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LABORATORY EVALUATION OF BIOTIC AND ABIOTIC FACTORS THAT MAY INFLUENCE LARVICIDAL ACTIVITY OF *BACILLUS THURINGIENSIS* SEROVAR. *ISRAELENSIS* AGAINST TWO FLORIDA MOSQUITO SPECIES

JAI K. NAYAR,[1] JUDY W. KNIGHT,[1] ARSHAD ALI,[2] DOUGLAS B. CARLSON[3] AND PETER D. O'BRYAN[3]

ABSTRACT. A technical powder of *Bacillus thuringiensis* serovar. *israelensis* (*B.t.i.*) (VectoBac® TP, 5,000 international toxic units [ITU]/mg), an aqueous suspension (VectoBac® 12AS, 1,200 ITU/mg), and a granular formulation (VectoBac® CG, 200 ITU/mg) were tested in the laboratory under different biotic and abiotic conditions for efficacy against larvae of saltwater (*Aedes taeniorhynchus*) and freshwater (*Culex nigripalpus*) mosquitoes. Second-, 3rd-, and 4th-instar larvae of *Cx. nigripalpus* were 1.3–3-fold more susceptible to both VectoBac TP and VectoBac 12AS than were the respective larval instars of *Ae. taeniorhynchus*. For each species, 2nd-instar larvae were several-fold more susceptible to these *B.t.i.* preparations than were the 4th instars. In test cups, larvae under lower densities exposed to *B.t.i.* concentrations sustained 5–9-fold higher mortalities than larvae under high-density conditions. VectoBac TP and VectoBac 12AS stayed in suspension for up to 24 h with similar larvicidal efficacy, which was greater at 32–35°C than at 15–20°C. At 60°C maintained for 24 h, VectoBac 12AS was adversely affected 2–3-fold in terms of potency, but VectoBac TP was not affected. Significant loss of potency of both VectoBac 12AS and VectoBac TP occurred when exposed to 35–37°C under high light intensity (140,000–170,000 lux) for 6 h. Increasing salinity levels from 0 (fresh water) to 50% sea water caused gradual efficacy declines of VectoBac 12AS and VectoBac TP against *Ae. taeniorhynchus* larvae. VectoBac CG caused insignificant initial and residual (up to 8 days) larval mortalities of both mosquito species. This formulation did not release the active ingredient of *B.t.i.* in any significant mosquito larvicidal concentration in surface layers of water, and the formulation was more effective in shallower water. Storage of all 3 formulations under constant laboratory and variable field conditions for up to 8 months did not produce detectable potency loss of these products.

KEY WORDS *Bacillus thuringiensis* serovar. *israelensis, Aedes taeniorhynchus, Culex nigripalpus,* bioassay, biotic and abiotic activity, larvicidal activity, laboratory evaluation

INTRODUCTION

In the state of Florida, 3 mosquito larval control materials are presently approved by the state's Department of Agriculture and Consumer Services. These materials include Abate® (temephos), Altosid® (s-methoprene), and *Bacillus thuringiensis* serovar. *israelensis* (*B.t.i.*). During 1994–95, nearly 459,000 acres of mosquito larval habitat in Florida were treated with these 3 materials, approximately half the acreage with Abate and a quarter each with Altosid and *B.t.i.* (Florida Coordinating Council on Mosquito Control 1998). Among the 3 materials, *B.t.i.* is not a contact insecticide and has to be ingested by mosquito larvae to produce toxic effects. It is considered to be the safest for the environment because of its specificity for some Nematocera, particularly simuliid blackflies, mosquitoes, and, to some degree, chironomid midges (Ali 1981, Merritt et al. 1989, Becker et al. 1992). This biocide is also compatible with integrated pest management (IPM) designs, is frequently highly toxic to target pests, and is readily available. Additionally, the development of resistance in mosquito larvae to *B.t.i.* is relatively much less pronounced (Georgiou and Wirth 1997). In spite of these attributes, the use of *B.t.i.* for mosquito larviciding purposes has increased at a rather low rate in recent years. The probable reasons for this slow increase of *B.t.i.* use in mosquito control are its significantly higher cost (Florida Coordinating Council on Mosquito Control 1998) and occasional control failures experienced by some mosquito control programs employing *B.t.i.* products for larval control in Florida freshwater and saltwater mosquito habitats (Van Handel et al. 1995).

Laboratory and field mosquito larvicidal efficacy of *B.t.i.* depends upon a number of biotic and abiotic factors. Besides susceptibility differences of various mosquito species, factors such as larval stage, larval feeding behavior, water temperature, water quality, light intensity, larval density, and vegetative cover play an important role in the success or failure of an application (Mulla et al. 1990, Becker et al. 1992, Becker and Rettich 1994, Consoli et al. 1995). Nature of formulation, potency, settling rate, shelf life, and method and timing of application also can influence effectiveness of *B.t.i.* (Becker and Rettich 1994, Consoli et al. 1995). The primary aim of the present study was to elucidate mosquito larvicidal efficacy of *B.t.i.* under different biotic and abiotic conditions created in the laboratory. Specifically, the influence of larval age (in-

[1] University of Florida, IFAS, Florida Medical Entomology Laboratory, 200 9th Street, SE, Vero Beach, FL 32960.

