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COMPARATIVE TOXICITY OF SELECTED LARVICIDES AND INSECT GROWTH REGULATORS TO A FLORIDA LABORATORY POPULATION OF *AEDES ALBOPICTUS*

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ABSTRACT. Five organophosphates (OPs) (chlorpyrifos, chlorpyrifos methyl, fenthion, malathion, and temephos), 3 pyrethroids (bifenthrin, cypermethrin, and permethrin), and 2 microbial pesticides (*Bacillus thuringiensis* serovar. *israelensis* [*B.t.i.*] and *Bacillus sphaericus*) were tested as larvicides against a Florida *Aedes albopictus* population colonized in the laboratory. In addition, 3 insect growth regulators (IGRS) (diflubenzuron, methoprene, and pyriproxyfen) were evaluated. All OPs, except for malathion, were highly effective as indicated by low LC_{90}s ranging from 0.0069 ppm (chlorpyrifos) to 0.026 ppm (fenthion); the larvae were considered tolerant to malathion (LC_{90} = 1.043 ppm). LC_{90} values of pyrethroids were: 0.0175 ppm (bifenthrin), 0.0079 ppm (cypermethrin), and 0.0031 ppm (permethrin). Commercial products of *B.t.i.*, Vectobac® and Bactimos® were considered economically effective against *Ae. albopictus* larvae but products of *B. sphaericus* were ineffective (LC_{90}s > 28 ppm). The IGRs showed exceptional activity. Pyriproxyfen (LC_{90} = 0.000376 ppm), was 2.23 and 21.5 times more toxic than diflubenzuron and methoprene, respectively. In general, toxicity ranking of chemicals and microbials tested was: IGRs > pyrethroids > OPs > microbials.

INTRODUCTION

Since the initial establishment of *Aedes albopictus* (Skuse) populations in Harris County, TX, in August 1985 (Sprenger and Wuithiranyagool 1986), this mosquito species has rapidly expanded its distribution in the continental USA. At present, established populations of *Ae. albopictus* occur in the mid-Atlantic and southeastern United States (C. G. Moore, personal communication). In Florida, *Ae. albopictus* was found for the first time in Jacksonville, Duval County, in 1986 (Peacock et al. 1988), and has since spread to all of the state's 67 counties (G. F. O'Meara, personal communication). This mosquito is most common throughout northern Florida, but is less abundant in the central part of the state, and is currently relatively rare in south Florida (O'Meara et al. 1993).

Presently, *Ae. albopictus* primarily poses only a biting nuisance in the USA. However, public health officials and agencies are concerned about the rapid spread of this species. North American strains of *Ae. albopictus* have experimentally shown a high degree of vector competence to several arboviruses that cause diseases, such as dengue hemorrhagic fever, Rift Valley fever, eastern equine encephalitis, yellow fever, and others (Mitchell 1991). The ability of this exotic mosquito to occupy a wide variety of habitats in urban, rural, and sylvan situations enhances its chances to be a true vector species (O'Meara et al. 1993).

Considering the rapid spread, escalating biting nuisance, and the vector potential of *Ae. albopictus* in the USA, it is essential to monitor susceptibility of this mosquito species to available insecticides. Khoo et al. (1988) and Robert and Olson (1989) reported the susceptibility of adult *Ae. albopictus* to various adulticides in the USA. Larval susceptibility of a Kentucky strain to selected insecticides was studied by Cilek et al. (1989). Recently, in field trials, Nasci et al. (1994) reported control of *Ae. albopictus* larvae in Louisiana using time-release larvicidal formulations. We evaluated several larvicides and insect growth regulators (IGRs) against a laboratory colonized population of *Ae. albopictus* collected from Vero Beach, FL. Such data on *Ae. albopictus* are needed from around the USA to establish localized baseline information and to formulate control criteria for this recently introduced mosquito.

