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

ARSHAD ALI,1 MANJUR A. CHOWDHURY,2 MOHAMMAD I. HOSSAIN,3 MAHMUD-UL-AMEEN,3 DILSHAD B. HABIBA3 AND ABU F. M. ASLAM4

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 LC50 values of all OPs, except for temephos (LC50 = 0.0096 ppm), were high, ranging from 0.13 ppm (fenthion) to 2.882 ppm (chlorpyrifos methyl). Pyrethroid LC50 values were 0.021 ppm (bifenthrin), 0.00061 (cypermethrin), and 0.017 ppm (permethrin). Fipronil exhibited a superior activity with LC50 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 (LC50 = 0.0011 ppm), being 3 times and 47 times more active than diflubenzuron (LC50 = 0.0034 ppm) and methoprene (LC50 = 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 biomics 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-
ve in a selected area of Dhaka to these possible control materials.

**MATERIALS AND METHODS**

For laboratory bioassays, field-collected *Culex 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. *israelesis* and *Bacillus sphaericus*), and 3 IGRs (diflubenzuron, methoprene, and pyriproxyfen) were tested against *Culex 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 3rd- and early 4th-instar *Cx. quinquefasciatus* were utilized. The IGRs, *B.t.i.*, and *B. sphaericus* were tested against late 3rd- and early 4th-instar *Culex 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 (*LC₉₀ = 0.0096 ppm*) and least susceptible to chlorpyrifos methyl (*LC₉₀ = 2.882 ppm*). In general, the *LC₉₀* 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, permethrin was the most toxic (*LC₉₀ = 0.0017 ppm*); followed by permethrin (*LC₉₀ = 0.021 ppm*); cypermethrin was 34 times and 28 times more toxic to *Culex quinquefasciatus* larvae than were bifenthrin and permethrin, respectively. Fipronil was highly active against *Culex quinquefasciatus* larvae, with an *LC₉₀* value of 0.000896 ppm. The level of activity of fipronil in the parts per billion range was comparable with the most toxic pyrethroid, cypermethrin (*LC₉₀ = 0.00061 ppm*).

The technical powder of *B.t.i.* (VectoBac TP) produced excellent larval mortality of *Culex quinquefasciatus*, with an *LC₉₀* 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 *LC₉₀* value of 0.222 ppm. All 3 IGRs were effective against *Culex quinquefasciatus* larvae, with *LC₉₀* 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 *Culex 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 *Culex 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.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>LC50  (ppm)</th>
<th>95% CL</th>
<th>LC90  (ppm)</th>
<th>95% CL</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.065</td>
<td>0.019-0.168</td>
<td>0.619</td>
<td>0.22-11.135</td>
<td>1.31</td>
</tr>
<tr>
<td>Chlorpyrifos methyl</td>
<td>0.164</td>
<td>0.118-0.227</td>
<td>2.882</td>
<td>1.775-5.840</td>
<td>1.03</td>
</tr>
<tr>
<td>Fenthion</td>
<td>0.062</td>
<td>0.053-0.069</td>
<td>0.130</td>
<td>0.111-0.170</td>
<td>3.96</td>
</tr>
<tr>
<td>Malathion</td>
<td>0.047</td>
<td>0.022-0.111</td>
<td>0.747</td>
<td>0.254-6.896</td>
<td>1.06</td>
</tr>
<tr>
<td>Temephos</td>
<td>0.0024</td>
<td>0.0016-0.0044</td>
<td>0.0096</td>
<td>0.006-0.216</td>
<td>2.32</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.009</td>
<td>0.006-0.021</td>
<td>0.021</td>
<td>0.012-0.198</td>
<td>2.74</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>0.00017</td>
<td>0.00012-0.00025</td>
<td>0.00061</td>
<td>0.00038-0.0017</td>
<td>2.33</td>
</tr>
<tr>
<td>Permethrin</td>
<td>0.005</td>
<td>0.003-0.009</td>
<td>0.017</td>
<td>0.009-0.079</td>
<td>2.43</td>
</tr>
<tr>
<td>Phenyl pyrazole1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.00035</td>
<td>0.00004-0.00066</td>
<td>0.000896</td>
<td>0.0005-0.0052</td>
<td>3.09</td>
</tr>
</tbody>
</table>

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

bility comparisons, it is difficult to clearly demonstrate whether the high LC90 values of these OPs resulted from natural or acquired resistance in the exposed *Culex 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 *Culex quinquefasciatus* in Dhaka by the Dhaka City Corporation. High LC90 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 *Culex 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 *Culex 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 LC90 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.

<table>
<thead>
<tr>
<th>Biological insecticide</th>
<th>LC50  (ppm)</th>
<th>95% CL</th>
<th>LC90  (ppm)</th>
<th>95% CL</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus thuringiensis</em> serovar. <em>israelensis</em> (24-h exposure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VectoBac® TP1 (8,300 ITU/mg)</td>
<td>0.006</td>
<td>0.004-0.009</td>
<td>0.024</td>
<td>0.017-0.042</td>
<td>2.17</td>
</tr>
<tr>
<td><em>Bacillus sphaericus</em> serotype H5a5b, strain 2362 (48-h exposure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VectoLex® TP2 (600 ITU/mg)</td>
<td>0.039</td>
<td>0.012-0.077</td>
<td>0.222</td>
<td>0.108-0.456</td>
<td>1.70</td>
</tr>
</tbody>
</table>

1 Lot no. 11-134-W5.
2 Lot no. 13-191-W5.
m ethrin) 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).

ACKNOWLEDGMENTS

The senior author expresses his sincere gratitude to faculty members of the Department of Zoology, University of Dhaka, and Safeway Pest Control (Manjur A. Chowdhury), Banani, Dhaka, for their hospitality and friendship during his visit to Dhaka. Laboratory and field assistance of M.S. (Zoology) students, Nasrin A. Snigdha, Saima H. Lisa, and M. Nuruzzaman Biplob, Department of Zoology, University of Dhaka, is deeply appreciated. This is Florida Agricultural Experiment Station Journal Series R-06361.

REFERENCES CITED

Mosquito breeding grounds in the city of Dhaka, Bangladesh. Bangladesh J. Life Sci. 8:41–47.