[2] University of Florida, IFAS, Central Florida REC, 2700 East Celery Avenue, Sanford, FL 32771-9608.

[3] Indian River Mosquito Control District, P. O. Box 670, 5655 41st Street, Vero Beach, FL 32961-0670.

star), larval feeding behavior, and efficacy loss of *B.t.i.* under different storage and environmental conditions was studied. This research was deemed necessary to improve understanding of *B.t.i.*, leading to its proper and efficacious handling and use in mosquito control programs. It should be pointed out that some studies of the nature proposed here were conducted more than 17 years ago (e.g., Van Essen and Hembree 1980, Dame et al. 1981). However, since these studies, substantial improvements in fermentation technology, toxicology, and quality enhancements of *B.t.i.* products, as well as an increase in commercial formulations (Wassmer 1995), have occurred, necessitating further efficacy research.

MATERIALS AND METHODS

Mosquito species

For these studies, saltwater (*Aedes taeniorhynchus* Wiedemann) and freshwater (*Culex nigripalpus* Theobald) mosquito species were used. For laboratory bioassays, different larval instars from laboratory-colonized *Ae. taeniorhynchus* at Florida Medical Entomology Laboratory (FMEL), Vero Beach, FL, and from wild-collected egg rafts of *Cx. nigripalpus* from the Vero Beach area were reared in artesian well water (pH 7.0) at 25 ± 1°C under 12 h light/dark cycle.

Test formulations

One technical powder and 2 formulations of *B.t.i.*, VectoBac® TP (technical powder, 5,000 international toxic units [ITU]/mg, lot no. 11-134-E5), VectoBac® 12AS (aqueous suspension, 1,200 ITU/mg, lot no. 13-348-N9), and VectoBac® CG (corncob granules, 200 ITU/mg, lot no. 03-573-N8) were tested. These materials were donated by Abbott Laboratories, Chicago, IL.

Laboratory procedures

All laboratory bioassays conducted with VectoBac 12AS and Vectobac TP followed the general guidelines of the World Health Organization (1981), with some modifications as described in Ali et al. (1984). These formulations were mixed with distilled water and suspended (magnetic stirrer) to make 1% stock suspensions (vol/vol or wt/vol). The suspensions were maintained while making serial dilutions as well as transfers for treatments. Six different concentrations of each formulation were used for treatment purposes. The bioassays were done in 120-ml disposable plastic cups. Twenty larvae of the instar(s) and species to be tested were transferred to the cups, each containing 100 ml of well water and 15 mg of larval food (liver powder and brewer's yeast, 1:3 mixture). Each treatment was replicated 3 times, and 3 untreated controls were maintained in each test. Larval mortality readings were noted at 24 h posttreatment. All bioassays, unless otherwise stated, were conducted at 25 ± 1°C maintained in controlled temperature rooms. Each experiment was repeated at least twice on different days. Larval response to exposed dosages of VectoBac TP and VectoBac 12AS was calculated by a log/dose/probit regression analysis (United States Environmental Protection Agency 1994).

Tests with VectoBac CG were conducted with nalgene high-density polypropylene pans (each 54 × 43.5 cm surface and 13 cm deep) slightly tapered from top to bottom (Fisher Scientific, Pittsburgh, PA). The bioassay method in these pans was generally similar to that of Ali et al. (1994) with the following modifications. A total of 100 3rd- and 4th-instar larvae of *Ae. taeniorhynchus* or *Cx. nigripalpus* were released in 6 liters of well water containing 100 mg of the mosquito larval food in each pan. VectoBac CG granules needed for a treatment rate of 2.5 kg/ha (typical field application rates are 8–11 kg/ha) were randomly distributed on the water surface in each pan at the beginning of the experiment. The experiment was replicated 2 times, and 2 untreated pans were maintained as controls. Larval mortality in the treated and control pans was checked at 24 h posttreatment, and all living and dead larvae from treated and control pans were carefully removed with a dropper, counted, and discarded. A new batch of 100 *Ae. taeniorhynchus* or *Cx. nigripalpus* 3rd- and 4th-instar live larvae and 50 mg of larval food were immediately introduced into the treated and control pans. This practice of daily larval introductions and 24-h postintroduction mortality checks was continued for 4 consecutive days (Monday–Thursday) for 2 wk; no larvae were introduced to the pans on Friday, Saturday, and Sunday of each week. Larval mortality data (corrected against controls) resulting from these tests were analyzed by ANOVA to elucidate residual activity of VectoBac CG utilized in various experiments.