MATERIALS AND METHODS

A laboratory colony from field-collected *Ae. albopictus* was established at the University of Florida Medical Entomology Laboratory (FMEL), Vero Beach. About 200 host-seeking females were collected near tires and artificial containers maintained on the grounds of FMEL on May 21, 1993. Females were bloodfed on a chicken and F_1 eggs were collected the following 4 wk. Eggs were periodically hatched as needed for larval bioassay purpose and larvae were reared

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to late 3rd, and early late 4th instars following standard mosquito rearing techniques.

Five organophosphates (OPs) (chlorpyrifos, chlorpyrifos methyl, fenthion, malathion, and temephos), 3 pyrethroids (bifenthrin, cypermethrin, and permethrin), 2 microbials (*Bacillus thuringiensis* serovar. *israelensis* [*B.t.i.*] and *B. sphaericus*), and 3 IGRs, (diflubenzuron, methoprene, and pyriproxyfen) were tested against *Ae. albopictus* 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%), diflubenzuron (90%), methoprene (95.6%), and pyriproxyfen (97%) were utilized in this study. The OPs, pyrethroids, and IGRs were dissolved in acetone to prepare 1% stock solution (w/v) and 6–9 serial dilutions. Two formulations of *B.t.i.*, a technical powder (TP) (Vectobac®, containing 5,000 International Toxic Units [ITU]/mg) and a flowable concentrate (FC) (Bactimos®, containing 1,200 ITU/mg), and 2 formulations of *B. sphaericus*, a TP (ABG-6184, containing 2,478 ITU/mg) and an FC (Spherimos®), containing 300 ITU/mg were also evaluated. All *B.t.i.* and *B. sphaericus* formulations were mixed in well water (pH 6.8) to prepare 1% (w/v) stock solutions and 4–7 serial dilutions.

Mosquito bioassay methods for OPs and pyrethroids were similar to those of Mulla et al. (1982). *B.t.i.* and *B. sphaericus* bioassay methods used the test procedures of Ali et al. (1981) and Ali and Nayar (1986). The IGRs were evaluated in the manner described by Mulla et al. (1974). For OPs and pyrethroids, late 4th-instar *Ae. albopictus* were utilized. The IGRs were tested against late 3rd and early 4th instars, and early 4th instar *Ae. albopictus* were exposed to *B.t.i.* and *B. sphaericus*. In all evaluations, 20 mosquito larvae were placed in 120-ml disposable paper cups containing 100 ml tap water. Four 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 the OP, pyrethroid, and IGR tests. Controls in *B.t.i.* and *B. sphaericus* tests did not require addition of acetone because their stock solutions and serial dilutions were prepared in well water. One ml of 1% beef liver + yeast (1:1) was added to each cup only once for cups receiving OPs, pyrethroids, *B.t.i.*, and *B. sphaericus*, and their respective controls; in IGR tests lasting for 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. *Bacillus sphaericus* tests were extended to 48 h to assess larval mortality. In IGR tests, 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 larvae or pupae remained. A 14-h photoperiod and 26 ± 2°C were maintained in the evaluation room during the tests. Mortality in treatments was corrected for control mortality and the data were subjected to a log-dose-probit regression analysis (U.S. Environmental Protection Agency 1994) to estimate larval dosage response to the larvicides and IGRs.

RESULTS

Susceptibility of *Ae. albopictus* larvae to the various OPs varied considerably (Table 1). Larvae were most susceptible to chlorpyrifos (LC_{90} = 0.0069 ppm) and least susceptible to malathion (LC_{90} = 1.043 ppm). Chlorpyrifos and chlorpyrifos methyl were almost equally toxic as indicated by LC_{90}s of 0.0069 ppm (chlorpyrifos) and 0.0087 ppm (chlorpyrifos methyl). Similarly, fenthion and temephos were almost equally toxic with LC_{90}s of 0.026 ppm (fenthion) and 0.021 ppm (temephos). Chlorpyrifos was 3 times more toxic than temephos and 151 times more toxic than malathion. The high LC_{90} of 1.043 ppm (malathion) as compared to other OPs suggested that the exposed larval population of *Ae. albopictus* was tolerant to malathion.

