XX.	0.30		-	-	--	-	-	-	-	+	-	
XXI.	0.31		-	-	-	-	-	+	-	-		
XXII.	0.32		-	-		+	-	-	+	-	-	
XXIII.	0.33		-	+	+	-	-	-	-	-	-	
XXIV.	0.34		+	-	-	--	+	-	-	-	-	
XXV.	0.35		-	-	-	-	-	-	-	-	+	+
XXVI.	0.36		-	-	-	-	-	+	-	-	-	
XXVII.	0.42		-	-	-	-	-	--	-	+	--	
XVIII.	0.43		+	-	+	-	-	-	+	-	-	-
XXIX.	0.44		-	+	-	-	-	-	-	-	-	-
XXX.	0.45		-	-	-	-	-	-	-	-		+
XXXI.	0.46		-	-	-	+	-	-	-	-	+	-
XXXII.	0.47		-	-	-	-	+	-	-	-	-	-
XXIII.	0.50		-	-	-	-	-	-	+	+	-	-
XXIV.	0.53		-	-	-	-	-	-	-	-	+	+
XXXV.	0.56		-	+	+	+	+	+	-	-	-	--
XXVI.	0.58		+	-	-	-	-	-	-	-	-	-
XXVII.	0.61		-	-	-	-	-	-	-	+	-	+
XVIII.	0.63		+	-	-	-	-	-	-	-	-	-
XXIX.	0.64		-	-	-	-	-	-	+	-	-	-
XL.	0.65		-	-	-	+	+	+	--	-	-	-

XLI.	0.66		-	-	+	-	-	-	-	-	-	-
XLII.	0.70		-	-	-	-	-	+	-	-	-	-
XLIII.	0.71		-	-	-	-	-	-	-	-	-	+
XLIV.	0.72		+	-	-	+	+	-	-	-	+	-
XLV.	0.73		-	-	-	-	-	-	+	-	-	-
XLVI.	0.75		-	+	-	-	-	-	-	-	-	-
XLVII.	0.76		-	-	-	-	-	-	-	+	-	-
XLVIII.	0.78		-	-	+	-	-	-	+	-	-	-
XLIX.	0.79		-	-	-	-	-	+	-	-	-	-
L.	0.81		-	-	-	+	-	-	-	-	+	-
LI.	0.84		-	-	-	-	+	-	-	-	-	-
LII.	0.85		-	-	-	-	-	-	-	-	-	+
LIII.	0.86		-	-	-	-	-	-	-	+	-	-
LIV.	0.87		+	+	+	-	-	-	+	-	-	-
LV.	0.89		-	-	-	-	-	+	-	-	-	-
LVI.	0.92		-	-	-	+	-	-	-	-	-	-
LVII.	0.93		-	-	+	-	+	-	+	-	+	-
LVIII.	0.94		-	-	-	-	-	-	-	+	-	-
LIX.	0.95		-	+	-	-	-	-	-	-	-	-
LX.	0.96		-	-	-	-	-	-	-	-	-	+
LXI.	0.97		+	-	-	-	-	-	-	-	-	-
LXII.	0.98		-	-	-	-	-	+	-	-	-	-

Table 7: values of various proteins observed in lead acetate treated and untreated *Bactrocera dorsalis* larvae.

Rf	Egg Albun	*Bactroca dorsalis* normal	*Bactrocera dorsalis treated*
0.03		+	-
0.04	+		+
0.05		+	-
0.06		-	+
0.09		-	+
0.21		+	-
0.22		-	+
0.33		-	+
0.34		+	-
0.43		+	-
0.44		-	+
0.56		-	+
0.58		+	-
0.63		+	-
0.72		+	-
0.75		-	+
0.95		-	+
0.97		+	-

Table 8: values of various proteins observed in lead acetate treated and untreated *Bactrocera zonata* larvae.

Rf	Egg Albun	Bactrocera Zonata normal	Bactrocera zonata treated
0.04	+	-	-
0.05		-	+
0.07		+	-
0.08		-	+
0.15		-	+
0.16		+	-
0.26		+	-
0.32		-	+
0.33		+	-
0.43		+	-
0.46		_	+
0.65		-	+
0.66		+	-
0.72		-	+
0.78		+	-
0.81		-	+
0.87		+	-
0.92		-	+
0.93		+	-

Table 9: 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		-	+
0.47		+	-
0.70		-	+
0.72		+	-
0.79		-	+
0.84		+	-
0.89		-	+
0.93		+	-
0.98		-	+

Table 10: values of various proteins observed in lead acetate treated and untreated *Drosophila melanogaster* larvae.

