

Susceptibility of third instar larvae of *Culex quinquefasciatus* Say (Culicidae: Insecta) against some commercial organophosphate and pyrethroid insecticides

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Abstract

Eight commercial insecticides-chlorpyrifos, bifenthrin, fenitrothion, cypermethrin, deltamethrin, cyhalothrin, dimethoate and malathion under the brand name of Dursban 20 EC, Bifenithrin 20 EC, Sumithion 50 EC, Relothrin 10 EC, Marker 2.5 EC, Reeva 2.5 EC, Tafgar 40 EC and Hilthion 57 EC, respectively were tested in laboratory at room temperature to evaluate the effective level against 3rd instar larvae of *Culex quinquefasciatus* Say from Savar area of Dhaka. The LC₅₀ values for chlorpyrifos, bifenthrin, fenitrothion, cypermethrin, deltamethrin, cyhalothrin, dimethoate and malathion were 0.127, 297.474, 0.308, 0.327, 0.198, 0.189, 0.054 and 0.031 ppm respectively and their corresponding LC₉₀ values were found to be 0.984, 2582, 0.041, 3.298, 0.728, 1.705, 0.192 and 0.030 ppm respectively. The relative potency of these insecticides tested to the larvae was found in respect to their LC₅₀ values in the order of Hilthion 57 EC (malathion) > Tafgar 40 EC (dimethoate) > Dursban 20 EC (chlorpyrifos) > Reeva 2.5 EC (cyhalothrin) > Marker 2.5 EC (deltamethrin) > Sumithion 50 EC (fenitrothion) > Relothrin 10 EC (cypermethrin) > Bifenithrin 20 EC. However, the relative potency of these insecticides tested to the larvae was found in respect to their LC₉₀ values in the order of Hilthion 57 EC (malathion) > Sumithion 50 EC (fenitrothion) > Marker 2.5 EC (deltamethrin) > Dursban 20 EC (chlorpyrifos) > Tafgar 40 EC (dimethoate) > Reeva 2.5 EC (cyhalothrin) > Relothrin 10 EC (cypermethrin) > Bifenithrin 20 EC. Taken together, the insecticide-malathion (Hilthion 57 EC) was found to be the most effective against the third instar larvae of *Cx. quinquefasciatus* in Savar area.

Key words: Insecticides third instar larvae, susceptibility, *Cx. Quinquefasciatus*.

INTRODUCTION

Culex quinquefasciatus Say is the most prevalent mosquito species in Dhaka city (Ameen & Moizuddin, 1973; Ameen *et al.*, 1982; 1984), which live in close association with human habitats. The adult mosquitoes are found hiding at day times in the darker places of bedroom, storeroom, kitchen, latrine etc. Due to their nocturnal feeding habits, they come out at the evening and suck blood and during their feeding transmit the disease to human and other animals. *Cx. quinquefasciatus* carry nematode parasite *Wuchereria bancrofti* causes *Bancroftian filariasis* in Bangladesh. (Wolfe & Aslamkhan., 1971; Barry *et al.*, 1971; Aslamkhan & Wolfe., 1972; Ahmed *et al.*, 1986). Filariasis is a kind of disease in which parasite worms invade the human lymphatic system swelling of surrounding tissue. In Dhaka city *Cx.* is also found transmitting 0.9% *Bancroftian*

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filariasis. Filariasis was detected among the human population at Mirpur in Dhaka city (Aslamkhan & wolfe., 1972; Ahmed *et al.*, 1986). Different organophosphorous and pyrethroid compounds are being tried as mosquito larvicide in Dhaka and adjacent areas in Bangladesh. Sometimes limited breeding place management (environmental management) has been taken in Dhaka city, such as filling, drainage, water flow management, improvement of sanitation, vegetation control, etc. Currently DCC is trying to educate the people on mosquitoes and its control. Dhaka City Corporation (DCC) currently spends over US\$ 2.5 million annually on various chemicals for larval and adult mosquito control attempts (Annual Budget, DCC, 2000-01). In towns and cities of Bangladesh mosquito coils, vaporizing mats and aerosol canisters are widely used by both underprivileged and rich communities for personal protection from mosquito bites.

