DETERMINATION OF LC_{50} OF CHLORPYRIFOS AND NEEM EXTRACT ON THIRD INSTAR LARVAE OF HOUSE FLIES AND THEIR EFFECT ON FECUNDITY

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Abstract

Two pesticides one from OP compound (Chlorpyrifos) and other from Biopesticides (Neem extract) were applied against 3rd instar larvae of house flies by contact method. LC_{50} of Chlorpyrifos and Neem extract was calculated as 0.25% for chlorpyrifos and 0.42% for Neem Extract. Treated larvae when succeeded to reach the adult stage, the effects of the mentioned insecticides were noted on fecundity of adults of *Musca domestica*. Chlorpyrifos delayed egg-laying while Neem extract completely inhibited the egg-laying. Moreover, the treated larvae when succeeded to pupate, they failed to emerge from the pupal case completely and the larvae got heavy melanization after 48 hours of treatment.

Keywords: Neem Extract, Chlorpyrifos, Fecundity, *Musca domestica*.

INTRODUCTION

The common housefly (*Musca domestica*) is found throughout the world. It is a domesticated insect. It acts as a vector of human and animal diseases.

Houseflies are naturally infected with the pathogens of more than twenty human diseases like Typhoid fever, epidemic or summer diarrhea, amoebic and bacillary dysentery, cholera, poliomyelitis etc. The housefly carries the germs of various diseases on its body parts such as wings, foot pads (pulvilli) and mouth parts (Metcalf and Flint, 1926). The larvae of *Musca domestica* can also cause myiasis in human and veterinary animals, and as a result of myiasis, economic loss particulary in farm animals occur (Zumpt, 1965; Zhu and Lin, 1999; Kumarsinghe et al., 2000; Jiang, 2002; Sehgal et al., 2002).The larvae also carry germs and retain them in living condition during pupal stage and transfer them after emergence.

By many ways entomologists have tried to control the population of houseflies. One way to control its density is to control the larvae, soon after hatching, by the application of various chemicals. Due to these chemicals, insect remains in their larval stage and become unable to reach the pupal or adult stage. If they succeded to overcome the effects of pesticides, then the effect of chemical retain even in their adult stage. Therefore, their fecundity may be affected.

Chemical treatment to flies larvae, on one hand, kills them and, on other hand, produces abnormalities in their biological activities. Chemicals, which belong to 3rd generation pesticides produce sterility in the gonads of insect. Nitrogen mustard, sulphonic acid esters and various amides (tepa and hempa) and plant products such as Shikonin and Azadirachtin, have been used in the past which make insects sterile. Very little is known about the effect of OP compounds on insect fecundity. In the present investigation OP pesticide (Chlorpyrifos) and plant product (Neem extract) were used to observe their effect on fecundity of houseflies. Several researchers have reviewed that the most of the synthetic conventional pesticides, particularly, OP, OC and carbamate groups are harmful to house flies.

Chlorpyrifos belongs to a group of pesticides, which is called OP i:e organophosphates. It is a broad spectrum and a potent insecticide, which kill a numbers of insect's pest species. It was used, for the first time, against developmental stages of mosquitoes, in the year 1965 (Hayes and Laws, 1990). Chlorpyrifos is available in many formulations such as EC i:e emulsifiable concentrate, dust, flowable, spray granular and wettable powder . It has stomach poison activity specially against Dipterous, Orthopterous and Lepidopterous insects. It is a non-systemic contact poison. It works by alteration with the function of cholinesterase enzyme this enzyme is important for the functioning of the brain in humans as well as, in insects. It is moderately poisonous to humans. According to a report of EPA the oral LD_{50} of Chlorpyrifos in rats is 82-270 mg/kg. It has been found that Chlorpyrifos does not badly affect upon reproduction. In one study, by the Environmental Protection Agency, in which rats were allowed to feed 1.0 mg/ kg/ day for two successive generations, then slight increase mortality was noted in newborn off springs. In the blood stream

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Chlorpyrifos may pass through placenta. Research shows that Chlorpyrifos does remaine in the body. It is very toxic to fresh water fish and marine organisms. In recent years many plant products specially neem compounds were applied for controlling insect pest species because they are biodegradable less persistant and least toxic to mammals and other non-target organisms. Moreover insects could not develop resistance against neem (Vollinger, 1987, 92, 95; Naqvi and Tabassum, 1992).

Many neem extracts were reported to have pesticidal activity (Naqvi et al., 1996).

Many Extract of Neem and their isolated products have been tested as an insecticide.