Laboratory experiments with VectoBac TP and VectoBac 12AS

Susceptibility of various larval instars: This experiment utilized newly molted 2nd-, 3rd-, and 4th-instar larvae and older 4th-instar larvae (24 h and 48 h old) of *Ae. taeniorhynchus* and *Cx. nigripalpus* to determine their susceptibility to different concentrations of VectoBac TP and VectoBac 12AS.

Effectiveness against various mosquito larval densities: In this evaluation, newly molted 3rd- or 4th-instar larvae of *Ae. taeniorhynchus* and *Cx. nigripalpus* were exposed to VectoBac TP and VectoBac 12AS at densities of 20, 50, and 100 larvae placed in 100 ml of well water in 120-ml disposable bioassay cups to elucidate impact of larval density on *B.t.i.* effectiveness.

Effect of water temperature: This study utilized

newly molted 3rd- and 4th-instar larvae of both mosquito species. Larvae of each species and instar were reared at 25°C and then separately acclimatized for 2 h at temperatures of 15, 25, and 35°C prior to bioassay under these temperatures with VectoBac TP and VectoBac 12AS to determine the effectiveness of these materials under various temperatures.

Effect of water salinity: Newly molted 3rd- and 4th-instar larvae of *Ae. taeniorhynchus* were utilized. The larvae were reared in well water (0 salinity). Sea salts (Sigma Chemical Co., St. Louis, MO) were used to make 12.5, 25, and 50% seawater-salt concentrations in distilled water. Larvae were acclimatized to these salinities for 2 h prior to exposing them to VectoBac TP and VectoBac 12AS prepared in different seawater concentrations to determine salinity effects on *B.t.i.* activity.

Effect of settling in water: One percent stock solutions of VectoBac TP and VectoBac 12AS were made. These solutions were utilized to make 4–5 serial dilutions of each formulation in 1-liter quantities of well water. The dilutions were left undisturbed at 25°C. From these dilutions, appropriate amounts of *B.t.i.*-treated water were carefully pipetted from the top 1 cm of each dilution at 0, 2, 6, and 24 h after preparation of the dilution. The pipetted water was utilized to bioassay against newly molted 3rd-instar larvae of *Ae. taeniorhynchus* and *Cx. nigripalpus* and assess the activity profile of *B.t.i.* toxin at or near the water surface, indicating its settling in time posttreatment.

Combined effect of sunlight and temperature: Three separate sets of 5–6 dilutions of each VectoBac 12AS and VectoBac TP in 100-ml clear glass bottles were made from respective 1% stock solutions. One set of serial dilutions of each material was exposed to direct sunlight for 6 h, while one similar set was kept in shade for 6 h, and another kept under laboratory conditions for the same time period. Light intensity and water temperature were recorded hourly for 6 h at the location where each set of bottles was placed; a light meter (Extech Instruments, Model 401025, Forestry Supplies, Inc., Jackson, MS) was used to measure the light intensity. After the exposures, the serial dilutions were utilized to run standard bioassays in the laboratory (25°C) against newly molted 3rd instar larvae of *Ae. taeniorhynchus.*

Product storage effects at different temperatures: VectoBac TP and VectoBac 12AS were stored at 5, 25, 40, and 60°C for a 24-h period. These products were previously stored at 5°C. Each formulation was then bioassayed at 25 ± 1°C against newly molted 3rd- and 4th-instar larvae of *Ae. taeniorhynchus* and *Cx. nigripalpus.* In another experiment, VectoBac TP and VectoBac 12AS were stored at 5 and 25°C for a 32-wk period. Samples of each formulation were taken at 8-wk intervals and tested in the laboratory against 3rd-instar *Ae. taeniorhynchus* and *Cx. nigripalpus.*

Laboratory experiments with VectoBac CG

Effect of storage at different temperatures: This experiment utilized 6 bags (each 20 kg) of VectoBac CG purchased by Indian River Mosquito Control District (IRMCD), Vero Beach, FL, from Abbot Laboratories, North Chicago, IL. One of these bags was opened and 2 samples (2.5 kg each) were transferred into ziplock plastic bags and stored at the FMEL at 5°C and at 25°C under 60% relative humidity. The remaining granules were kept under variable temperature (13–38°C) and humidity (40–80%) conditions prevailing at the IRMCD's pesticide storage facility. Samples of VectoBac CG granules from each portion were taken at 0, 8, 16, 24, and 32 wk after storage and tested for efficacy against 3rd-instar larvae of *Ae. taeniorhynchus* and *Cx. nigripalpus.* The closed bags containing VectoBac CG stored at the IRMCD's facility under the variable temperature and humidity conditions were opened 1 by 1 at 8-wk intervals, and a sample of the granules taken from each bag was tested against 3rd- and 4th-instar larvae of both mosquito species at a rate of 2.5 kg/ha; the granules from these bags were tested at 0, 8, 16, 24, and 32 wk after storage.