Among the pyrethroids, permethrin was 2–3 times more toxic than cypermethrin and 5–6 times more toxic than bifenthrin (Table 1). Permethrin was 2–3 times more toxic than chlorpyrifos or chlorpyrifos methyl whereas the LC_{90} of cypermethrin was similar to that of chlorpyrifos and chlorpyrifos methyl.

Both formulations of *B.t.i.* were effective against *Ae. albopictus* with LC_{90}s of 0.38 ppm (Vectobac®) and 1.913 ppm (Bactimos®) (Table 2). A comparison of the larvicidal activity, keeping in consideration the potency (ITU/mg) difference of the 2 *B.t.i.* formulations, indicated that Vectobac® was slightly superior in activity than Bactimos®. Larvae were tolerant to both formulations of *B. sphaericus* (Table 2).

The IGRs showed exceptionally superior activity against *Ae. albopictus* as indicated by low LC_{90}s in the ppb range (Table 3). The juvenoid, pyriproxyfen (LC_{90} = 0.000376 ppm) was 2.23 times and 21.5 times more active than diflubenzuron and methoprene, respectively. Diflubenzuron was 9.6 times more active than methoprene. However, methoprene in general had a similar level of activity against *Ae. albopictus* when compared with the most toxic OP, chlorpyrifos and the pyrethroid, permethrin.

Table 1. Comparative laboratory toxicity of various organophosphate and pyrethroid larvicides to laboratory-reared[1] late 4th-instar *Aedes albopictus*.

Larvicides	24-h lethal concentration (ppm)				
	LC_{50}	95% CL	LC_{90}	95% CL	Slope
Organophosphates					
Chlorpyrifos	0.0033	0.0014–0.0052	0.0069	0.0044–0.0193	4.00
Chlorpyrifos methyl	0.0043	0.00069–0.0069	0.0087	0.0059–0.106	4.22
Fenthion	0.012	0.011–0.014	0.026	0.022–0.032	4.09
Malathion	0.379	0.338–0.421	1.043	0.917–1.209	2.92
Temephos	0.010	0.009–0.011	0.021	0.017–0.027	4.08
Pyrethroids					
Bifenthrin	0.0052	0.0045–0.0060	0.0175	0.0143–0.0224	2.45
Cypermethrin	0.0026	0.0016–0.0040	0.0079	0.0049–0.0189	2.63
Permethrin	0.00095	0.00082–0.0011	0.0031	0.0025–0.0040	2.48

[1] Colony maintained from field-caught adults collected in May 1993, Vero Beach, FL.

DISCUSSION

Limited laboratory data exist for comparing susceptibility of various populations of *Ae. albopictus* in the USA to larvicides and IGRs. However, some *Ae. albopictus* larval studies from Asia showing temephos LC_{50}s of <0.017 ppm (Toma et al. 1992, Wu et al. 1992), and fenthion LC_{50}s of 0.0055–0.006 ppm (Herbert and Perkins 1973, Toma et al. 1992) are compatible with the Vero Beach, FL, population (temephos LC_{50} = 0.01 ppm; fenthion LC_{50} = 0.012 ppm). Our study and several previous laboratory bioassays with malathion against *Ae. albopictus* larvae (Herbert and Perkins 1973, Cilek et al. 1989, Toma et al. 1992) have indicated the possibility of resistance to this insecticide. No data are available in the literature on larval susceptibility of *Ae. albopictus* to chlorpyrifos and chlorpyrifos methyl. Among pyrethroids, only permethrin has been previously evaluated against *Ae. albopictus* larvae in the USA, with an LC_{90} of 0.0028 ppm (Cilek et al. 1989), a value very close to the LC_{90} of 0.0031 ppm permethrin in our study. However, a wide range (0.003–0.663 ppm) of larval LC_{50}s was reported for permethrin against various geographical strains of *Ae. albopictus* in China (Wu et al. 1992).

