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Laboratory evaluation of two organophosphate and one pyrethroid insecticide against the *Culex quinquefasciatus* (Say) (Diptera: Culicidae) mosquito larvae

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Abstract
Two organophosphate (OP) insecticides (Chlorpyrifos and Fenitrothion) and one synthetic pyrethroid insecticide (Deltamethrin) were evaluated against 4th instar larvae of *Culex quinquefasciatus* (Diptera: Culicidae) under laboratory condition. The larvae were collected from nearby area of Bangladesh Agricultural University, Mymensingh 2202 campus and reared at the Insect Biotechnology and Biopesticide Laboratory of Department of Entomology. For bioassay, five concentrations (viz. 10, 30, 50, 70 and 90ppm) of each of the insecticide were prepared. Ten actively swimming 4th instar larvae of *C. quinquefasciatus* were added into respective concentration. Mortality was recorded and the LC_{50} and LC_{90} values were determined by probit analysis. The LC_{50} values for Fenitrothion, Deltramethrin Chlorpyrifos, and were found to be 8.88, 26.73 and 71.14ppm, respectively. Thus, the Fenitrothion exhibited comparatively higher toxicity about 3 and 8 times more toxic compared to deltamthrin and chlorpyrifos. The order of the tested insecticides was found to be Fenitrothion > Deltamethrin > Chlorpyrifos. The LC_{90} values were also followed similar trend. Hence, the Fenitrothion and Deltamethrin could be suggested for *Culex* mosquito larvae management.

Keywords: efficacy, mosquito larvae, organophosphate, pyrethroid, chlorpyrifos, fenitrothion, deltamethrin

1. Introduction
The mosquitos belong to the diptera order are a group of tiny but very important insect pests. They serve as vector to transmit of many medically important pathogens and parasites such as viruses, bacteria, protozoans, and nematodes, which are responsible for causing deadly diseases such as malaria, dengue, yellow and Chikungunya fever, encephalitis or filariasis [3, 7]. As vectors of many zoonotic diseases, they are involved in human health problems in many countries including Bangladesh. The *Anopheles*, *Aedes* and *Culex* are the most three important genera of mosquito. Among these three genera, the *Culex* is one of the important genus serve as vector of many important diseases of humans, birds, and other animals such as West Nile virus, Japanese encephalitis, or St. Louis encephalitis, filariasis, and avian malaria. Apart from these, they cause biting annoyance and irritation through sucking blood. They are the most nuisance agents in both urban and rural areas predominantly in the developing countries including Bangladesh. A survey conducted by Khan *et al.* (2014) [11] reported presence of 13 species of mosquitos but the *Culex quinquefasciatus* became the predominant ones in Dhaka, Bangladesh.

Despite considerable national and international efforts aimed at suppressing these vector-borne diseases, they still obstruct improvement in health and socio-economical development in Bangladesh and other countries. Vector control is one of the important and most effective protective measures which attack the problem at its root. Vector control programs, however, mostly rely on use of synthetic chemical insecticides such as organochlorines, organophosphates and carbamates. Use of chemical insecticides already created a cascade of undesirable effects, for example, vectors become resistant to them [2, 3]. At the same time many of these insecticides are regarded as environmental pollutants responsible for residue problem and creating bio-hazards to human being, beneficial organisms, etc. Thus, mosquito control is becoming difficult day by day and it demands testing new insecticides against them. Quicker remedies are also the demand in epidemic condition. Hence, it is necessary to check the susceptibility of the commonly used chemical insecticides against the larvae of mosquitoes so that the most effective one insecticide could be identified as well as development of resistance, if any could also be ascertained. The present study was therefore undertaken to study the effectiveness of three commonly used chemical insecticides from two different groups (organophosphate and pyrethroid) against *Culex quinquefasciatus* mosquito larvae and to evaluate their relative toxicity for controlling the *Culex* larvae.