According to Rembold, (1995) Neem products are effective on growth and metamorphosis of insects. They reported that the Neem products have adverse effect on endocrine system of Bean beetles and may cause stetrility in the female insects (Schmutterer et al., 1981)

MATERIALS AND METHODS

A: Collection of Insect

Initial population of house flies (*Musca domestica*) was collected from Empress Market Saddar, Karachi.

B: Insects Rearing

Rearing of *Musca domestica* was carried out according to Ashrafi *et al***.** **(**1966) with some modification, in the insectory of the Department of Zoology University of Karachi. The insects were collected from Empress Market and released in the cage. The bottom of the cage was made of hardboard. One side of the cage had a long sleeve of muslin cloth for handling and cleaning purpose. These insects were provided the milk solution (soaked in cotton) in small plastic bowls (6cm). On the other hand, their larvae were given wheat bran as food in bowls of 15cm diameter.

C: Preparation of Chemicals

Many preliminary trials were done for the selection of the specific concentration and doses. After such preliminary experiments, a stock solution of 1% was prepared by mixing 1ml of chlorpyrifos with 99ml of distilled water and then 0.5%, 0.25%, 0.125% and 0.0625% were prepared. For preparing these dilutions, 0.1ml and 1ml pipettes were used which were prewashed and sterilized before use in order to make an accurate concentration and to avoid impurities.

The second chemical was Neem Extract (BIOSAL A). This extract was obtained from Dr. Beena Shaheen Siddiqui of H.E.J University of Karachi. Main chemical constituents of BIOSAL were as follow. Azadirachtin 0.32%, Salannin 1.06%, Nimbin 0.75, Deacetylnimbin 0.31%, other terpenoids 1.5%, Neem oil 7.5. The Neem extract was applied, directly, in following doses as 01 ml 0.8ml , 0.6ml , 0.4ml and 0.2ml.

D: Method of Treatment

1.35 g sterilized wheat bran was taken in a small bowl of 6cm in diameter. The test chemicals were applied by mixing the specific quantity of each insecticide in the bran. The dose was kept constant (1ml) in case of chlorpyrifos. The 3rd instar larvae (10 in number) were released to each bowl. The mouth of the bowl was covered with a piece of muslin cloth held down by a rubber band in order to pass the fresh air inside. Mortality was noted after 48 hours of treatment. Each experiment was repeated 5 times and the mean values were taken for analysis and LC_{50} was determined.

To observe the effect of fecundity on *Musca domestica*, five pairs of insects were released in the cage . These flies had been treated with insecticides in their larval stages. The adult flies were provided with milk for feeding. A control batch of five pairs of adult house flies was also maintained. The adults were later observed for their mating and egg laying processes.

RESULTS

The LC_{50} of Chlorpyrifos and that of Neem extract against 3rd instar larvae of *Musca domestica* was determined and are interpreted in Fig #1 and #2 which indicate that LC_{50} of chlorpyrifos as 0.25% and that of Neem extract as 0.42%.

The highest mortality, of the larvae, caused by Chlorpyrifos was 82% and the highest mortality caused by Neem extract was 96% as given in Table#1 and #2. As far as, fecundity is concerned, both compounds affected fecundity. It was completely inhibited by the Neem extract while insects treated with Chlorpyrifos, laid limited number of eggs and their hatching was completely inhibited (Tables 3 and 4).

DISCUSSION

Jahan *et al.* (1990) reported that preimaginal stages of houseflies failed to emerge after treatment with Margosan-O (0.03% Azadirachtin).

Our finding is in accordance with the previous report (Jahan et al., 1990) as larvae, treated with Neem extract, were failed to come out of the pupal case (photo:1).

Khan *et al.* (1991) applied Neem extract against 2nd instar larvae of house flies. They calculated LC_{50} as 3.7%. The decrease in weight and deformation, in various developmental stages of flies, was also reported. This report is comparable to our report as the sub-lethal

concentration caused deformed pupa (photos:2&5) and adults. The highest LC_{50} in the previous report (3.7%) may be due to different developmental stage of insect and different method of Neem extract application.

Jamal and Qamar, (2002) reported the egg production and hatching of female *Dysdercus cingulatus* emerged from the treated nymph. Egg hatching decreased linearly and the application of higher concentration resulted in the gradual degeneration of the oocyte.

The present report shows that Chlorpyrifos delayed egg-laying it decreased the egg production and even the egg failed to hatch. It is necessary to study the oocyte development within the ovary after Chlorpyrifos treatment to the female houseflies to extend the present research.