The mosquito control activity revolves around a small number of pesticides over the year. In fact mosquito control programmes were based on exclusive use of pesticide (Kabir, 1987). This exclusive reliance on pesticides for controlling mosquito population failed often with disastrous consequences. Today's extreme severity of mosquito problem in Dhaka city is largely a result of over reliance on pesticides. (Ameen *et al.*, 1994) identified the main causes of this failure as: (a) sole dependence on chemical insecticides and the use of inappropriate insecticides, (b) untrained and inadequate manpower, and (c) lack of proper equipment. Since larvicides kill larvae in the breeding habitat before they can emerge into mature adult mosquitoes, larvicide treatment of breeding habitats helps reduce the adult mosquito population. Ahmed *et al.* (1986) suggested the following aspects to take under consideration when the insecticides are being used: (a) the susceptibility of the target species to the insecticides available for use, (b) the stability and the residual activity of the insecticide, and (c) the safety of the insecticide formulation for human and non-target organisms.

Since resistance to pesticides is common among the mosquitoes, an approach that is receiving renewed attention as a possible resistance-delaying tactic is the use of insecticides in mixtures or in rotation. This concept assumes that the mechanisms for resistance to each member chemical exist in such frequency that they do not occur together in any single individual in the population. Thus insects that may survive one of the chemicals in the combination are killed by the others. Use of the insecticides in mixtures, rotation or in specified sequences, however, will be limited by economic or practical considerations and also by the non-availability of appropriate adulticides (Kabir, 1987).

The most important factors that influence the development of resistance are the chemical diversity of the insecticides (Kabir, 1987) and the resistance phenomenon poses a threat for the future for three reasons: firstly, the number of cases of resistance continues to increase; secondly, when a pest strain has become resistant to a particular pesticide, no way has yet been found to restore the original effectiveness of the pesticide. Even when the use of that pesticide is discontinued for many years, resistance rapidly reappears when it is reapplied; and thirdly, the full effects of pest resistance have not yet been felt worldwide, because the areas involved are still limited (Rumkar & Horay 1972).

However, the development of resistance has led to serious problems in the mosquito control programme of Dhaka city (Kabir, 1987). To avoid the insecticide resistance problem investigation on the resistant populations of mosquitoes are necessary. Very few works were studied on mosquito population in Bangladesh. Although some experiments were carried out in the past to study the susceptibility level of mosquitoes to commonly used insecticides but insects are capable to change their susceptibility level within a few generations. In view of the above facts the present work was undertaken to screen out eight commercial insecticides to recheck their susceptibility levels against the larval populations of *Cx. quinquefasciatus* say in Dhaka District.

MATERIALS AND METHODS

Mosquito Collection: In the present study 3rd instar larvae of *Culex quinquefasciatus* were collected from Savar area of Dhaka District. The study was conducted from October 2016 to August 2017 in the laboratory condition at the temperature of 27°C and 77% humidity. The larvae were obtained from suitable breeding places of *Culex* mosquitoes such as drains, ditches, derelict ponds, stagnant drains and lakes of those locations. To collect test insect, various equipment were used such as Dipper, Enamel pan, Sweeping net, Ladle spoon, Dropper, Plastic jar etc. The larvae along with the water from the source area were kept in two-litre plastic jars covered with mosquito-proof netting. The open ends of the jars were covered with mosquito netting to avoid oviposition by other mosquitoes. The collected larvae were then brought to the laboratory and poured into a clean earthen bowl or enamel plate. The larvae were washed gently with tap water to clean them from adherent substances. The larvae were supplied with yeast and kept into mosquitoes cages.

Insecticides: Chlorpyrifos, Bifenithrin, Fenitrothion, Tafgar, Relothrin, Reeva, Markar, Hilthion-all these commercial insecticides were procured from local market and used to test the susceptibility level of third instar larvae of *Culex quinquefasciatus*. The insecticides were organophosphates (OPs) and pyrethroids (PY) in commercial formulations. Brief descriptions of these insecticides are given in Table 1.

Test procedure: The larvae were collected from the field and were kept in a jar with mosquito net cage at the opening so that the adults of another species of mosquitoes did not lay eggs and up to 20 3rd instar larvae were collected and kept in another plate. Then the larvae were introduced into the test dose concentration supplied with food (yeast). These dose concentrations were kept for 24 h/48 h and 72 h in secluded. Mortality counts were made after 24 hour, 48 hour and 72 hour. The results were recorded on specially designed table. Five replicates were done with each of the dose concentrations and as well five controls. When the control mortality was between 5%-20% it was corrected by Abbott's (1925) formula. The formula is

$$\frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100 - \% \text{ Control mortality}} \times 100$$

Dose Preparation: Insecticides were measured by a pipette and taken into a glass beaker of 1000ml. Then serial dilutions of these formulated products were by adding water. Five to seven concentrations were used. Different test doses were prepared making reduction of stock concentrations in the order of ¼ (250 ml in 1000 ml). For each test fresh solutions of insecticides were prepared and used on the same day. During the preparation of successive dilutions active ingredient (AI) of the insecticides were taken into consideration. The doses were used in terms of ppm (parts per million). Care was taken to avoid contamination of the laboratory equipment with insecticides.

