

TOXICOLOGICAL EVALUATION OF CHLORPYRIFOS AND NEEM EXTRACT (BIOSAL B) AGAINST 3RD INSTARS LARVAE OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Third instars larvae of *Drosophila melanogaster* were assessed for toxicological evaluation of Chlorpyrifos and Neem extract. The larvae were found to be melanized and failed to pupate when both the compounds were applied. Lethal concentration (LC₅₀) of chlorpyrifos (40EC) 0.0127% and of neem extract (Biosal B) were found to be 2.4%. LC₅₀ were determined to be higher in Neem extract as compared to chlorpyrifos. Treatment of *Drosophila* with chlorpyrifos and Neem extract suggested that chlorpyrifos is very effective pesticide than Neem extract. Further, the melanization and antifeedent activity revealed that Neem extract may affect up to hormonal level of insect. Both compounds were found to inhibit the level of cholinesterase in treated insects as compared to control.

Key words: Chlorpyrifos, Cholinesterase, *Drosophila melanogaster*, Neem extract, Toxicity

INTRODUCTION

Drosophila melanogaster is the most commonly used insect for various experimental purposes. It occasionally becomes a pest in home, restaurant, and fruit markets. Some species are attracted to human and animal excrement; they also feed on uncooked foods serving as disease carriers. *Drosophila* S2 cells mimic early events in *Chlamydia trachomatis* host cell (Elwell and Engel 2005), *Drosophila* as a vector of life threatening causative pathogen, *Staphylococcus aureus* (Nedham *et al.* 2004). In the present investigation the two compounds have been used to produce mortality in late 3rd instar larvae of *Drosophila melanogaster*. One of them is an organophosphate compound (Chlorpyrifos) which is most widely used as a second generation pesticide (Naqvi, *et al.*, 1989) and the other is neem extract (Biosal B). Neem is the name of indigenous tree (*Azadiracta indica*), which is found to be of many advantages to people. The inhibition of cholinesterase activity leads to accumulation of acetylcholine at synapse, causing over stimulation and disruption of neurotransmission in both central and peripheral nervous systems. (Namba *et al.* 1971; Menzoni *et al.*, 2004) Neem is currently one of the world's most researched trees and believed to help solve global environmental and health concerns. The neem has been reported to contain several biological active compounds such as Azadiractin, Salanin, Nimbin and Nimbidin (Nakanishi, 1975; Warthen *et al.* 1978). All four Neem tree compounds (azadiractin, salannin, nimbin, and 6-desacetylnimbin) have been found to inhibit, in a dose-dependent fashion, the ecdysone 20-monooxygenase (E-20-M) activity in the third instar larvae of *Drosophila melanogaster*. (Mitchell *et al.*, 1997). Among biopesticides Neem extract are successfully used to date.

Schluter and Schutz, (1983) has also reported this compound to preventing the larvae from molting. Azadiractin is biodegradable and shows very low toxicity to mammals, thus being environmentally sound. The present study was designed to compare the efficacy of the chlorpyrifos and the Neem extract against the *D. melanogaster* 3rd instar larvae.

MATERIALS AND METHODS

A group of *D. melanogaster* were reared at room temperature 30-35°C and their sufficient number was obtained between 25-30 °C. The medium was kept hydrated for easy movement of larvae and kept away from dirt, direct sun light and other heat sources. Initial population of *D. melanogaster* was obtained from Saith Syfullah cold stores of banana in new fruit mandi situated at national high way, at a distance of 10km from Karachi city. The mass population was then brought to Toxicology laboratory, Zoology Department, and kept on natural diet (banana). The glass bottles were used for constant rearing of 3rd instar larvae. Among OP chlorpyrifos (40EC) and among biopesticide neem extract were used as test chemicals. Chlorpyrifos 40EC (kapadan) was purchased from market and Biosal "B" (neem extract) was obtained from Research institute of Chemistry, University of Karachi. Different concentration of chlorpyrifos, 0.1%, 0.05%, 0.025%, 0.0125%, 0.00625% and 0.003125% were prepared from 1% stock solution. Different concentrations of neem extract 5%, 4%, 3%, 2%, 1% and 0.5% were prepared from stock solution.