2. Materials and Methods

2.1 Collection and rearing of test insect's larvae

For laboratory bioassay, the larvae of the *C. quinquefasciatus* were used as test insect. The 1st instar larvae of the *C. quinquefasciatus* were collected from drains, pits, etc. of Keatkhali near Bangladesh Agricultural University campus, Mymensingh and brought to the laboratory during January to June, 2017. They were on goldfish feed in a plastic tray at the Insect Biotechnology and Biopesticide Laboratory of the Department of Entomology, Bangladesh Agricultural University. The larvae of *Culex quinquefasciatus* were identified following the identifying characteristics suggested by Bram (1967) [4]. The 4th instar larvae were used for bioassay test in an ambient environment (25±2˚C and 75-85% RH) of the laboratory.

2.2 Tested insecticides

Two commonly available organophosphate insecticides, Chlorpyrifos (trade name: Dursban 20EC, Auto Crop Care Limited) and Fenitrothion (trade name: Sumithion 50EC, Shetu Agro Industries Limited) and one synthetic pyrethroid insecticide, Deltamethrin (trade name:
Decis 2.5EC, Bayer Crop Science Limited) were selected to test their efficacy against 4th instar *C. quinquefasciatus* larvae.

2.3 Preparation of stock and tested concentration

At first a stock solution of 5000ppm concentration of the commercial product was prepared for each of the tested insecticide using 100ml of water. Then 100ml solutions for each of ten test concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100ppm) were made from pre-prepared stock solution by dilution method. Before the final tests, preliminary screenings using different concentrations (ppm) of the insecticides were conducted which helped to ascertain the dose ranges for obtaining 1-99% mortality.

2.4 Larvicidal bioassay tests

The efficacy of the selected insecticides against the larvae of *C. quinquefasciatus* was assessed by using the method suggested by WHO (1996) [15] and Dua *et al.* (2009) [6] with slight modification. Briefly, ten actively swimming 4th instar larvae were added into a beaker (250ml) containing 100ml along with one of the respective tested concentration. Three replicates of each concentration were prepared and three untreated controls were also prepared with water only.The experimental setup was kept at an ambient room temperature environment (14L: 10D photoperiod, 26±2ºC temperature and 80±5% relative humidity) in the laboratory during the test.
The number of larvae died at each of the concentrations at the end of the stipulated exposure period was recorded and the percentage mortality was calculated using the formula:

$$\text{Percentage of mortality} = \frac{\text{Number of larvae died}}{\text{Total number of test larvae}} \times 100$$

When the mortality in control was more than 5%, the percentage of mortality was corrected as per Abbott's (1925) [1] formula.

2.5 Statistical analysis

The toxicity of those insecticides was calculated as LC_{50} and LC_{90} values at 95% confidence intervals, lower and upper confidence limits, slopes, Chi-square values were determined by probit analysis [8] using Ldp line software (http://www.ehabsoft.com/ldpline/).

3. Results and Discussion

3.1 Efficacy of chlorpyrifos, fenitrothion and deltamethrin

The larvicidal efficacy of Fenitrothion, Deltamethrin, and Chlorpyrifos against *C. quinquefasciatus* larvae is shown in Figure 1(a), (b) and (c). It is found that the tested three insecticides were found effective against the *C. quinquefasciatus* larvae but exhibited varied mortality (Fig.1).
The mortality caused by Fenitrothion, Deltamethrin, and Chlorpyrifos was proportional to concentration and time i.e. the larval mortality percentage increased with the increase of concentration and time.
The Fenitrothion caused sharp increase of mortality of mosquito larvae. Initially, the mortality was slow but after 10 minutes of application exhibit dramatic increase from the beginning. More than eighty percent mortality was observed within 30 minutes of application of insecticide for all the concentration tested. All the larvae died (100% mortality) in case of 30ppm after 30 minutes of insecticide application (Fig. 1a).