Preparation of Stock Solution: The required amount of commercial insecticides for 100 ml of dilute (0.1% concentration) solution is obtained from following formula-

$$\text{Commercial insecticide (ml)} = \frac{\text{Quantity of diluted solution} \times \% \text{ of solution desired}}{\text{Strength (\%)} \text{ of commercial insecticide}}$$

Laboratory Bioassay: In all evaluations, a batch of 20 healthy, field collected 3rd instar larvae of *Cx. quinquefasciatus* were placed in 120 ml of disposable plastic cup containing 100 ml different concentrations of diluted insecticides with the help of dropper. The larvae were transferred carefully to avoid physical trauma. Any larva showing abnormalities e.g., a fuzzy appearance due to the presence of parasites on the body surface was discarded. For each larvicide, the larvae were exposed to serial doses from lower to higher concentrations. Some exploratory screening tests were performed to find out the range of dose concentrations and corresponding mortality rates. After the screening tests doses were prepared in such a way that, mortality in the tests were in the range from below 50% to above 50%. In each set of bioassay 5 to 7 different concentrations were used. Thus a total of 100 mosquito larvae were exposed to each dose of insecticides and five untreated controls without any insecticide were maintained during the test. Mortality counts were made 24 hours, 48 hours and 72 hours after the treatment. The larvae that did not move after probing with a glass rod were scored as dead. The moribund larvae (incapable of rising to the surface or of showing the characteristic diving reaction when the water was disturbed; they may also show discoloration, unnatural positions, tremors, incoordination or rigor) were not scored as dead.

Data Analysis: The dose-response data were analyzed using a probit analysis programme (computer software) developed by the Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio-45268. The title of the programme was EPA Probit Analysis Programme, Version 1.5, which was used to determine Chi-square values, Intercept values, Slope values, LC₅₀ and LC₉₀ values. Computer programme plotted a predicted regression line of percentage mortality against dose concentrations in ppm. Computations performed by this programme are based on Finney (1971). Graphical presentations were done using Microsoft Excel Programme 2010.

RESULTS AND DISCUSSION

The third instar larvae of *Cx. quinquefasciatus* from Savar area were exposed with different concentrations of eight commercial insecticides and their efficacies were evaluated at the laboratory.

Susceptibility of *Cx. quinquefasciatus* larvae against Dursban 20 EC: Chlorpyrifos (CPS), sold under many brand name of Dursban 20EC, is an organophosphate pesticide used to kill a number of pests including insects and worms (Rathold & Garg, 2017). The susceptibility of 3rd instar larvae of *Cx. quinquefasciatus* against six different doses of chlorpyrifos at different hours demonstrated that at 24 hours 95 and 5 percent mortality occurred at the doses of 1 ppm and 0.004 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes. After 48 hours 95 and 15 percent mortality resulted at doses 1 ppm and 0.004 and 0.001 ppm as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes. And after 72 hours 100 and 40 percent mortality resulted at doses 1 ppm and 0.004 ppm as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes.

Table 1. Efficacy of various insecticides at different concentrations against *Cx. Quinquefasciatus* larvae

Name of insecticide	Dose Concentration (ppm)	Percent (%) of mortality after 24hours	Percent (%) of mortality after 48hours	Percent (%) of mortality after 72hours
Dursban 20 Ec	1ppm	95	95	100
	0.25 ppm	40	55	90
	0.063 ppm	30	40	70
	0.016 ppm	15	25	50
	0.004 ppm	5	15	45
	0.001 ppm	10	15	40
	Control	5	5	10
Bifenithrin 20 EC	1ppm	35	50	60
	0.25 ppm	20	35	45
	0.063 ppm	25	45	55
	0.016 ppm	5	30	50
	0.004 ppm	10	15	35
	0.001 ppm	25	25	50
	Control	5	5	10
Sumithion 50 EC	1ppm	100	100	100
	0.25 ppm	100	100	100
	0.063 ppm	90	95	100
	0.016 ppm	65	75	85
	0.004 ppm	30	40	50
	0.001 ppm	10	30	45
	Control	5	5	5