Rf	Egg Albumin	Drosophila melanogasr Untreated Control	Drosophila melanogastr treated
0.04	+	-	-
0.05		-	+
0.09		+	-
0.14		-	+
0.22		-	+
0.23		+	-
0.30		-	+
0.32		+	-
0.42		-	+
0.43		+	-
0.61		-	+
0.64		+	-
0.73		+	-
0.76		-	+
0.78		+	-
0.86		-	+
0.87		+	-
0.93		+	-
0.94		-	+

Table 11: values of various proteins observed in lead acetate treated and untreated *Musca domestica* larvae.

Rf	Egg Albun	Musca domestica normal	Musca domestica treated
0.03		+	-
0.04	+	-	-
0.28		-	+
0.45		-	+
0.46		+	-
0.61		-	+
0.71		-	+
0.72		+	-
0.81		+	-
0.85		-	+
0.93		+	-
0.96		-	+

GRAPHS

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

Fig. 2: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Bactrocera zonata*

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

Fig. 4: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Drosophila melanogaster*

Fig. 5: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Musca domestica*

PLATES/FIGURE

Fig I.*Bactrocera dorsalis* (larval stage),in normal condition.Body elongated cylindrical fusiform in shape creamy white in colour, spinules band encircling the body, the larva is 10 mm. in length.

Fig II. *Bactrocera dorsalis* (larval stage) effected, with acetate, 0.125 mg at 48 hours. Showing curved structure of larvae and pigmentation.

Fig III. *Bactrocera dorsalis* (larval stage) effected with lead acetate, 0.25 mg at 48 hours. Showing hazardous toxic on larval structure.

Fig IV.*Bactrocera dorsalis*(larval stage), effected with lead acetate 0.5 mg at 48 hours. Showing almost normal.

Fig V. *Bactrocera dorsalis*(larval stage), effected with lead acetate 1.0 mg at 48 hours. Showing almost normal.

Fig VI. *Bactrocera dorsalis*,(larval stage) effected with lead acetate 2.0 mg

at 48 hours. Showing no toxic effect almost normal.

Fig VII. *Bactrocera dorsalis* (pupal stage) in normal condition like structure, elastic cover, 4mm. in length,yellowish brown pigmentation, slightly segmented body.

Fig VIII. *Bactrocera dorsalis* pupal stage effected with lead acetate 0.125 mg at 48 hours. Showing structural change in pupae and effected melanization.

Fig IX. *Bactrocera dorsalis*(pupal stage) effected with lead acetate 0.25 mg at 48 hours. Showing morphological change in structure due to toxic effect of lead acetate.

Fig X. *Bactrocera dorsalis* (pupal stage) effected with, lead acetate 0.5 mg at 48 hours. Toxic effect of lead acetate showing structural changes in of pupae.

Fig XI. *Bactrocera dorsalis* (pupal stage) effected with, lead acetate 1.0 mg at 48 hours. Showing toxic effect on structure of pupae.

Fig XII B*actrocera dorsalis* (pupal stage) effected with lead acetate 2.0 mg at 48 hours.Showing toxic effect on structural abnormalities and melanization.

Fig XIII. *Bactrocera dorsalis* (Adult stage) in normal condition, fly is triangular in shape, body length 8 mm. The wings are about 7.3 mm in length, yellow, green brown pigmentation, black marking on the thorax, and abdomen.

Fig XIV. *Bactrocera dorsalis* (Adult stage), effected with lead acetate 0.125 mg, at 48 hours. Showing slightly curved wing, folded legs and melanization.

Fig XV. *Bactrocera dorsalis* (Adult stage), effected with lead acetate, 0.25 mg, at 48 hours. Showing toxic effect of lead acetate as cutting edges of wings.

Fig XVI. *Bactrocera dorsalis* (Adult stage), effected with lead acetate, 0.5 mg, at 48 hours. Showing toxic effect as absence of one wings, and folded legs.