Effect of water depth: VectoBac CG granules were evaluated against 3rd-instar larvae of both species at 2.5 kg/ha in 3 and 9 cm deep water in Nalgene pans maintained at 25°C. One hundred larvae of each species were introduced to the treated pans for 4 days per week; dead and living larvae were carefully removed from each pan after 24-h exposure, and a new batch of 100 larvae was immediately introduced. The experiment was repeated twice to determine water depth effect on activity of VectoBac CG.

Settling effect of granules: VectoBac CG granules were applied to 6 plastic pans at 2.5 kg/ha. The pans contained 9-cm-deep water and were left undisturbed at 25°C. After 30 min and 1, 2, 4, 24, and 48 h of application, 300 ml of water from the surface (upper 10 mm) of each pan was carefully removed with a 50-ml syringe and tested for toxicity to 3rd-instar larvae of *Ae. taeniorhynchus* and *Cx. nigripalpus* in test cups to elucidate any *B.t.i.* activity in top layers of water.

RESULTS

VectoBac TP and VectoBac 12AS

Susceptibility of various larval instars: Comparative susceptibility data of newly molted 2nd-, 3rd-, and 4th (N)-instar larvae and older 4th-instar larvae (24-h-old 4th [M] and 48-h-old 4th [L]) of *Cx. nigripalpus* and *Ae. taeniorhynchus* to VectoBac TP and VectoBac 12 AS are presented in Table 1. All tested larval instars of *Cx. nigripalpus* were nearly 1.3–3-fold more susceptible to both *B.t.i.* materials than were comparable larval instars of *Ae. taeniorhynchus.* Among the 2 materials, the LC_{50} and LC_{90}

Table 1. Susceptibility of laboratory-reared *Aedes taeniorhynchus* and *Culex nigripalpus* (various larval instars) to *Bacillus thuringiensis* serovar. *israelensis* formulations, VectoBac® 12AS and VectoBac® TP, in the laboratory.

	24-h lethal concentration (LC) in ppm			
	Cx. nigripalpus		*Ae. taeniorhynchus*	
Larval instar[1]	LC_{50}	LC_{90}	LC_{50}	LC_{90}
VectoBac-12AS (1,200 ITU/mg)				
2nd	0.016 (0.010–0.023)[2]	0.041 (0.028–0.079)	0.022 (0.012–0.036)	0.055 (0.034–0.167)
3rd	0.053 (0.038–0.066)	0.102 (0.079–0.191)	0.090 (0.080–0.103)	0.220 (0.180–0.293)
4th (N)	0.088 (0.081–0.098)	0.159 (0.137–0.200)	0.164 (0.135–0.205)	0.328 (0.252–0.503)
4th (M)	0.131 (0.118–0.148)	0.207 (0.179–0.257)	0.208 (0.184–0.240)	0.374 (0.310–0.503)
4th (L)	0.165 (0.145–0.186)	0.272 (0.237–0.329)	0.317 (0.279–0.365)	0.664 (0.564–0.884)
VectoBac TP (5,000 ITU/mg)				
2nd	0.002 (0.001–0.002)	0.004 (0.003–0.006)	0.005 (0.004–0.006)	0.011 (0.009–0.016)
3rd	0.005 (0.005–0.007)	0.016 (0.013–0.022)	0.012 (0.009–0.016)	0.040 (0.017–0.042)
4th (N)	0.009 (0.008–0.011)	0.019 (0.016–0.024)	0.026 (0.022–0.030)	0.068 (0.056–0.088)
4th (M)	0.010 (0.008–0.011)	0.025 (0.020–0.032)	0.036 (0.013–0.061)	0.060 (0.042–0.084)
4th (L)	0.017 (0.015–0.019)	0.037 (0.024–0.034)	0.032 (0.007–0.072)	0.067 (0.040–0.080)

[1] 4th instar, newly molted (N), 24 h old (middle, M), and 48 h old (late, L).
[2] 95% fiducial limits in parentheses.

values of both mosquito species resulting from VectoBac TP were 4–13 times lower than those achieved by VectoBac 12AS. This result is to be expected because VectoBac 12AS was 4.2-fold less potent than VectoBac TP in terms of ITU/milligram. The trend of declining susceptibility of each species with increasing larval age (instar) is obvious from data in Table 1. For example, newly molted 2nd-instar larvae of *Cx. nigripalpus* and *Ae. taeniorhynchus* were 5.5- and 7.5-fold, respectively, more susceptible to VectoBac 12AS than were their respective newly molted 4th-instar counterparts, as indicated by the LC_{50} values of these larval instars. Also, within 4th-instar larvae, newly molted 4th instars of both species were generally more susceptible than late 4th instars with the exception of *Ae. taeniorhynchus* exposed to VectoBac TP. Specifically, the LC_{90} values of newly molted and late 4th-instar larvae of *Cx. nigripalpus* for VectoBac 12AS were 0.159 and 0.272 ppm, respectively. Similarly, for VectoBac TP, these values for *Cx. nigripalpus* were 0.019 and 0.037 ppm; values (LC_{90}) for *Ae. taeniorhynchus* and VectoBac 12AS were 0.328 ppm (newly molted 4th instar) and 0.664 ppm (late 4th instar).