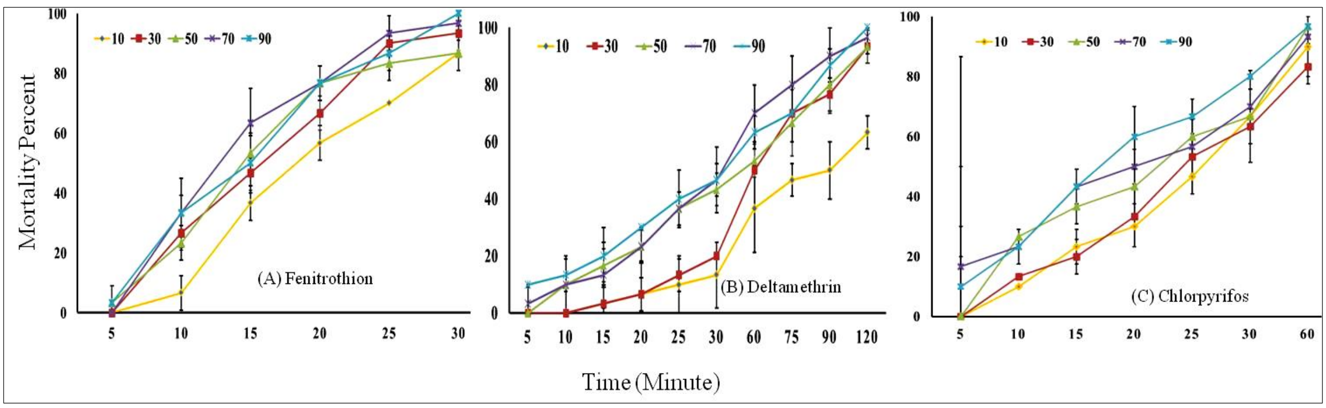

Fig 1: The larvicidal effectiveness of different concentrations (ppm) of Fenitrothion (a), Deltamethrin (b) and Chlorpyrifos (c) at different exposure time against *Culex quinquefasciatus* larvae

In case of Deltamethrin, the effect was initially very slow effect but after 30 minutes mortality rate increased gradually. It took about 60 minutes to cause 50% mortality. Compared to Fenitrothion, more than 90% mortality was observed after two hours (120 minutes) of insecticide application for all the concentrations tested except 10ppm (Fig. 1b).
On the other hand, the effect of Chlorpyrifos showed classic that the exposed larvae responded to the treatments in a concentration dependent manner. Compared to Fenitrothion, it requires at least 20 minutes exerting 50% mortality which was 15 minutes for the Fenitrothion. After 60 minutes of insecticide treatment, all the tested concentration caused more than 90% mortality of *C. quinquefasciatus* larvae.
Thus, our results indicates that the Fenitrothion is the quickest in causing mortality followed by the Deltamethrin while the Chlorpyrifos required most delayed effect on *C. quinquefasciatus* larvae.

3.2 Lethal concentration of chlorpyrifos, fenitrothion and deltamethrin and their relative toxicity

Based on the percentage of the larval mortalities, LC_{50} and LC_{90} values, Chi-square, slope and 95% confidence limits were calculated with Ldp line software and are shown in Table 1.

Table 1: Comparative toxicities of fenitrothion, deltamethrin and chlorpyrifos insecticides to 4th instar larvae of *Culex quinquefasciatus*

Insecticide	LC_{50} value (ppm) (95% CL*)	LC_{90} value (ppm) (95% CL)	Chi-square value	Slope
Fenitrothion	8.88 (3.19-14.36)	491.68 (233.46-2578.44)	13.679	0.735
Deltamethrin	26.73 (18.70-33.94)	969.80 (448.90-4229.64)	7.277	0.821
Chlorpyrifos	71.14 (51.61-148.80)	2296.53 (1708.29-151147.90)	18.931	0.849

*CL= Confidence upper and lower limits

Culex quinquefasciatus susceptibility to the tested insecticides varied considerably (Table 1). The larvae were least susceptible to Chlorpyrifos (LC_{50} = 71.144ppm and LC_{90} = 2296.532ppm). Deltamethrin was moderate in action; it showed LC_{50} value 26.727ppm and LC_{90} value 969.796ppm. Fenitrothion showed highest toxicity to the larvae. The LC_{50} value of Fenitrothion was 8.877ppm which was about 3 and 8 times more toxic compared to Deltamthrin and Chlorpyrifos, respectively. At the same time, the LC_{90} value for Fenitrothion was 491.675ppm which was around 2 times and 5 times more toxic compared to Deltamthrin and Chlorpyrifos, respectively (Fig.2).

