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LABORATORY EVALUATION OF SELECTED LARVICIDES AND INSECT GROWTH REGULATORS AGAINST FIELD-COLLECTED *CULEX QUINQUEFASCIATUS* LARVAE FROM URBAN DHAKA, BANGLADESH

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ABSTRACT. Five organophosphates (OPs) (chlorpyrifos, chlorpyrifos methyl, fenthion, malathion, and temephos), 3 pyrethroids (bifenthrin, cypermethrin, and permethrin), 1 phenyl pyrazole (fipronil), 2 microbial pesticides (*Bacillus thuringiensis* serovar. *israelensis* [*B.t.i.*] and *Bacillus sphaericus*), and 3 insect growth regulators (IGRs) (diflubenzuron, methoprene, and pyriproxyfen) were evaluated against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. The LC_{90} values of all OPs, except for temephos (LC_{90} = 0.0096 ppm), were high, ranging from 0.13 ppm (fenthion) to 2.882 ppm (chlorpyrifos methyl). Pyrethroid LC_{90} values were 0.021 ppm (bifenthrin), 0.00061 (cypermethrin), and 0.017 ppm (permethrin). Fipronil exhibited a superior activity with LC_{90} value of 0.000896 ppm. Technical powders of *B.t.i.* and *B. sphaericus* (VectoBac® TP and VectoLex® TP) were considered highly effective against the *Cx. quinquefasciatus* larvae. The IGRs also were effective with pyriproxyfen (LC_{90} = 0.0011 ppm), being 3 times and 47 times more active than diflubenzuron (LC_{90} = 0.0034 ppm) and methoprene (LC_{90} = 0.052 ppm), respectively. In general, toxicity ranking of chemicals and microbials tested was phenyl pyrazole > IGRs > pyrethroids > microbials > OPs.

KEY WORDS Bioassays, mosquito larvae, organophosphates, pyrethroids, fipronil, *Bacillus thuringiensis* serovar. *israelensis, Bacillus sphaericus,* insect growth regulators

INTRODUCTION

In recent years, populations of the mosquito *Culex quinquefasciatus* Say in Bangladesh, and particularly in the capital city of Dhaka, have increased dramatically (Ameen et al. 1984, Hossain et al. 1996). An extremely dense human population of nearly 7 million people dwelling in a 225-km² area (Department of Geography, University of Dhaka 1994), lack of proper and/or adequate disposal of wastes associated with human and animal activities, poor city planning combined with relatively rapid growth and development, and resource limitations are some of the contributing factors encouraging development and propagation of this mosquito associated with polluted waters. Favorable climatic conditions, such as rainfall, cyclones, floods, etc., that are suitable for the creation of new mosquito habitats and for the recharging of nutrients and water in the existing habitats are also conducive to increasing the mosquito populations. The higher temperatures in the tropics may also have a positive influence on mosquito productivity through accelerated developmental rates resulting in increased numbers of mosquito generations in a year as well as increased mosquito larval food supplies because of more efficient photosynthesis.

A rather rigorous larval population survey conducted by Ameen et al. (1994) had identified several types of *Cx. quinquefasciatus* habitats in the Dhaka municipality city area. This survey examined 1,742 mosquito breeding sites covering a total water surface area of 530 ha (nearly 2% of the total Dhaka municipality area at the time of survey) and showed that the lowest mean mosquito density of 22 larvae/m² (range 0–258 larvae/m²) occurred in lakes and the highest (11,283 larvae/m²; range 0–286,950 larvae/m²) in derelict ponds. The high larval densities and scattered habitats of *Cx. quinquefasciatus* throughout Dhaka often result in widespread and large adult populations. *Culex quinquefasciatus* adults become a severe biting nuisance, especially during the winter months (Ameen et al. 1982, Hossain et al. 1996). The role of *Cx. quinquefasciatus* as a vector of bancroftian filariasis in many districts of Bangladesh (Wolfe and Aslamkhan 1971) and in the Mirpur area of Dhaka, with 1.5% filariasis endemicity, has been reported (Ahmed et al. 1986).

The city of Dhaka currently spends over 1 million U.S. dollars annually in various (primarily chemical) larval and adult mosquito control attempts (Ameen et al. 1994). However, these attempts often produce inadequate control, and the biting nuisance continues despite these relatively costly measures; perhaps because of nonsystematic and haphazard control approaches as well as presently insufficient knowledge of the mosquito's bionomics and its susceptibility status to various insecticides (Ameen et al. 1994). We conducted laboratory bioassays of selected larvicides and insect growth regulators (IGRs) in an attempt to determine the susceptibility status of *Cx. quinquefasciatus* lar-

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vae in a selected area of Dhaka to these possible control materials.