Influence of larval densities: Efficacy of VectoBac 12AS and VectoBac TP against *Cx. nigripalpus* and *Ae. taeniorhynchus* 3rd-instar larvae declined with increased larval densities (Table 2). For example, LC_{50} values of 3rd-instar larvae of *Cx. nigripalpus* against VectoBac 12AS were 0.053, 0.141, and 0.423 ppm for the larval densities of 20, 50, and 100/cup, respectively, an 8-fold increase of LC_{50} value between 20 larvae/cup and 100 larvae/cup. The trend of increase in LC_{50} values (VectoBac 12AS) of 4th-instar larvae of *Cx. nigripalpus* was similar to that of the 3rd-instar larvae, with a 7-fold increase of LC_{50} value in 100 larvae/cup over 20 larvae/cup (data not included). Bioassays of 3rd-instar larvae of *Ae. taeniorhynchus* with VectoBac 12AS followed a similar pattern of LC_{50} value increases with increased larval densities as observed for *Cx. nigripalpus*. VectoBac TP essentially revealed a similar pattern of dosage increase with increased larval densities of 3rd instars of each species. A nearly 9-fold increase of LC_{50} values of 3rd-instar larvae of *Cx. nigripalpus* occurred when per cup density was increased from 20 to 100; this increase for *Ae. taeniorhynchus* amounted to nearly 5-fold. In general, the increases of LC_{50} values of both mosquito species with increased larval densities were significantly correlated ($r = 0.80$, $P < 0.05$, $n = 24$).

Effect of water temperature: Susceptibility of

Table 2. Influence of various larval densities of *Aedes taeniorhynchus* and *Culex nigripalpus* 3rd-instar larvae on effectiveness of *Bacillus thuringiensis* serovar. *israelensis* formulations, VectoBac® 12AS and VectoBac® TP, in the laboratory.

Species	24-h LC_{50} (ppm) values at various larval densities/cup[1]		
	20/cup	50/cup	100/cup
VectoBac 12AS (1,200 ITU/mg)			
Cx. nigripalpus	0.053 (0.038–0.066)[2]	0.141 (0.095–0.259)	0.423 (0.401–0.444)
Ae. taeniorhynchus	0.090 (0.080–0.103)	0.321 (0.257–0.416)	0.499 (0.475–0.524)
VectoBac TP (5,000 ITU/mg)			
Cx. nigripalpus	0.005 (0.005–0.007)	0.016 (0.010–0.019)	0.047 (0.060–0.260)
Ae. taeniorhynchus	0.012 (0.001–0.069)	0.036 (0.025–0.045)	0.055 (0.046–0.065)

[1] 100 ml water in each cup.
[2] 95% fiducial limits in parentheses.

3rd-instar *Cx. nigripalpus* and *Ae. taeniorhynchus* to both VectoBac 12AS and VectoBac TP generally increased with increased temperature in the laboratory. Third-instar larvae of both species were 1.4–3.0-fold more susceptible to both *B.t.i.* materials at 35°C than at 15°C (Table 3). In contrast, the increase in susceptibility of 4th-instar larvae of both mosquito species with temperature increase was inconsistent and showed maximum susceptibility of 4th-instar larvae of both species at 25°C to VectoBac 12AS as well as to VectoBac TP.

Effect of water salinity: Susceptibility of 3rd- and 4th-instar larvae of *Ae. taeniorhynchus* to VectoBac 12AS as well as to VectoBac TP gradually decreased with increased salinity (Table 4). The LC_{50} values (VectoBac 12AS) in well water to 50%

Table 3. Susceptibility of *Aedes taeniorhynchus* and *Culex nigripalpus* 3rd- and 4th-instar larvae exposed to *Bacillus thuringiensis* serovar. *israelensis* formulations, VectoBac® 12AS and VectoBac® TP, and maintained under different temperatures during bioassays in the laboratory.

Species and larval instar	24-h LC_{50} (ppm) values at various temperatures		
	15°C	25°C	35°C
VectoBac 12AS (1,200 ITU/mg)			
Cx. nigripalpus			
3rd	0.094 (0.087–0.101)[1]	0.074 (0.069–0.080)	0.066 (0.057–0.077)
4th	0.152 (0.123–0.168)	0.139 (0.091–0.157)	0.140 (0.127–0.159)
Ae. taeniorhynchus			
3rd	0.072 (0.064–0.080)	0.056 (0.049–0.063)	0.052 (0.045–0.059)
4th	0.292 (0.268–0.330)	0.202 (0.186–0.221)	0.224 (0.203–0.247)
VectoBac TP (5,000 ITU/mg)			
Cx. nigripalpus			
3rd	0.018 (0.016–0.020)	0.011 (0.010–0.013)	0.006 (0.002–0.012)
4th	0.017 (0.016–0.019)	0.013 (0.010–0.016)	0.016 (0.014–0.017)
Ae. taeniorhynchus			
3rd	0.009 (0.008–0.010)	0.010 (0.007–0.011)	0.005 (0.001–0.160)
4th	0.049 (0.020–0.067)	0.028 (0.025–0.030)	0.036 (0.033–0.039)

[1] 95% fiducial limits in parentheses.