Relothrin	1 ppm	70	80	90
10 EC	0.25 ppm	30	50	75
	0.063 ppm	10	20	30
	0.016 ppm	10	20	35
	0.004 ppm	5	15	30
	0.001 ppm	10	15	30
	Control	5	5	5
Reeva 2.5	1 ppm	75	95	100
EC	0.25 ppm	50	65	85
	0.063 ppm	15	30	45
	0.016 ppm	15	20	35
	0.004 ppm	5	25	35
	0.001 ppm	10	20	45
	Control	5	5	5
Marker 2.5	1 ppm	90	90	100
EC	0.25 ppm	55	70	85
	0.063 ppm	10	20	45
	0.016 ppm	10	15	30
	0.004 ppm	5	10	25
	0.001 ppm	10	25	50
	Control	5	5	5
Tafgar 40	1 ppm	100	100	100
EC	0.25 ppm	90	90	95
	0.063 ppm	60	70	90
	0.016 ppm	15	15	45
	0.004 ppm	15	15	25
	0.001 ppm	30	30	60
	Control	10	10	10
Hilthion	1 ppm	95	95	100
57 EC	0.25 ppm	90	90	95
	0.063 ppm	60	70	85
	0.016 ppm	15	35	70
	0.004 ppm	15	35	55
	0.001 ppm	10	15	35
	Control	5	10	10

Susceptibility of *Cx. quinquefasciatus* larvae against Bifenithrin 20 EC: Bifenithrin is a pyrethroid insecticide used primarily against aquatic organisms. Twenty-four hours exposure response of 3rd instar larvae of *Cx. quinquefasciatus* to six different doses of bifenithrin is presented in Table 1, which shows that 35 and 5 percent mortality resulted at doses 1 ppm and 0.016 ppm. Other insecticidal doses caused mortalities in between these two extremes. After 48 hours of exposure, 50 percent and 15 percent mortality were found at doses 1 ppm and 0.004 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes. And after 72 hours 60 percent and 35 percent mortality were found at doses 1 ppm and 0.004 ppm, respectively as given in Table 1. Other insecticidal doses caused mortalities in between these two extremes.

Susceptibility of *Cx. quinquefasciatus* larvae against Sumithion 50 EC: Fenitrothion is a phosphorothioate (organophosphate) insecticide; cheap and widely used worldwide. Response of 3rd instar larvae of *Cx. quinquefasciatus* to six different doses of fenitrothion is presented in Table 1, in which 100, 100 and 10 percent mortality occurred at doses 1 ppm, 0.25 ppm and 0.001 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes.

After 48 hours 100 percent and 30 percent mortality occurred at doses 1 ppm, 0.25 ppm and 0.001 ppm, respectively as displayed in Table 3. Other insecticidal doses caused mortalities in between these two extremes. After 72 hours 100 percent and 45 percent mortality occurred at doses 1 ppm, 0.25 ppm, and 0.001 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes.

Susceptibility of *Cx. quinquefasciatus* larvae against Relothrin 10 EC: The susceptibility of 3rd instar larvae of *Cx. quinquefasciatus* against six different doses of relothrin, a Cypermethrin insecticide, are presented in Table 1, which indicates that after 24 hours 70 and 5 percent mortality resulted at doses 1 ppm and 0.004 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes. After 48 hours 80 percent and 15 percent mortality resulted at doses 1 ppm, 0.004 ppm and 0.001 ppm, respectively as represented in Table 1. Other insecticidal doses caused mortalities in between these two extremes. After 72 hours 90 percent and 30 percent mortality resulted at doses 1 ppm, 0.004 ppm and 0.001 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes.

Susceptibility of *Cx. quinquefasciatus* larvae against Reeva 2.5 EC: Response of 3rd instar larvae of *Cx. quinquefasciatus* to six different doses of reeva, a pyrethroid, is shown in Table 1, which shows that after 24 hours 75 and 5 percent mortality resulted at doses 1 ppm and 0.004 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes. After 48 hours 95 percent and 20 percent mortality resulted at doses 1 ppm, 0.016 ppm and 0.001 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes. After 72 hours 100 percent and 35 percent mortality resulted at doses 1 ppm, 0.016 ppm and 0.004 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes.