According to previous report (Ghoneim et al., 2007) extract of Margosan-O produced inhibitory action on fecundity, of houseflies, while Jojoba produced relatively slight effect on fecundity. It shows that Margosan-O (a product of Neem extract) has comparatively greater strength towards sub-lethal effects to houseflies than other plant material like Jojoba.

Further, the teratomorphic effect such as deformed pupae and partial emergence of adult house flies treated with Neem extract (N-9), was reported (Naqvi et al., 2007).

In our study deformed pupae and partially emerged flies have also been found when Neem extract was applied on 3rd instar larvae of *Musca domestica*.

The present research shows that Chlorpyrifos and Neem extract kill insects in their immature stage of development and if the larvae succeede to pupate, then insecticide convert some of the pupae in deformed condition which become unable to produce adult insects. If the pupae succeeded to produce the adult insect then adults laid limited number of eggs and the eggs later failed to hatch. Since the last two decades Neem and its extracts have been applied against variety of insect pest species. The target pest includes both holometabolous and hemimetabolous insects (Jahan et al., 1990; Khan et al., 1991; Jamal and Qamar, 2002; Ghoneim et al., 2007; Naqvi et al., 2007). Many reports proved the Neem to have strong efficacy against the insect emergence, reproduction and many biological and biochemical activities. There is no doubt to say that Neem and its products is a best replacement of synthetic conventional pesticides. In the present investigation the lethal chemicals that are Chlorpyrifos and Neem extract were found to have substantial capability to kill the larvae of *Musca domestica*. The fecundity and mating were reduced when treated with sublethal doses of Chlorpyrifos and NE (Neem Extract)

Table 1. Percent mean mortality of third instar Larvae of *Musca domestica* at various concentration of Chlorpyrifos

S.No.	Concentration (%)	Mean Mortality (%)
1	1.0	82
2	0.5	48
3	0.25	34
4	0.125	32
5	0.0625	16
6	Control	2

Table 2. Percent mean mortality of third instar Larvae of *Musca domestica* at various concentration of Neem Extract.

S.No.	Concentration (%)	Mean Mortality (%)
1	1	96
2	0.8	78
3	0.6	56
4	0.4	54
5	0.2	38
6	Control	4

Table 3. Effect of Chlorpyrifos on Fecundity of *Musca domestica L*.

Days	No of Eggs		Hatching	
	Control	Treated	Control	Treated
1	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil
4	60	Nil	45	Nil
5	40	Nil	35	Nil
6	30	Nil	20	Nil
7	50	18	35	Nil
8	Nil	Nil	Nil	Nil
9	Nil	10	Nil	Nil
10	40	Nil	25	Nil

Table 4. Effect of Neem Extract on Fecundity of *Musca domestica L*.

Days	No of Eggs		Hatching	
	Control	Treated	Control	Treated
1	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil
4	50	Nil	35	Nil
5	30	Nil	25	Nil
6	40	Nil	35	Nil
7	50	Nil	35	Nil
8	Nil	Nil	Nil	Nil
9	Nil	Nil	Nil	Nil
10	Nil	Nil	Nil	Nil

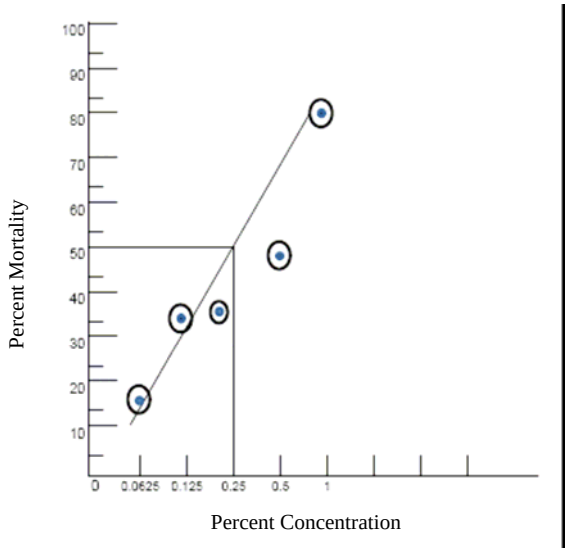

Fig. 1. Graph showing LC50 of chlorpyrifos against 3rd master larvae of *Musca domestica*.