Table 4. Susceptibility of *Aedes taeniorhynchus* 3rd- and 4th-instar larvae exposed for 24 h to *Bacillus thuringiensis* serovar. *israelensis* formulations, VectoBac® 12AS and VectoBac® TP, in different water salinities in the laboratory.

Larval instar	24-h LC_{50} (ppm) values in well water and seawater			
		Seawater		
	Well water	12.5%	25%	50%
VectoBac 12AS (1,200 ITU/mg)				
3rd	0.107 (0.098–0.117)[1]	0.153 (0.139–0.170)	0.194 (0.136–0.261)	0.226 (0.196–0.259)
4th	0.219 (0.200–0.224)	0.312 (0.264–0.355)	0.334 (0.301–0.368)	0.444 (0.390–0.493)
VectoBac TP (5,000 ITU/mg)				
3rd	0.026 (0.015–0.037)	0.035 (0.029–0.040)	0.040 (0.031–0.044)	0.042 (0.036–0.047)
4th	0.031 (0.028–0.034)	0.045 (0.041–0.049)	0.061 (0.047–0.083)	0.107 (0.096–0.129)

[1] 95% fiducial limits in parentheses.

sea water showed a nearly 2-fold increase for both larval instars (0.107 to 0.226 ppm [3rd instar] and 0.219 to 0.444 ppm [4th instar]); the increase was more pronounced (3.5-fold) in 4th-instar larvae exposed to VectoBac TP, where LC_{50} values of 0.031 ppm in well water and 0.107 ppm in 50% sea water were observed.

Effect of settling in water: The LC_{50} data of both mosquito species (Table 5) indicate the settling property difference of VectoBac 12AS and VectoBac TP in water. The nearly 2-fold increase of LC_{50} values of 3rd-instar *Ae. taeniorhynchus* (LC_{50}: 0.111 to 0.216 ppm) and *Cx. nigripalpus* (LC_{50}: 0.045 to 0.103 ppm) at 0 to 24 h is indicative of diminishing *B.t.i.* active ingredient in VectoBac 12AS from surface water. VectoBac TP was relatively slower in settling, as shown by *Ae. taeniorhynchus* LC_{50} values of 0.019 ppm at 0 h and 0.028 ppm at 24 h; *Cx. nigripalpus,* however, showed no change of LC_{50} values between 0 and 24 h with VectoBac TP.

Combined effect of sunlight and temperature: The LC_{50} values of 3rd-instar *Ae. taeniorhynchus* with VectoBac 12AS and VectoBac TP kept under shade (24–28°C, 800–1700 lux) and in the laboratory (25°C, 800 lux) were similar. For example, VectoBac 12AS LC_{50} values were 0.062 ppm (shade) and 0.061 ppm (laboratory), and VectoBac TP LC_{50} values were 0.023 ppm (shade and laboratory) (Table 6). However, the activity loss of both *B.t.i.* materials exposed to sunlight (25–37°C, 110,500–164,500 lux) is evident from the nearly 12- and 3-fold increases of LC_{50} values with VectoBac 12AS and VectoBac TP, respectively.

Effect of product storage at different temperatures: No susceptibility difference between 3rd- as well as 4th-instar larvae of *Ae. taeniorhynchus* and *Cx. nigripalpus* was noted with VectoBac 12AS and VectoBac TP stored at 5 and 25°C; the LC_{50} values of each instar and each species against each of these products for the 2 storage temperatures were the same or very similar (Table 7). At 40 and 60°C

Table 5. Effect of *Bacillus thuringiensis* serovar. *israelensis* formulations, VectoBac® 12AS and VectoBac® TP, settling in water allowed for various time durations on its larvicidal activity against 3rd-instar larvae of *Aedes taeniorhynchus* and *Culex nigripalpus* in the laboratory.

Species	24-h LC_{50} (ppm) values for various settling time durations			
	0 h	2 h	6 h	24 h
VectoBac 12AS (1,200 ITU/mg)				
Ae. taeniorhynchus	0.111 (0.099–0.123)[1]	0.089 (0.059–0.136)	0.157 (0.142–0.173)	0.216 (0.197–0.297)
Cx. nigripalpus	0.045 (0.041–0.049)	0.063 (0.051–0.072)	0.079 (0.055–0.125)	0.103 (0.090–0.116)
VectoBac TP (5,000 ITU/mg)				
Ae. taeniorhynchus	0.019 (0.013–0.025)	0.021 (0.014–0.027)	0.026 (0.023–0.029)	0.028 (0.017–0.035)
Cx. nigripalpus	0.008 (0.005–0.011)	0.008 (0.006–0.010)	0.007 (0.006–0.008)	0.008 (0.002–0.015)

[1] 95% fiducial limits in parentheses.