Susceptibility of *Cx. quinquefasciatus* larvae against Marker 2.5 EC: The susceptibility of 3rd instar larvae of *Cx. quinquefasciatus* against six different doses of Marker, which shows that after 24 hours 90 and 5 percent mortality resulted at doses 1 ppm and 0.004 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes. After 48 hours 90 percent and 10 percent mortality resulted at doses 1 ppm and 0.004 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes. And after 72 hours 100 percent and 25 percent mortality resulted at doses 1 ppm and 0.004 ppm, respectively as shown in Table 1. Other insecticidal doses caused mortalities in between these two extremes.

Susceptibility of *Cx. quinquefasciatus* larvae against Tafgar 40 EC: Response of 3rd instar larvae of *Cx. quinquefasciatus* to six different doses of Tafgar, as shown in Table 1., which indicates that after 24 hours 100 and 15 percent mortality resulted at doses 1 ppm, 0.016 ppm and 0.004 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes. After 48 hours 100 percent and 15 percent mortality resulted at doses 1 ppm, 0.016 ppm and 0.004 ppm, respectively which is represented in Table 1. Other insecticidal doses caused mortalities in between these two extremes. And after 72 hours 100 percent and 25 percent mortality resulted at doses 1 ppm and 0.004 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes.

Susceptibility of *Cx. quinquefasciatus* larvae against Hilthion 57 EC: The susceptibility of 3rd instar larvae of *Cx. quinquefasciatus* against six different doses of hilthion are given in Table 2, which indicates that after 24 hours 95 and 10 percent mortality resulted at doses 1 ppm and 0.001 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes.

Estimated LC₅₀ and LC₉₀ values for Hilthion were found to be 0.061 ppm and 0.370 ppm, respectively are represented in Table 2. After 48 hours 95 and 15 percent mortality resulted at doses 1 ppm and 0.001 ppm, respectively. Other insecticidal doses caused mortalities in between these two extremes. Chi-square value, intercept and slope values along with 95% confidence limits obtained using computer programme. Estimated LC₅₀ and LC₉₀ values for hilthion were found to be 0.028 ppm and 0.424 ppm, respectively. Estimated LC₅₀ and LC₉₀ values for Hilthion were found to be 0.005 ppm and 0.110 ppm, respectively as shown in Table 2.

Table 2. Estimated LC Values and Confidence Limits for Hilthion 57 EC after exposure of 24, 48 and 72 hours

LC Values	After exposure of 24 hours			After exposure of 48 hours			After exposure of 72 hours		
	Exposure Concentration (ppm)	95% confidence Limits		Exposure Concentration (ppm)	95% confidence Limits		Exposure Concentration (ppm)	95% confidence Limits	
		Lower	Upper		Lower	Upper		Lower	Upper
LC ₅₀	0.061	0.028	0.107	0.028	0.008	0.062	0.005	0.001	0.011
LC ₉₀	0.370	0.197	1.255	0.424	0.177	2.173	0.110	0.043	0.567
LC ₉₅	0.619	0.298	2.915	0.917	0.332	7.585	0.268	0.091	2.295
LC ₉₉	1.618	0.619	14.824	3.899	1.003	85.337	1.434	0.332	34.689

According to the potency, the insecticides were compared that is a reciprocal of an equitoxic dose. Of these insecticides, Hilthion 57 EC (malathion) was found to be most toxic against the tested larvae (Table 3.), LC₅₀ being 0.054 ppm. Simultaneously, Tafgar 40 EC (dimethoate) whose LC₅₀ value was 0.054 ppm, Dursban 20 EC (chlorpyrifos) was placed third in position of toxicity with the LC₅₀ value of 0.127 ppm. Reeva 2.5 EC (cyhalodrin) ranked fourth in position of toxicity with the LC₅₀ value of 0.189 ppm. Marker 2.5 EC (deltamethrin) was found to be fifth in the position of toxicity with the

LC₅₀ value of 0.198 ppm. Sumithion 50 EC (fenitrothion) was sixth in the ranked of toxicity with the LC₅₀ values of 0.308 ppm. Relothrin 10 EC (cypermethrin) was seventh in the position of toxicity with the LC₅₀ value of 0.327 ppm. And Bifenithrin 20 EC was found to be the least toxic against the treated larval population, of all insecticides. The LC₅₀ value of Bifenithrin was 297.474 ppm. The relative potency of eight tested insecticides to larvae of *Cx. quinquefasciatus* on the basis of LC₅₀ values (Table 3.) in order of decreasing toxicity was as follows: Hilthion 57 EC (malathion)> Tafgar 40 EC (dimethoate)> Dursban 20 EC (chlorpyrifos)> Reeva 2.5 EC (cyhalothrin)> Marker 2.5 EC (deltamethrin)> Sumithion 50 EC (fenitrothion)> Relothrin 10 EC (cypermethrin)> Bifenithrin 20 EC.