Fig XVII. *Bactrocera dorsalis* (Adult stage), effected with lead acetate, 1.0 mg at 48 hours. Showing toxic effect as lengthy wings and folded legs.

Fig XVIII. *Bactrocera dorsalis* (Adult stage), effected with lead acetate, 2.0 mg, at 48 hours. Toxic effect showing absence of one wing and elongated legs.

Fig XIX. *Bactrocera zonata* (Larval stage), in normal condition. Elongated body,fusiform in shape, pointed at anterior end,broad at posterior end, slightly segmented body.dull white colour.

Fig XX. *Bactrocera zonata* (larval stage), effected with lead acetate, 0.125 mg, at 48 hours. Showing hazardous toxic effect of lead acetate on structure of larvae, such as corved and banded boby, rough surface, shrinkage body, dull white brown colour.

Fig XXI. *Bactrocera zonata* (larval stage) effected with lead acetate, 0.25 mg at 48 hours. Showing structural abnormalities and curved larvae.

Fig XXII. *Bactrocera zonata* (larval stage) effected with lead acetate 0.5 mg, at 48 hours.Showing toxic effect on larva No. 4.as curved and banded body.

Fig XXIII. *Bactrocera zonata* (Larval stage) effected with lead acetate 1.0 mg, at 48 hours. Showing toxic effects and abnormal structure of larvae.

Fig XXIV. *Bactrocera zonata* (Larval stage) effected with acetate 2.0 mg at 48 hours. Showing structural abnormalities as curved and banded bodies, rough surface dull white brown colour.

Fig XXV.*Bactrocera zonata* (Pupal stage) in normal condition.Yellowis brown colour, 4 to 5 mm in length, elastic body cover, round at both the end, slightly segmented body, small capsule shape.

Fig XXVI. *Bactrocera zonata* (pupal stage) effected with lead acetate, 0.125 mg at 48 hours. Toxic effect of lead acetate showing the abnormalities on pupal structures, and pigmentation.

Fig XXVII. *Bactrocera zonata* (pupal stage) effected with lead acetate 0.25 mg at 48 hours.Showing effect as abnormal structure of pupae and spoted body.

Fig XXVIII *Bactrocera zonata* (pupal stage) effected with lead acetate 0.5 mg at 48 hours.Slightly effected boby shape and colour.

Fig XXIX. *Bactrocera zonata* (pupal stage) effected with lead acetate 1.0 mg at 48 hours. Showing abnormalities in body structure and spotted body.

Fig XXX.*Bactrocera zonata* (pupal stage) effected with lead acetate 2.0 mg at 48 hours. Showing structural change in body and melanization.

Fig XXXI *Bactrocera zonata* (Adult stage) in normal condition. red brown in colour, marking on abdomen and thorax can be seen, transparen expanded wing. a pair of dark marks on tergite .Body length 8 mm and wings are 7 mm in length.

Fig XXXII.*Bactrocera zonata* (Adult stage) effected with lead acetate, 0.125 mg at 48 hours. Slightly toxic effect on wing legs and abdomen.

Fig XXXIII. *Bactrocera zonata* (Adult stage) effected with lead acetate, 0.25 mg at 48 hours. Almost no toxic effect.

Fig XXXIV. *Bactrocera zonata* (Adult stage) effected with lead acetate, 0.5 mg at 48 hours.Showing slightly toxic effecty of lead acetate as elongated wings and legs.

Fig. XXXV. *Bactrocera cucurbitae*(larval stage) in normal condition. Cigar shape maggot, anterior portion of larva is narrow, with pointed mouth hook, slightly curved at anterior end, elongated fusiform in shape and flattened caudal end,7 to 10 mm , in length.

Fig.XXXVI. *Bactrocera cucurbitae* (larval stage) effected with lead acetate 0.125 mg, at 48 hours. Showing hazardous toxic effect of lead acetate and abnormalities on structure of larvae body.

Fig. XXXVII. *Bactrocera cucurbitae* (larval stage) effected with lead acetate 0.25 mg at 48 hours. Abnormal effect of lead acetate on body structure of larvae.

Fig.XXXVIII. *Bactrocera cucurbitae* (larval stage) effected with lead acetate 0.5 mg at 48 hours. Showing toxic effect of lead acetate on morphological

structural abnormalities as curved and shrinkage body.