Table 6. Laboratory susceptibility of 3rd-instar *Aedes taeniorhynchus* larvae to *Bacillus thuringiensis* serovar. *israelensis (B.t.i.)* formulations, VectoBac® 12AS and VectoBac® TP, exposed to various light and temperature conditions prior to bioassays.

Formulation	24-h LC_{50} (ppm) values with *B.t.i.* formulations exposed to various light and temperature conditions		
	Sunlight[1]	Shade[2]	Laboratory[3]
VectoBac 12AS	0.742 (0.669–0.822)[4]	0.062 (0.058–0.067)	0.061 (0.057–0.064)
VectoBac TP	0.067 (0.062–0.073)	0.023 (0.017–0.034)	0.023 (0.016–0.032)

[1] 25–37°C, 110,500–164,500 lux.
[2] 24–28°C, 800–1,700 lux.
[3] 25°C, 800 lux.
[4] 95% fiducial limits in parentheses.

storage temperatures, susceptibility of 3rd- and 4th-instar larvae of *Ae. taeniorhynchus* to VectoBac 12AS and VectoBac TP decreased 1.6–3.7- and 1.2–2.1-fold, respectively, as compared with the susceptibility levels at 5 or 25°C. Susceptibility declines of *Cx. nigripalpus* larvae ranged from 1.1- to 5.7-fold (VectoBac 12AS) and 1.6- to 2.7-fold (VectoBac TP) at storage temperatures of 40 and 60°C as compared with 5 and 25°C.

Storage of VectoBac 12AS and VectoBac TP at 5 and 25°C for 0–32 wk produced no susceptibility declines of 3rd-instar *Cx. nigripalpus* larvae with either formulation stored at 5°C for 32 wk (Table 8). However, at 25°C, susceptibility of *Cx. nigripalpus* declined 1.3- and 4.2-fold against VectoBac 12AS and VectoBac TP, respectively, at 32 wk of storage. Susceptibility changes of 3rd-instar *Ae. taeniorhynchus* larvae at the 8-wk evaluations also revealed some decline in effectiveness of both *B.t.i.* formulations at 5°C as well as 25°C due to storage (Table 8).

VectoBac CG

Effect of storage at different temperatures: Table 9 presents data on larval mortality of *Ae. taeniorhynchus* exposed to VectoBac CG stored for various lengths of time at 5°C, 25°C, and variable temperature and humidity conditions. Under different storage conditions, larval mortalities of *Ae. taeniorhynchus* during the 32-wk storage period ranged between 94 and 100% at 1 day posttreatment, with significant ($P < 0.05$) differences breaking out after 8 days posttreatment. At 10 days posttreatment, larval mortality with granules stored under all 4 conditions dropped significantly ($P < 0.001$), with a range from 45 to 85% for the 32-wk storage period. Larvae of *Cx. nigripalpus* revealed relatively less residual mortality effects of VectoBac CG under different storage conditions studied in this experiment (data not included). However, VectoBac CG stored at 5°C and 25°C produced overall higher larval mortalities in *Cx. nigripalpus* than did the granules exposed to variable temperature and humidity conditions.

Effect of water depth: Larval mortality of *Ae. taeniorhynchus* ranged from 95 to 100% at 1 day posttreatment in both 3-cm and 9-cm water depths treated with VectoBac CG at 2.5 kg/ha. Larval mortality showed a significant decrease (95% to 70%) during subsequent 3 days in pans containing 9 cm of water, whereas no such larval mortality declines in pans containing 3 cm deep water were noted (data not included). Larval mortality of *Cx. nigripalpus* ranged from 93 to 100% at 1 day posttreatment in both 3-cm and 9-cm depths. In subsequent days, a significant decline of larval mortality in both water depths was noted (data not included).

Settling effect of granules: The bioassay results showed no larval mortality of either species (data not presented). These results suggested that when VectoBac CG was applied to the water surface and the water remained undisturbed, the VectoBac CG settled to the bottom of the pans; the active ingredient did not go into suspension at the surface and, thus, did not become available in any significant mosquito larvicidal concentrations at or near surface of the treated water.

DISCUSSION

Growth and development of mosquito larvae including *Cx. nigripalpus* and *Ae. taeniorhynchus* are affected by both biotic and abiotic factors, such as nutrition, population density, salinity of the rearing medium, light–dark regimes, and temperature (Nayar 1967, 1968; Clements 1992). It is therefore logical that larval susceptibility of these species to a control agent would also be affected by these factors. However, in the case of *B.t.i.*, efficacy of a formulation may also be affected by other abiotic factors, such as physical nature and release mechanism of active ingredient, potency, settling rate, and shelf life. The effects of these biotic and abiotic factors on the mosquito larvicidal efficacy of tested *B.t.i.* formulations is discussed.