Inadequate laboratory data is the main obstacle for comparing susceptibility of various populations of *Cx. quinquefasciatus* in Bangladesh to insecticides. It is hard to clearly demonstrate whether the high LC values of these insecticides resulted from tolerance or acquired resistance (due to prolonged exposure to the same group of insecticides) in the exposed *Cx. quinquefasciatus* larvae, as no susceptible laboratory population of *Cx. quinquefasciatus* was maintained in Dhaka or in Any other place of Bangladesh for purposes of susceptibility comparisons. In a word, it is assumed that this may be due to acquired resistance as Dhaka City Corporation previously sprayed insecticides like chlorpyrifos, malathion, deltamethrin, cypermethrin, cyhalothrin, bifenithrin, fenitrothion and dimethoate as larvicides against *Cx. quinquefasciatus* in Dhaka.

Table 3. LC₅₀ and LC₉₀ values with 95% confidence limits of test insecticides against 3rd instar larvae of *Cx. Quinquefasciatus*

Name of insecticides	LC values with 95% confidence limits					
	LC ₅₀	95% confidence limits		LC ₉₀	95% confidence limits	
		Lower	Upper		Lower	Upper
Dursban 20 EC	0.127	0.054	0.240	0.984	0.440	7.27
Bifenithrin 20 EC	297.474	0.16	410	2582	59.26	100
Sumithion 50 EC	0.308	0.002	0.010	0.041	0.022	0.131
Relothrin 10 EC	0.327	0.145	0.76	3.298	1.049	145.7
Reeva 2.5 EC	0.189	0.079	0.379	1.705	1.557	22.02
Marker 2.5 EC	0.198	0.092	0.329	0.728	0.467	2.91
Tafgar 40 EC	0.054	0.018	0.072	0.192	0.089	0.512
Hilthion 57 EC	0.031	0.012	0.06	0.030	0.139	1.33

In the present study, the test insects from Savar area showed the LC₅₀ values of chlorpyrifos was 0.127 ppm which indicates that *Cx. quinquefasciatus* of Savar area are going to be resistant to chlorpyrifos. (Ali *et al.*, 1999) tested five organophosphates including chlorpyrifos and obtained the LC₅₀ value of chlorpyrifos 0.065 ppm against the late 3rd and early 4th instar larvae of *Cx. quinquefasciatus* from Karwan Bazar area of Dhaka city. This may be due to the fact that chlorpyrifos is no longer used as mosquito control insecticide in Dhaka city resulting in the consequent increase of susceptibility levels.

During the present investigation Savar area are going to be resistant to malathion. Hashem, 2001 tested malathion, deltamethrin and DDT against adult mosquito populations from 9 locations of Dhaka city and one location in Tongi and recorded the level of resistance to malathion which was moderate among mosquitoes from most locations. A large volume of malathion was used in Dhaka city as an adulticide during the year of 2002 and also early 2003. This excessive use of malathion might have caused the rapid loss of susceptibility among mosquito populations.

In the present investigation, 3rd instar larvae of *Cx. quinquefasciatus* from Savar area were also found to be resistant to deltamethrin. But Hashem, (2001) observed that only the population from Jatrabari was resistant to deltamethrin. Begum (2001) showed that the test population of *Cx. quinquefasciatus* from Mohakhali and Khilgaon were highly resistant to malathion. In this study, larvae from Savar area, the LC₅₀ values of chlorpyrifos was 0.127 ppm, which showed that the larvae of *Cx. quinquefasciatus* are going to be resistant to chlorpyrifos. Begum, (2001) observed that LC₅₀ value of chlorpyrifos was 0.065 ppm against 3rd and 4th instar larvae of *Cx. quinquefasciatus*.