MATERIALS AND METHODS

For laboratory bioassays, field-collected *Cx. quinquefasciatus* larvae were utilized. These larvae, as needed, were collected from mosquito habitats in the Kawran Bazar area of urban Dhaka in May 1997.

Five organophosphates (OPs) (chlorpyrifos, chlorpyrifos methyl, fenthion, malathion, and temephos), 3 pyrethroids (bifenthrin, cypermethrin, and permethrin), 1 phenyl pyrazole (fipronil), 2 microbials (*Bacillus thuringiensis* serovar. *israelensis* [*B.t.i.*] and *Bacillus sphaericus*), and 3 IGRs (diflubenzuron, methoprene, and pyriproxyfen) were tested against *Cx. quinquefasciatus* larvae.

Technical grade materials of chlorpyrifos (99%), chlorpyrifos methyl (99.8%), fenthion (96.5%), malathion (95%), temephos (96.5%), bifenthrin (93.7%), cypermethrin (92.3%), permethrin (94.6%), fipronil (97.1%), diflubenzuron (90%), methoprene (95.6%), and pyriproxyfen (97%) were utilized in this study. The OPs, pyrethroids, fipronil, and IGRs were individually dissolved in acetone to prepare a 1% stock solution (w/v) of each material and its 6–9 serial dilutions. Technical powders of *B.t.i.* (VectoBac® TP, containing 8,300 international toxic units [ITU]/mg) and *B. sphaericus* serotype H5a5b, strain 2362 (VectoLex® TP, containing 600 ITU/mg) were also evaluated. All *B.t.i.* and *B. sphaericus* formulations were mixed with tap water (pH 7.1) to prepare 1% (w/v) stock solutions and 4–7 serial dilutions.

Mosquito bioassay methods for OPs, pyrethroids, and fipronil were generally similar to those of Mulla et al. (1982). For *B. sphaericus* and *B.t.i.* bioassay methods, we used the test procedures of Ali and Nayar (1986) and Ali et al. (1981). The IGRs were evaluated in the manner described by Mulla et al. (1974). For OPs, pyrethroids, and fipronil, late 4th-instar *Cx. quinquefasciatus* were utilized. The IGRs, *B.t.i.*, and *B. sphaericus* were tested against late 3rd- and early 4th-instar *Cx. quinquefasciatus*. In all evaluations, 20 mosquito larvae were placed in 120-ml disposable paper cups containing 100 ml tap water. Five to 9 different concentrations of each larvicide or IGR were tested on at least 3 different occasions. Each concentration was replicated 3 times, and 3 untreated controls receiving only 1 ml of acetone were maintained during tests with the OPs, pyrethroids, fipronil, and IGRs. Controls in *B.t.i.* and *B. sphaericus* tests did not require addition of acetone because their stock solutions and serial dilutions were prepared with tap water. One milliliter of 1% beef liver and yeast (1:3) was added to each cup once for cups receiving OPs, pyrethroids, fipronil, *B.t.i.*, *B. sphaericus*, and their respective controls. In IGR tests lasting 7–10 days, larval food was added to each cup at 2-day intervals. Larval mortality in the tests of OPs, pyrethroids, and *B.t.i.* was scored after 24 h of exposure. Fipronil and *B. sphaericus* tests were extended to 48 h to assess larval mortality. In experiments with IGRs, treated and control cups were examined daily for any larval, pupal, or adult mortality, and cumulative mortality was recorded at the termination of the test when adult emergence was completed in control cups and no living larva or pupa remained. A 14-h photoperiod and 28 ± 3°C temperature were maintained in the evaluation room during the tests. Mortality in treatments was corrected for any control mortality, and the data were subjected to a log-dose-probit regression analysis (United States Environmental Protection Agency 1994) to estimate larval dosage response to the test materials.