Fig.XXXIX. *Bactrocera cucurbitae* (larval stage) effected with lead acetate 1.0 mg, at 48 hours. Toxic effect of lead acetate showing curve, shrinkage and rough. Body structural abnormalities on larvae.

Fig. XL. *Bactrocera cucurbitae* (larval stage) effected with lead acetate 2.0 mg at 48 hours. Showing abnormal structural change as rough and compressed curved body.

Fig. XLI. *Bactrocera cucurbitae* (pupal stage) in normal condition. cylindrical maggot shape, anterior portion of larva is narrow,with pointed mouth hook, slightly curved at anterior end, elongated fusiform in shape, body is dull white in colour, and segmented.

Fig. XLII. *Bactrocera cucurbitae* (pupal stage) effected with lead acetate, 0.125 mg at 48 hours. Some slight change in structure except No.4 where a class of curved and thin pupae was obtained.

Fig.XLIII. *Bactrocera cucurbitae* (pupal stage) effected with lead acetate, 0.25 mg at 48 hours. Showing toxic effect on melanization and slightly elongated pupae (No.3).

Fig. XLIV. *Bactrocera cucurbitae* (pupal stage) effected with lead acetate, 0.5 mg at 48 hours. Showing minute change in structure such as curved and pointed body (No.2) and elongated body (No.4).

Fig. XLV. *Bactrocera cucurbitae* (pupal stage) effected with lead acetate 1.0 mg at 48 hours. Abnormal effect on structure of pupae as curved and shrinkage body (No. 1) rough and changed structural abnormality with melanization (No.4).

Fig. XLVI. *Bactrocera cucurbitae* (pupal stage) effected with lead acetate, 2.0 mg at 48 hours. Almost no effect.

Fig.XLVII. *Bactrocera cucurbitae* (Adult stage) in normal condition. 8 mm in ength, wing pattern, long third antennal segment,the dorsum of the thorax redish yellow with light yellow markings, and the head yellowish with black spots, head and eyes are dark brown, abdomens are redish yellow with darker bands.

Fig.XLVIII. *Bactrocera cucurbitae* (Adult stage) effected with lead acetate 0.125 mg, at 48 hours. Showing toxic effect on spoted wings and legs.

Fig.XLIX. *Bactrocera cucurbitae* (Adult stage) effected with lead acetate 0. 25 mg at 48 hours.Showing effect of lead acetate on wings and abdomen.

Fig.L. *Bactrocera cucurbitae* (Adult stage) effected with lead acetate 0.5 mg at 48 hours. Showing almost no toxic effect.

Fig.LI. *Bactrocera cucurbitae* (Adult stage) effected with lead acetate 1.0 mg at 48 hours. Showing elongated expanded wings

Fig.LII. *Bactrocera cucurbitae* (Adult stage) effected with lead acetate 2.0 mg at 48 hours.almost no toxic effect.

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

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

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

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

Fig. LXXV. *Drosophila melanogaster* (Larval stage), effected with

lead acetate 1.0 mg at 48 hours. Minute effect on melanization.

Fig. LXXVI. *Drosophila melanogaster* (larval stage) effected with lead acetate 2.0 mg at 48 hours. Minute change in structure and melanization.

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

Fig. LXXVIII. *Drosophila melanogaster*, pupal stage, effectedwith lead acetate,0.125 mg at 48 hours.Minute change on morphological structure.

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

Fig. LXXX. *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. LXXXI. *Drosophila melanogaster,* effected with lead aceta 1.0 mg at 48 hours.Showing toxication on morphological structural abnormalities and pigmentation.

Fig.LXXXII. *Drosophila melanogaster* (pupal stage) effected with lead acetate 2.0 mg at 48 hours. Showing toxic effect on structure and size of pupae.

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

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

Fig. 33.1. *Drosophila melanogaster* (Adult stage) effected with lead acetate, 0.25 mg at 48 hours. Showing toxic effect on expanded wings and abdomen.

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

Fig. LXXXVII. *Drosophila melanogaster* (Adult stage) effected with lead acetate,1.0 mg at 48 hours. Minute effect on abdomen and folded legs.

Fig.LXXXVIII. *Drosophila melanogaster* (Adult stage) effected with lead acetate,2.0 mg at 48 hours. Showing toxic effect on wings and abdomem.