In case of present study, the LC₅₀ value of chlorpyrifos was found to be 0.127 ppm, which indicates that *Cx. quinquefasciatus* larvae of Savar area of Dhaka city are now going to become resistant to chlorpyrifos. Saha, (2003) tested five organophosphate insecticides including chlorpyrifos and obtained the LC₅₀ value of chlorpyrifos 0.022 ppm against the 3rd instar larvae of *Cx. quinquefasciatus* from Gulshan area of Dhaka city. The increased resistance towards chlorpyrifos in larval populations may have resulted due to continuous use of this insecticide by the Dhaka city corporation (DCC) for larval control.

Saha, (2003) recorded the LC₅₀ value of fenitrothion 0.032 ppm against the late 3rd instar larvae of *Cx. quinquefasciatus* from Gulshan area of Dhaka city. Although fenitrothion has been widely used for agricultural pest control, its use against public health pest such as mosquitoes in the past has been limited. The *Cx. quinquefasciatus* larvae developed resistance to fenitrothion due to cross-resistance by other OP insecticides.

Das *et al.* (1982) reported the susceptibility of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. Culicifacies* against four insecticides. Bendiocarb, primiphos-methyl, permethrin and phendal were evaluated in the laboratory against larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. Culicifacies*. It was found that bendiocarb was the most effective adulticide and permethrin, the most effective larvicide. Begum & Bhuiya (1983) observed the effect of DDT, gammaxene, aldrin, dieldrin, heptachlor, abate dipterex and imidan against *Cx. fatigans* larvae to find out the susceptibility level. Abate was most toxic to the larvae, whereas dipterex and imidan exhibited very low degree of toxicity. The relative toxicities of eight insecticides to the larvae were found to be in the order: Abate (LD₅₀ = 0.441) > Aldrin (LD₅₀ = 1.05) > Dieldrin (LD₅₀ = 1.53) > DDT (LD₅₀ = 1.75) > Heptachlor (LD₅₀ = 1.95) > Gemmaxane (LD₅₀ = 2.35) > Dipterex (LD₅₀ = 3.35) > Imidan (LD₅₀ = 4.44).

Verma & Rajvanshi (1983) observed the larvicidal efficacy of two synthetic pyrethroids against mosquitoes. From LC₅₀ values, they observed that the tested synthetic pyrethroids

were most effective against *Cx. quinquefasciatus*, followed by *Ae. aegypti* and *An. stephensi*. Cypermethrin was found to be more potent than permethrin against the three species of mosquitoes. Against *Cx. quinquefasciatus*, cypermethrin was found to be 6.59 and 19.36 times more effective than permethrin at LC₅₀ and LD₉₀ levels respectively. Zannat (2003) recorded the susceptibility status of the field collected late 3rd and early 4th instar larvae of *Culex quinquefasciatus* from seven different locations of Dhaka city (five locations from the inside part and two from the peripheral region) to commonly used five organophosphates larvicides (Chlorpyrifos, fenitrothion, malathion, diazinon and primiphos-methyl) were evaluated in the laboratory at room temperature (26 to 30^o C). The susceptibility of the seven larval populations to the tested organophosphates (OPs) varied considerably. Among five OPs the highest LC₅₀ value was recorded for malathion (1.157 ppm) against larvae from Dhanmondi and the lowest LC₅₀ value was recorded for chlorpyrifos (0.004 ppm) against the larvae from Nikunja. The highest range of toxicity was obtained for diazinon (LC₅₀ values ranging from 0.008 to 0.261 ppm). Larvae from the inside part of the city showed least susceptibility to all OPs than larvae from peripheral region. Against the seven larval populations, chlorpyrifos was the most effective and malathion was the least effective among insecticides test.

From this investigation, it is clear that mosquito population is going to be resistance to a number of organophosphates and pyrethroids insecticides. For this reason it greatly complicates the control operation and also increases operational cost. To prevent the growing of resistance several steps should be taken such as putting more emphasis on non-chemical control of mosquitoes using bio-control agents, environmental management, creating public awareness. Insecticides should be used as the least resort. When used, all insecticides should be used judiciously. Repeated use of same insecticide should be avoided at all cost. Rotational use of insecticide delays the development of resistance.

