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

ARSHAD ALI, MANJUR A. CHOWDHURY, MOHAMMAD I. HOSSAIN, MAHMUD-UL-AMEEN, DILSHAD B. HABIBA AND ABU F. M. ASLAM

ABSTRACT. Five organophosphates (OPs) (chlorpyrifos, chlorpyrifos methyl, fenitrothion, malathion, and temephos), 3 pyrethroids (bifenthrin, cypermethrin, and permethrin), 1 phenyl pyrazole (fipronil), 2 microbial isolates (IGRs) (dichlorantruron, 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 = 0.021 ppm), pyrethroid LC50 values were 0.021 ppm (bifenthrin), 0.00061 ppm (cypermethrin), and 0.017 ppm (permethrin). Fipronil exhibited a superb activity with LC50 value of 0.000896 ppm. Technical powders of B.t.i. and B. sphaericus (VectoBac®) were highly effective against Culex quinquefasciatus larvae. The IGRs also showed significant activity against Culex quinquefasciatus larvae. The IGRs also showed significant activity against Culex quinquefasciatus larvae.

KEY WORDS. Bioassays, mosquito larvae, organophosphates, pyrethroids, fipronil, Bacillus thuringiensis, Culex quinquefasciatus, 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, Hojsain 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 population. 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 Culex 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 dierelict ponds. The larval densities and scattered habitats of Culex 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, Hojsain et al. 1996). The role of Culex 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 biometrics 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 Culex quinquefasciatus lar-
larvae in a selected area of Dhaka to these possible control materials.

**MATERIALS AND METHODS**

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

Five organophosphates (OPs) (chlorpyrifos, chlorpyrifos methyl, fenithion, 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 (dilubenzuron, methoprene, and pyriproxyfen) were tested against *C. quinquefasciatus* larvae.

Technical grade materials of chlorpyrifos (99%), chlorpyrifos methyl (99.8%), fenithion (96.5%), malathion (95%), temephos (96.5%), bifenthrin (93.7%), cypermethrin (92.3%), permethrin (94.6%), fipronil (97.1%), dilubenzuron (90%), methoprene (95.6%), and pyriproxyfen (37%) 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 6-7 serial dilutions. Technical powders of *B.t.i.* (VectoBac® TP) containing 8,300 international toxic units (ITU)/mg and *B. sphaericus* serotype HSa5b, 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 Nayak (1986) and Ali et al. (1981). The IGRs were evaluated in the manner described by Muller et al. (1974). For OPs, pyrethroids, and fipronil, late 4th-instar *C. quinquefasciatus* were utilized. The IGRs, *B.t.i.*, and *B. sphaericus* were tested against late 3rd- and early 4th-instar *C. 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 larvicidal 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 (LC₅₀ = 0.0096 ppm) and least susceptible to chlorpyrifos methyl (LC₅₀ = 2.882 ppm). In general, the LC₅₀ values of chlorpyrifos, chlorpyrifos methyl, fenithion, and malathion were rather high, ranging from 0.13 ppm (Fenithion) to 2.882 ppm (chlorpyrifos methyl). Among the pyrethroids, cypermethrin was the most toxic (LC₅₀ = 0.00061 ppm), followed by permethrin (LC₅₀ = 0.017 ppm) and bifenthrin (LC₅₀ = 0.021 ppm); cypermethrin was 34 times and 28 times more toxic to *C. quinquefasciatus* larvae than were bifenthrin and permethrin, respectively. Fipronil was highly active against *C. quinquefasciatus* larvae, with an LC₅₀ 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 (LC₅₀ = 0.00061 ppm).

The technical powder of *B.t.i.* (VectoBac TP) produced excellent larval mortality of *C. quinquefasciatus*, with an LC₅₀ 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 LC₅₀ value of 0.222 ppm. All 3 IGRs were effective against *C. quinquefasciatus* larvae, with LC₅₀ values of 0.0011 ppm (pyriproxyfen), 0.0034 ppm (dilubenzuron), and 0.052 ppm (methoprene) (Table 3). Thus, among the IGRs tested, pyriproxyfen was 3 times and 47 times more active than dilubenzuron and methoprene, respectively.

**DISCUSSION**

It is evident from these laboratory studies that *C. quinquefasciatus* larval populations in the vicinity of Dhaka tolerated rather high concentrations of OPs (chlorpyrifos, chlorpyrifos methyl, fenithion, and malathion). Because no susceptible laboratory population of *C. quinquefasciatus* is maintained in Dhaka or in Bangladesh for purposes of suscepti-
the tests of OPs, pyrethroids after 24 h of exposure. Tests were extended to study. In experiments with 50% were examined daily, and curleld mortality, and curleld at the termination once was completed in wet or dry pupa remained. Thus the temperature were room during the tests. Corrected for any conc are subjected to a logarithmic (United States Environmental 1994) to estimate test materials.

### 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% CI</th>
<th>LC₉₀ (ppm)</th>
<th>95% CI</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organophosphates</strong></td>
<td></td>
<td></td>
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<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><strong>Pyrethroids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bifenthrin</td>
<td>0.009</td>
<td>0.008-0.012</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><strong>Phenyl pyrazole</strong></td>
<td></td>
<td></td>
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<tr>
<td>Fipronil</td>
<td>0.00035</td>
<td>0.00004-0.00066</td>
<td>0.000896</td>
<td>0.00005-0.00052</td>
<td>3.09</td>
</tr>
</tbody>
</table>

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

### 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% CI</th>
<th>LC₉₀ (ppm)</th>
<th>95% CI</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus thuringiensis serovar. israelensis (24-h exposure)</strong></td>
<td></td>
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<tr>
<td>VectorBac® TP¹</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><strong>Bacillus sphaericus serotype H5aSb, strain 2362 (48-h exposure)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>VectorBac® TP²</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. 14-134-W3.
2 Lot no. 14-191-W3.
methrin) are annually used by the Dhaka City Corporation for mosquito adulticide purposes.

The phenyl pyrazone, 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, 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. 1999, 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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