RESULTS

Culex quinquefasciatus susceptibility to the tested OPs varied considerably (Table 1). Larvae were most susceptible to temephos (LC_{90} = 0.0096 ppm) and least susceptible to chlorpyrifos methyl (LC_{90} = 2.882 ppm). In general, the LC_{90} values of chlorpyrifos, chlorpyrifos methyl, fenthion, and malathion were rather high, ranging from 0.13 ppm (Fenthion) to 2.882 ppm (chlorpyrifos methyl). Among the pyrethroids, cypermethrin was the most toxic (LC_{90} = 0.00061 ppm), followed by permethrin (LC_{90} = 0.017 ppm) and bifenthrin (LC_{90} = 0.021 ppm); cypermethrin was 34 times and 28 times more toxic to *Cx. quinquefasciatus* larvae than were bifenthrin and permethrin, respectively. Fipronil was highly active against *Cx. quinquefasciatus* larvae, with an LC_{90} value of 0.000896 ppm. This level of activity of fipronil in the parts per billion range was comparable with the most toxic pyrethroid, cypermethrin (LC_{90} = 0.00061 ppm).

The technical powder of *B.t.i.* (VectoBac TP) produced excellent larval mortality of *Cx. quinquefasciatus*, with an LC_{90} value of 0.024 ppm (Table 2). *Bacillus sphaericus* technical powder (VectoLex TP) of only 600 ITU/mg potency also gave a positive result, with an LC_{90} value of 0.222 ppm.

All 3 IGRs were effective against *Cx. quinquefasciatus* larvae, with LC_{90} values of 0.0011 ppm (pyriproxyfen), 0.0034 ppm (diflubenzuron), and 0.052 ppm (methoprene) (Table 3). Thus, among the IGRs tested, pyriproxyfen was 3 times and 47 times more active than were diflubenzuron and methoprene, respectively.

DISCUSSION

It is evident from these laboratory studies that *Cx. quinquefasciatus* larval populations in the vicinity of Dhaka tolerated rather high concentrations of OPs (chlorpyrifos, chlorpyrifos methyl, fenthion, and malathion). Because no susceptible laboratory population of *Cx. quinquefasciatus* is maintained in Dhaka or in Bangladesh for purposes of suscepti-

Table 1. Comparative laboratory toxicities of selected organophosphates, pyrethroids and a phenyl pyrazole insecticide to field-collected 4th-instar larvae of *Culex quinquefasciatus* from Kawran Bazar area, Dhaka, May 1997.

	24-h lethal concentration (ppm)				
Insecticide	LC_{50}	95% CL[1]	LC_{90}	95% CL	Slope
Organophosphates					
Chlorpyrifos	0.065	0.019–0.168	0.619	0.22–11.135	1.31
Chlorpyrifos methyl	0.164	0.118–0.227	2.882	1.775–5.840	1.03
Fenthion	0.062	0.053–0.069	0.130	0.111–0.170	3.96
Malathion	0.047	0.022–0.111	0.747	0.254–6.896	1.06
Temephos	0.0024	0.0016–0.0044	0.0096	0.006–0.216	2.32
Pyrethroids					
Bifenthrin	0.009	0.006–0.021	0.021	0.012–0.198	2.74
Cypermethrin	0.00017	0.00012–0.00025	0.00061	0.00038–0.0017	2.33
Permethrin	0.005	0.003–0.009	0.017	0.009–0.079	2.43
Phenyl pyrazole[2]					
Fipronil	0.00035	0.00004–0.00066	0.000896	0.0005–0.0052	3.09

[1] CL = confidence limits.
[2] 48-h lethal concentration.

bility comparisons, it is difficult to clearly demonstrate whether the high LC_{90} values of these OPs resulted from natural or aquired resistance in the exposed *Cx. quinquefasciatus* larvae. However, acquired resistance is a good possibility because OPs, such as chlorpyrifos, chlorpyrifos methyl, diazinon, and malathion, have been previously used as larvicides of *Cx. quinquefasciatus* in Dhaka by the Dhaka City Corporation. High LC_{90} values of fenthion may be a result of cross-resistance within the OP group because fenthion has not been used in the Dhaka area as a larvicide or an adulticide of mosquitoes in the past, but fenthion and other OPs were probably used extensively against agricultural pests on lands surrounding Dhaka. It is interesting and rather surprising to note that temephos was highly effective against the exposed *Cx. quinquefasciatus* larvae despite its occasional use as a mosquito larvicide in Dhaka. The level of susceptibility of larvae to temephos indicated that temephos would be economically effective against the exposed larvae. However, this study did not test larvae from different locations (habitats) in Dhaka for their susceptibility to temephos. Temephos, because of its relative safety to most fish species (Sanders et al. 1981, Pierce et al. 1989), extremely low avian and mammalian toxicity, (Hill 1971, Laws et al. 1967) and nonpersistence in aquatic environments, is registered as a mosquito larvicide in the USA.