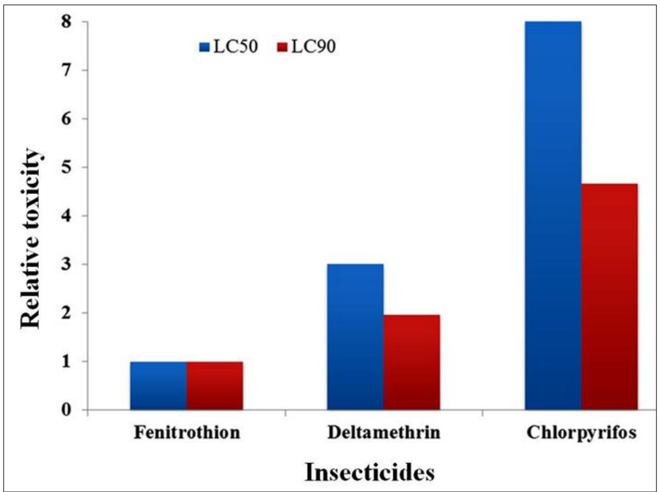

Fig 2: Relative toxicity of Fenitrothion, Deltamethrin and Chlorpyrifos against the*Culex quinquefasciatus* larvae based on the calculated LC_{50} and LC_{90} values

Dorta *et al.* (1993) [5] tested three organophosphorus compounds- malathion, folithion and temephos and two synthetic pyrethroids- alphamethrin and deltamethrin to six vector mosquito species. They reported that all mosquito species including the field strains of *C. quinquefasciatus* and *Ae. albopictus* were highly susceptible to the insecticides tested. The Deltamethrin was more effective against *C. quinquefasciatus* (lab) strain than the other mosquito species tested.
Two organophosphorous insecticides of Chlorpyrifos group, Reldan 40EC and Dursban 50EC were tested against the larvae of *C. quinquefasciatus* [10]. Dursban and Reldan were equally effective against *Aedes aegypti*, *Annopheles stephensi* and *An. culicifacies*. Reldan was about nine times more effective than Dursban against *C. quinquefasciatus*. Hossain *et al.* (1995) [9] and Kumar *et al.* (2011) [12] also reported efficacy of Deltamethrin on *C. quinquefasciatus* larvae. Our results also showed that Deltamethrin was effective against *C. quinquefasciatus* but less effective than Fenitrothion. We found that the efficacy of Fenitrothion to *C. quinquefasciatus* was higher than the others tested insecticides which are in agreement of the previous findings reported by Rettich (1997) [14] and Parsons and Surgeoner (1991) [13] who reported that the susceptibility of mosquito larvae including *Culex* spp. to Fenitrothion with several other insecticides was very high. This suggests that the use of theses insecticides especially the Fenitrothion for the management of C. *quinquefasciatus* larvae.

4. Conclusion

Laboratory evaluation were conducted to determine the efficacy of two organophosphate (OP) insecticides (Chlorpyrifos and Fenitrothion) and one synthetic pyrethroid insecticide (Deltamethrin) were evaluated against 4th instar larvae of *C. quinquefasciatus* by bioassay Mortality was recorded and the lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis. The calculated LC_{50} values for Fenitrothion, Deltramethrin and Chlorpyrifos were 8.88, 26.73 and 71.14ppm, respectively. Thus, the order of the tested insecticides was found to be Fenitrothion > Deltamethrin > Chlorpyrifos. The LC_{90} values were also followed similar trend. Based on our results it could be suggested that the Fenitrothion and Deltamethrin could be used for *Culex* larvae management.

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