Fig.LIII. *Musca domestica* (larvalstage) normal in condition, narrow at the mouth end, larvae are 3 to 9 mm. in length, typical creamy whitish in color, cylindrical but tapering toward the head.The head contains one pair of dark hooks.

Fig.LIV. *Musca domestica* (larval stage) effected with lead acetate 0.125 mg at 48 hours. Showing some abnormalities and morphological changes.

Fig. LV. *Musca domestica* (larval stage) effected with lead acetate

0.25 mg at 48 hours. Toxicity of lead acetate showing the abnormalities on different larvae.

Fig. LVI. .*Musca domestica* (larval stage), effected with lead acetate 0.5 mg at 48 hours, showing the abnormal morphological effect on different larvae.

Fig. LVII. *Musca domestica* (larval stage) effected with lead acetate 1.0 mg at 48 hours. Showing abnormal effect of lead acetate on larval structure and morphology.

Fig.LVIII. *Musca domestica* (larval stage), effected with lead acetate,2.0

mg at 48 hours. Showing structural and morphological changes on different larvae.

) in normal condition. The pupal

is 8 mm in length , is passed in a pupal case formed from the last larval skin which varies in color from yellow,red, brown, to black as the pupa ages.The shape of the pupa bluntly rounded at both ends.

Fig.LX.*Musca domestica* (Pupal stage), effected with lead acetate 0.125 mg, at 48 hours. Showing the toxigenous morphological effect on different pupal structures.

Fig.LXI. *Musca domestica* (Pupal stage) effected with lead acetate 0.25 mg at 48 hours. Showing toxic effect of lead acetate on melanization and structure.

Fig. LXII. *Musca domestica* (pupal stage) effected with lead acetate 0.5 mg, at 48 hours.Showing hazardous effect of lead acetate on their morphological structure.

Fig. LXIII. *Musca domestica* (Pupal stage) effected with Lead acetate 1.0 mg at48 hours. Showing the toxic effect of lead acetate in the on their structure and spotted melanization.

Fig. LXIV. *Musca domestica* (Pupal stage), effected with Lead acetate 2.0 mg at 48 hours. Toxic effect of lead acetate showing the abnormal structure and different melanization on pupae.

Fig. LXV *Musca domestica* ,(adult fly) in normal condition. Brown redisd eyes, spongy mouth part, thorax bears four narrow black strip, abdomen dull white in colour transparent veinleted wings.

Fig.LXVI. *Musca domestica* (Adult stage), effected with lead acetate 0.125 mg at 48 hours. Showing the abnormal toxic effect on their structure and wings.

Fig. LXVII. *Musca domestica* (Adult stage), effected with lead acetate 0.25 mg at 48 hours.Toxic effect of lead showing slightlymorphological change in their structure.

Fig. LXVIII. *Musca domestica* (Adult stage), effected with lead acetate 0.5 mg at 48 hours. Showing toxic effect of lead on their wings and legs.

Fig. LXIX. *Musca domestica*,(Adult stage) effect with lead Acetate 1.0 mg, at 48 hours. Showing slightly curve on their wings and folded legs.

Fig.LXX.*Muscadomestica* effected with lead acetate 2.0 mg at 48 hours.

Showing slightly morphological change on their one side wing and legs.

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PUBLICATIONS

LIST OF PUBLICATIONS:

1) Effect of lead on external morphology and polytene chromosomes of *Tabanus bovines. Int.J.Biol.,Biotech*.,2(3): 589-595, 2005.

   1)M.Jamal Haider(2)H.R. Siddiqui(3)Rizwan ul Haq (4)Ehtesham ul Haq(5)Rukhsana Talat

2) Teratogenic effect of lead acetate on *Bactrocera cucurbitae* (COQ). Pakistan Entomologist, , vol. 33, No. 1, 2011, ISSN 1017-1827.

   1)Rizwan ul Haq (2) M.Farhan ullah Khan (3) Ehtesham ul Haq

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1) Rizwan ul Haq (2) M.Farhan ullah Khan (3) Ehtesham ul Haq

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1) Rizwan ul Haq (2) M.Farhan ullah Khan (3) M.Faheem (4) Habibullah Rana (5) S.N.H.Naqvi.