Cypermethrin was highly active against *Cx. quinquefasciatus* larvae. Generally, pyrethroids are not recommended for use as mosquito larvicides because of their known adverse effects on aquatic nontarget organisms. However, pyrethroids as mosquito larvicides may be utilized in some highly polluted habitats where nontarget organisms are of minimal or no concern. The LC_{90} values of permethrin and bifenthrin in our study were high, possibly because of low levels of resistance in the exposed larvae to these compounds. Pyrethroids, such as allethrin, S-bioallethrin, permethrin, resmethrin, etc., in aerosol products are extensively used in Dhaka by the general public, with annual sales of such mosquito adulticide products amounting to 3 million U.S. dollars. In addition, nearly 125,000 liters of fogging concentrate (S-bioallethrin + per-

Table 2. Comparative laboratory toxicities of technical powders of biological insecticides, *Bacillus thuringiensis* serovar. *israelensis* and *Bacillus sphaericus*, to field-collected 3rd-instar *Culex quinquefasciatus* larvae from Kawran Bazar area, Dhaka, May 1997.

Biological insecticide	Lethal concentration (ppm)				
	LC_{50}	95% CL	LC_{90}	95% CL	Slope
Bacillus thuringiensis serovar. *israelensis* (24-h exposure)					
VectoBac® TP[1] (8,300 ITU/mg)	0.006	0.004–0.009	0.024	0.017–0.042	2.17
Bacillus sphaericus serotype H5a5b, strain 2362 (48-h exposure)					
VectoLex® TP[2] (600 ITU/mg)	0.039	0.012–0.077	0.222	0.108–0.456	1.70

[1] Lot no. 11-134-W5.
[2] Lot no. 13-191-W5.

Table 3. Comparative toxicity of 2 juvenile hormones (methoprene and pyriproxyfen) and one chitin synthesis inhibitor (diflubenzuron) insect growth regulators (IGRs) to field-collected[1] late 3rd- and early 4th-instar *Culex quinquefasciatus* larvae exposed continuously to the IGRs in the laboratory, May 1997.

IGR	Lethal concentration (ppm)				
	LC_{50}	95% CL[2]	LC_{90}	95% CL	Slope
Methoprene	0.017	0.009–0.029	0.052	0.031–0.096	2.61
Pyriproxyfen	0.00029	0.00023–0.00035	0.0011	0.00084–0.0013	2.39
Diflubenzuron	0.0014	0.0012–0.0017	0.0034	0.0027–0.0048	3.24

[1] Kawran Bazar area, Dhaka.
[2] CL = confidence limits.

methrin) are annually used by the Dhaka City Corporation for mosquito adulticiding purposes.

The phenyl pyrazole, fipronil, may offer an excellent potential for *Cx. quinquefasciatus* control in Dhaka because of its attributes of high toxicity to a wide variety of mosquito larvae, relative safety to aquatic nontarget organisms, and novel mode of action (Ali et al. 1998).

The IGRs (methoprene, pyriproxyfen, and diflubenzuron) were effective against *Cx. quinquefasciatus* larvae and may be useful in some permanent habitats of *Cx. quinquefasciatus* in Dhaka.

Our laboratory data concerning *B.t.i.* and *B. sphaericus* indicate high levels of susceptibility of *Cx. quinquefasciatus* larvae from Dhaka to both microbial larvicides. Although *B.t.i.* previously has been successfully field tested against *Cx. quinquefasciatus* in Dhaka (Ahmed et al. 1980), significant improvements of fermentation technology to enhance toxicity as well as formulation improvements of this microbial mosquito larvicide in the past decade warrant new field studies testing the improved product(s). Field studies on *B. sphaericus* have shown this microbial pesticide to be highly active against *Culex* spp. in a variety of rather challenging situations with various degrees of pollution (Ali et al. 1989, Mulla et al. 1997). Therefore, field testing of *B. sphaericus* against *Cx. quinquefasciatus* in Dhaka is warranted. This microbial pesticide is presently registered for mosquito larval control in the USA.

This preliminary laboratory study has identified some promising chemical and biological control agents that may be considered in the development of an integrated approach to population management of *Cx. quinquefasciatus* in Dhaka. Specifically, the activity profile of *B.t.i.* and *B. sphaericus* is encouraging because the latter mosquito larvicide has been shown to provide relatively long-term control of *Cx. quinquefasciatus* in a variety of habitats (Mulla et al. 1997).

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