The Vero Beach strain of *Ae. albopictus*, with a larval LC_{90} of 0.38 ppm *B.t.i.* in the present study, was 9 times more tolerant to this microbial larvicide than the Kentucky strain (LC_{90} = 0.0449 ppm) (Cilek et al. 1989) when compared on equal potency basis of International Toxic Units (ITU)/mg. Our study on *B. sphaericus*

Table 2. Comparative laboratory toxicity of *Bacillus thuringiensis* serovar. *israelensis* and *Bacillus sphaericus* in various formulations of different potencies to laboratory-reared[1] early 4th-instar *Aedes albopictus*.

Formulation (potency)[2]	Lethal concentration (ppm)				
	LC_{50}	95% CL	LC_{90}	95% CL	Slope
B. thuringiensis israelensis (24-h exposure)					
Vectobac®, TP (5,000 ITU/mg)	0.181	0.149–0.219	0.380	0.302–0.536	3.98
Bactimos®, FC (1,200 ITU/mg)	0.849	0.789–0.914	1.913	1.717–2.176	3.63
B. sphaericus (48-h exposure)					
ABG-6184, TP (2,478 ITU/mg)	5.90	2.34–14.81	28.09	14.20–261.34	1.89
Spherimos®, FC (300 ITU/mg)	36.96	32.78–41.62	176.51	145.11–224.13	1.89

[1] Colony maintained from field-caught adults collected in May 1993, Vero Beach, FL.
[2] TP = technical powder; FC = flowable concentrate; ITU/mg = International Toxic Units/mg.

Table 3. Comparative toxicity of 2 juvenile hormone (methoprene and pyriproxyfen) and one chitin synthesis inhibitor (diflubenzuron) insect growth regulators (IGRs) to laboratory-reared[1] late 3rd- and early 4th-instar *Aedes albopictus* exposed continuously to the IGRs in the laboratory.

IGRs	Lethal concentration (ppm)				
	LC_{50}	95% CL	LC_{90}	95% CL	Slope
Diflubenzuron	0.00045	0.00039–0.00049	0.00084	0.00076–0.00097	4.72
Methoprene	0.0022	0.0014–0.0029	0.0081	0.0068–0.01	2.29
Pyriproxyfen	0.00011	0.000074–0.000143	0.000376	0.000257–0.000692	2.31

[1] Colony maintained from field-caught adults collected in May 1993, Vero Beach, FL.

showing L_{90}s of 28.09 ppm (ABG-6184) and 176.51 ppm (Spherimos®) confirmed the reports of Dagnogo and Coz (1982) and Ren et al. (1987) that *Ae. albopictus* larvae were tolerant to this microbial larvicide.

Our laboratory data on IGRs are in general agreement with those of Kawada (1993) who reported 50% emergence inhibition of *Ae. albopictus* caused by methoprene at 1.1 ppb, diflubenzuron at 0.3 ppb, and pyriproxyfen at 0.024 ppb. In our study the same level of emergence inhibition was caused by methoprene at 2.2 ppb, diflubenzuron at 0.45 ppb, and pyriproxyfen at 0.11 ppb.

We observed that the OPs (except for malathion), pyrethroids, and IGRs were highly effective against the larval *Ae. albopictus* population. Products of *B.t.i.* appeared to be economically effective against this population. These larvicides and IGRs could be safely used in *Ae. albopictus* control programs because adverse effects on associated aquatic nontarget organisms in the various habitats of *Ae. albopictus*, such as small containers, tires, etc. would be of minimal concern. Insect growth regulators, particularly diflubenzuron and pyriproxyfen, offer an excellent potential for the control of *Ae. albopictus* and warrant further laboratory and field studies on formulation research to elucidate long-term effectiveness and residual activity.

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