Experiments were conducted on 3rd instar larvae of *D. melanogaster* reared in the Insectory of Zoology Department University of Karachi. Seven sets of Petri dishes (One for control and six for treated) were taken

and placed on a laboratory bench. Control set was applied for environmental effects, and no check set was taken because the solution was prepared in distilled water. The two chemicals were applied on petri dishes containing 1gm of insect food (banana). For the provision of free space for pupation and roaming behavior of larvae, petri dishes of 12 mm diameter were selected. The petri dishes were labeled, viz., control, 0.1%, 0.05%, 0.025%, 0.0125%, 0.00625%, and 0.003125% for chlorpyrifos testings. By using a fine brush, 10 batches of larvae of *D. melanogaster* were released from bottles into each of the seven petri dishes.

The aforementioned concentration was then added to the respective petri dishes, with the help of pipette. After 24 hours of treatment mortality caused by the two compounds was noted. Five replicates of the same experiments were conducted and the mean % mortality was then determined. For neem compounds, the same method was applied for toxicity determination. Average values of mortality were plotted for mortality curve and determination of LC₅₀ values.

ENZYM ASSAY: For cholinesterase activity colorimetric method (Kit No.CE:190,041070B7AM) was applied. We are followed strictly the kit instruction. To calculate the activity of cholinesterase, the following formula was applied,

$$\% \text{ inhibition} = \frac{\text{CT-T}}{\text{CT}} \times 100$$

Remaining activity = 100- % inhibition

RESULTS AND DISCUSSION

Percent mortality due to the chlorpyrifos in 3rd instar larvae of *Drosophila melanogaster* is presented in Table 1, which shows that 0.1% of the pesticide caused 90% mortality during 24 hours, decline in chlorpyrifos concentration resulted in low larval mortality as at 0.05% concentration the percent mortality reduced to 66%.Decreases in mortalities as for 0.003125 % concentration the mortality was observed as 26%. Figure 1, shows the LC₅₀ value of chlorpyrifos (40EC) i.e. 0.0127%. For neem extract percent mortality at specific dose is represented in the table 2. When 5% dose was applied to 10 batches of 3rd instar larvae, the percent mortality after five trials was found to be 98%. To analyze the effect of neem extract below 98% mortality, we reduced the dose at various steps and finally when 0.5% dose was applied, it gave 12% mortality The effect of LC₅₀ value was found to be 2.4% as indicated in Fig 2.The cholinesterase activity in both the tested chemicals (chlorpyrifos & neem extract) was inhibited up to some extent. (Table 3 & 4)

Table 1: Percent mortality at various concentrations of chlorpyrifos and its statistical interpretation

Concentration%	Mortality mean %
Control	00
0.003125	26
0.00625	30
0.0125	42
0.025	58
0.05	66
0.1	90

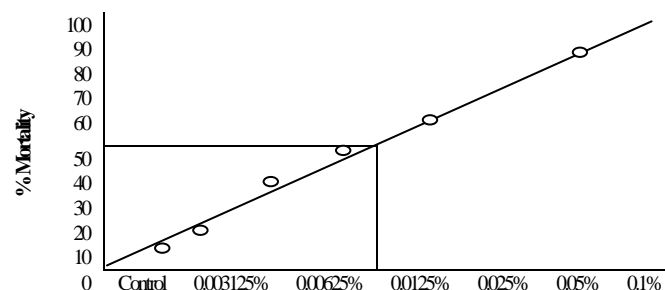


Fig. 01. Linear regression showing LC50 between percent concentration (chlorpyrifos) and percent mortality of *D. melanogaster*.

Table 2. Percent mortality at various concentrations of neem extract and its statistical interpretation

No	Concentration%	Mortality mean %
1	Control	00
2	0.5	12
3	1	24
4	2	56
5	3	62
6	4	88
7	5	98

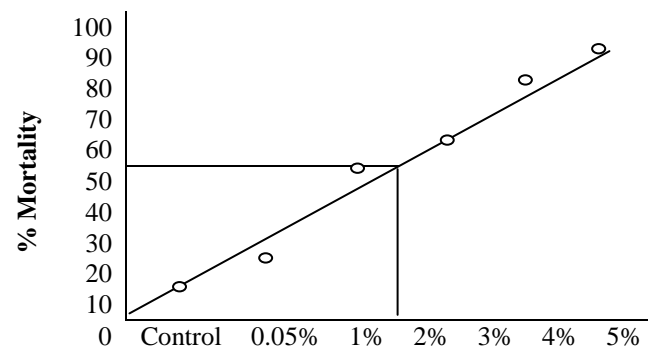


Fig # 02: Linear regression showing LC50 between percent concentration (Neem extract) and percent mortality of *D. melanogaster*.