REFERENCES

- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**:265-267.
- Ahmed, T.U. Maheswary, N.P. and Khan, N.I. 1986. Filariasis in Mirpur area of Dhaka City. *Bangladesh Med. Res. Counc. Bull.* **12**(2):83-94.
- Ali, A., Chowdhury, M.A., Hossain, M.I., Ameen, M., Habiba, D.B. and Aslam, A.F.M. 1999. Laboratory evaluation of selected larvicides and insect growth regulators against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. *J. Am. Mosq. Control Assoc.* **15**(1):43-47.
- Ameen, M. and Moizuddin, M. 1973. Bionomics of the common mosquitoes of Dacca. *J. Nat. Hist.* **7**: 1-21.
- Ameen, M., Hossain, M.I. and Khan, M.D.H. 1982. Resting behavior, biting activity pattern and host preference of the common mosquitoes of Dacca city. *Bangladesh. J. Zool.* **10**(1):35-48.
- Ameen, M., Hossain, M.I. and Khan, M.D.H. 1984. Seasonal prevalence of the common mosquitoes of Dhaka city. *Dhaka University Studies*, B, **32**(2):79-89.
- Ameen, M., Chowdhury, M.A. and Hossain, M.I. 1984. Survey of mosquito breeding sites in the city of Dhaka: a report submitted to the Dhaka City Corporation. Dhaka. 77pp.

- Amin, A.M. and White, G.B. 1985. Resistance spectra and allelism of chlorpyrifos resistance factors in *Culex quinquefasciatus* populations from Colombo (Srilanka) and Dar-us-salaam (Tanzania). *Insect Sci. Appl.* **6(1)**:97-104.
- Andrade, C.F.S., Moolo, M. and De-Andrade, C.F.S. 1991. Susceptibility of *Aedes aegypti* larvae to temephos and *Bacillus thuringiensis* var. *israelensis* in integrated control. *Revista-de-publica.* **25(3)**:184-187.
- Anonymous. 1991. Studies on the susceptibility of *Culex quinquefasciatus* Say larvae of Dhaka city to some organophosphorous and pyrethroid insecticides. Unpublished M.Sc. Thesis. Dept. Zool. Univ. Dhaka. 89 pp.
- Anonymous. 1997. Larvicidal effects of three common insecticides against mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) at different temperature. Unpublished M.Sc. Thesis. Dept. Zool. Jahangirnagar University, Savar, Dhaka, Bangladesh. 83 PP.
- Aslamkhan, M. and Wolfe, M.S. 1972. *Bancroftian filariasis* in two villages in Dinajpur district, East Pakistan. II Entomological investigation. *Am. J. Trop. Med. Hyg.* **21**:30-37.
- Begum, A. and Bhuiya, M.M.B. 1983. The susceptibility of *Culex fatigus* larvae to insecticides. *Bangladesh J. Zool.* **11(1)**:39-41.
- Begum, D.H. 2001. Determination of susceptibility levels to pesticides on mosquito *Culex quinquefasciatus* Say of Dhaka city. Unpublished M.Sc. Thesis. Dept. Zool. Dhaka university, Bangladesh. 156 pp.
- Das, P.K., Mariappan, T. and Reddy, C.B.S. 1982. Susceptibility of *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi* against insecticides. *Indian J. Med. Res.* **75(Apr.)**: 529-533.
- Finney, D.J. 1971. Probit analysis. Third ed. Cambridge University Press, Cambridge. 333pp.
- Hashem, A. 2001. Susceptibility in *Culex quinquefasciatus* Say populations to some selected pesticides. Unpublished M.Sc. Thesis. Dept. Zool. Jahangirnagar University, Savar, Dhaka, Bangladesh. 121 pp.
- Kabir, S.M.H. 1987. Role of insecticides in mosquito control and resistance problem. *Bangladesh J. Zool.* **2**: 43-49.
- Rathod, AL; Garg, RK (April 2017). "Chlorpyrifos poisoning and its implications in human fatal cases: A forensic perspective with reference to Indian scenario". *Journal of forensic and legal medicine.* **47**: 29–34.
- Saha, P. 2003 effectiveness and residual action of five selected organophosphate larvicides against third instar larvae of *Cx. Quinquefasciatus*. Unpublished M. Sc. Thesis. Dept. Zool. Jahangirnagar University, Savar, Dhaka, Bangladesh. 84 pp.
- Verma, B.L. and Rajvanshi, A.C. 1983. Larvicidal efficacy of two synthetic pyrethroids against mosquitoes. *J. Com. Dis.* **15(3)**: 219-221.
- Zannat, T. 2003. Susceptibility levels of larvae of *Culex quinquefasciatus* to commonly used insecticides in Dhaka city. Unpublished M. Sc. Thesis. Dept. Zool. Jahangirnagar University, Savar, Dhaka, Bangladesh. 161 pp.