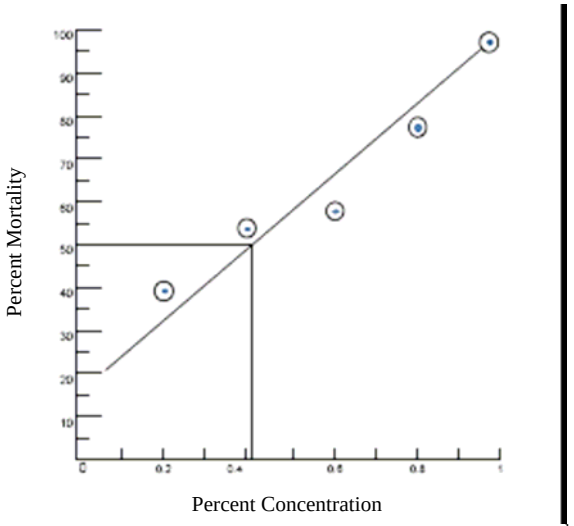

Fig. 2. Graph showing LC_{50} of Neem extract against 3rd inaster larvae of *Musca domestica*.

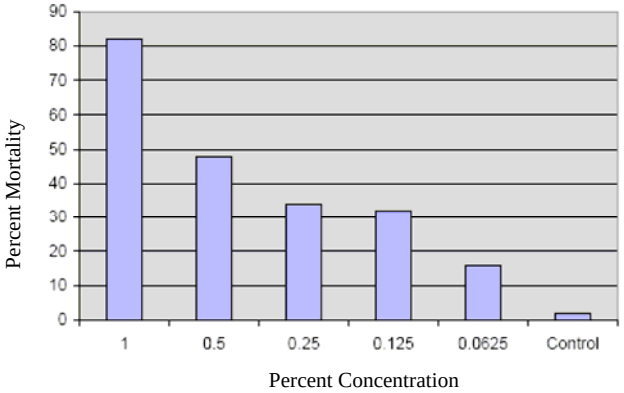

Fig. 3. Effect of chlorpyrifos on mortality of 3rd master larvae of *Musca domestica*.

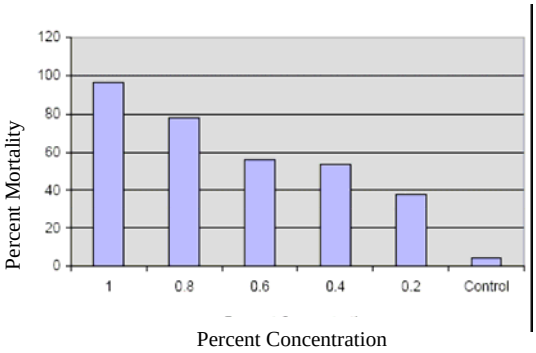

Fig. 4. Effect of Neem extract on mortality of 3rd master larvae of *Musca domestica*.

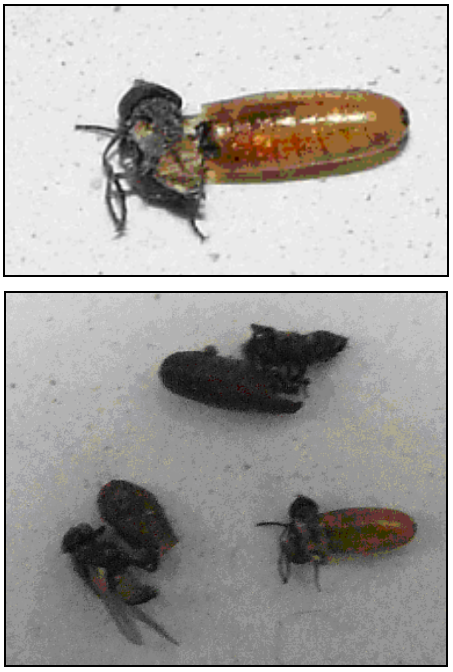

Photo 1. Pharate adults are unable to come out of the puparium.

Photo 2. Deformed pupa (treated with neem extract).

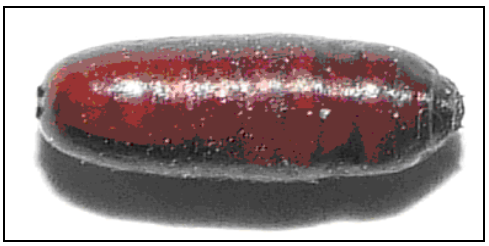

Photo 3. Untreated pupa (control).

Photo 4. Dead larvae show heavy melanization, treated with neem extract.

Photo 5. Deformed pupae treated, during larval stage, with neem extract.

Photo 6. Pupae (control) references.

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