Table 3: Percent Activity of Cholinesterase after LC₅₀ Treatment of Chlorpyrifos in 3rd Instar Larvae of *Drosophila melanogaster*

Duration	Control	0	30	60	90
		Second	Second	Second	Second
%Inhibition	0	5.63	7.33	8.96	9.67
Remaining Activity	100	94.37	92.67	91.04	90.33

Table 4. Percent Activity of Cholinesterase after LC₅₀ Treatment of Neem Compound in 3rd Instar Larvae of *Drosophila melanogaster*

Duration	Control	0	30	60	90
		Second	Second	Second	Second
%Inhibition	0	0.16	0.02	0.04	0.02
Remaining Activity	100	99.84	99.98	99.96	99.98

Comparison of melanized and normal larvae: In the present investigation chlorpyrifos (40 EC) gave noticeable mortality of the larvae at 0.1% concentration. Its LC₅₀ was found to be 0.0127%, which shows that this compound is very effective in controlling *Drosophila* larvae as it resulted in 50% death of larvae at lesser percentage of chlorpyrifos (Fig 2). In contrast to this the LC₅₀ of neem extract was 2.4% (Fig 4) and it gave maximum control at 4% and 5% concentration by producing 88% and 98% mortalities, respectively.

The above findings indicate that chlorpyrifos is more effective than neem extract. The difference may be due to immediate neurotoxic effect of organophosphate pesticides that kill the insect by directly inhibiting neuromuscular enzymes (Yasmin *et al.*, 1995; Gupta *et al.*, 2005) In the present work the organophosphate compound, chlorpyrifos inflict the same level of mortality at very low doses as compare to neem compound. (Naqvi *et al.*, 1989; Naqvi and Tabassum, 1992; Azmi *et al.* 1993). They all found RB-a (neem extract) to be less toxic killing agent than other tested compound. This is probably because of RB-b, being a crude extract contain very little active ingredient (Khan and Ahmad, 2000).

Naqvi *et al.* (1989) and Nurulain *et al.* (1994) worked on efficacy of neem fraction on white fly, and house fly respectively. They reported the immediate effects of organophosphate insecticides at low doses whereas they found a persistent toxicity of neem extracts. They reported that in terms of acute toxic effects neem extract, was required in higher doses as compared to conventional pesticides. Present finding is in accordance with their results and it confirmed that in addition to Hymenopterous pest species, Dipterous insects require lesser amount of organophosphate to produce toxic results as compared to neem extract. In the present work the treatment of *Drosophila* larvae with neem extract produced non specific development due to which the

pupation was delayed. In the present investigation not only the pupation of 3rd instar larvae of *Drosophila* was delayed but the treated larvae were also observed dead without pupation and with greater melanization. It may be due to disturbed process of ecdysis, by neem extract treatment due to which the pupation was delayed by 24 hours and before pupation their death occurred. (Naqvi, 1987).

Naqvi *et al.* (1995) reported the teratogenic effects of coopex pyreth and neem extract against 3rd instar larvae of *Musca domestica* and the larvae after 24 hours of treatment were found to be highly melanized. In the present study heavily pigmented larvae were observed but their number was insignificant. These differences may be due to the fact that in the present investigation the larvae were treated with organophosphate (chlorpyrifos) and neem extract whereas in the previous investigation pyrethroid (Coopex) was applied

Reports on larval stages of insects tested by neem extracts are few, but all of them revealed different states of toxicity against various larval stages and adults of different insect species. Use of different solvent in extraction, parts of the plant used for extraction whether leaves, barks, whole fruits, only seeds or kernels and these all may be responsible for the variation in the results. The kind and age of under test insects, and method of application is also valid reason for the difference in toxicity level (Naqvi *et al.*, 1995).

Uptill now resistance developed by insects against neem products have not been reported (Naqvi and Tabassum, 1992). Although organophosphate insecticides resistance have been widely known. Organophosphates produced immediate knockdown effect and very low dose can be applied for the 100 percent eradication of pest due to its neurotoxic activity. However, the cholinesterase level inhibited for some extent. So the accumulation of acetylcholine interfere with nerve impulse transmission (Namba *et al.*, 1971). The present findings of toxicity data are an addition to the scanty work on toxicological studies of *Drosophila melanogaster*.

The food deterioration is generally more by *Drosophila* larvae than adults, so the present report may provide an idea of its control, notably, by the greater melanization and developmental inhibition due to chlorpyrifos and neem extract. Neem and its products have been reported to be non toxic for human and domestic animals and have not or only relatively negligible side effects on beneficial organisms. These are out standing criteria of neem products which make their use desirable wherever possible.

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