Mosquito larvae feed constantly and grow continuously throughout each instar except for very brief periods just before and after each molt (Clements 1992). Because 2nd-instar *Ae. taeniorhyn-*

Table 7. Laboratory susceptibility of *Aedes taeniorhynchus* and *Culex nigripalpus* 3rd- and 4th-instar larvae to *Bacillus thuringiensis* serovar. *israelensis* formulations, VectoBac® 12AS and VectoBac® TP, stored at different temperatures immediately prior to the bioassays.

Species and larval instar	24-h LC_{50} (ppm) values for various temperatures			
	5°C	25°C	40°C	60°C
VectoBac 12AS (1,200 ITU/mg)				
Cx. nigripalpus				
3rd	0.053 (0.038–0.066)[1]	0.048 (0.038–0.058)	0.058 (0.052–0.069)	0.124 (0.113–0.139)
4th	0.088 (0.081–0.098)	0.088 (0.081–0.098)	0.126 (0.106–0.146)	0.500 (0.461–0.541)
Ae. taeniorhynchus				
3rd	0.090 (0.080–0.103)	0.090 (0.080–0.103)	0.140 (0.130–0.151)	0.334 (0.221–0.646)
4th	0.164 (0.135–0.148)	0.164 (0.135–0.205)	0.288 (0.225–0.322)	0.569 (0.355–0.715)
VectoBac TP (5,000 ITU/mg)				
Cx. nigripalpus				
3rd	0.005 (0.004–0.007)	0.005 (0.004–0.007)	0.008 (0.005–0.010)	0.010 (0.009–0.011)
4th	0.009 (0.008–0.011)	0.009 (0.008–0.011)	0.019 (0.008–0.025)	0.024 (0.022–0.027)
Ae. taeniorhynchus				
3rd	0.012 (0.009–0.016)	0.012 (0.001–0.069)	0.023 (0.016–0.030)	0.025 (0.019–0.033)
4th	0.026 (0.022–0.030)	0.026 (0.022–0.030)	0.032 (0.026–0.037)	0.038 (0.034–0.043)

[1] 95% fiducial limits in parentheses.

chus and *Cx. nigripalpus* larvae are much smaller than 4th-instar larvae and differ in their feeding behavior and mechanics of filtration, they were 6–8-fold more susceptible to VectoBac 12AS and VectoBac TP than were their respective 4th-instar larvae. Their 3rd-instar larvae were 2–6-fold more susceptible to these *B.t.i.* formulations than were the 4th-instar larvae. Similar patterns of larval susceptibility were observed in other mosquito species with different *B.t.i.* formulations (Van Essen and Hembree 1980, Lacey and Oldacre 1983, Becker and Rettich 1994). Therefore, it is advisable to make field applications with the aqueous suspension and the technical powder formulations of *B.t.i.* to 2nd- and/or 3rd-instar larvae rather than 4th-instar larvae. Regarding the susceptibility difference between *Ae. taeniorhynchus* and *Cx. nigripalpus* to a *B.t.i.* formulation, the results are in agreement with previous observations of Ali et al. (1984), where *Cx. nigripalpus* larvae were found to be nearly 2-fold more susceptible than *Ae. taeniorhynchus* larvae of the same instar to a VectoBac wettable powder tested in freshwater. Similar results were reported by Becker and Rettich (1994) when they tested *Aedes cantans* (Meigen), *Aedes vexans* (Meigen), and *Culex pipiens* L. larvae, with *Cx. pipiens* being more susceptible than the *Aedes* larvae.

High larval density of mosquitoes per unit area (volume) results in longer development time, reduced pupal success, and reduced pupal weight (Clements 1992). Hence, density of larvae in a habitat can affect susceptibility of larvae to the *B.t.i.* Especially in the case of *B.t.i.*, under high larval density conditions, competition for ingestion of *B.t.i.* active ingredient (δ-endotoxin) will be greater. Our observations of lower larval densities of both species requiring 2–6-fold less *B.t.i.* than the higher densities agree with similar reports on other species of mosquitoes (Mulla et al. 1990, Becker et al. 1992). Therefore, it is necessary in the field to make subjective adjustments of rates of *B.t.i.* applications depending upon prevailing larval densities.

Growth and development of mosquito larvae occur only within a temperature range that is defined by lower development threshold and upper lethal limit, and with this temperature range, the rates of growth and development are positively correlated with temperature (Clements 1992). This temperature range is species specific. In both *Cx. nigripalpus* and *Ae. taeniorhynchus,* the lower developmental threshold and lethal limits are 15 and 35°C, respectively (Nayar 1967, 1968). Lower temperature (15°C) of water slows the development of larvae, with the result that larvae consume fewer nutrients (and also less δ-endotoxin) and apparently become less susceptible. Higher temperatures

Table 8. Laboratory susceptibility of *Aedes taeniorhynchus* and *Culex nigripalpus* 3rd-instar larvae to *Bacillus thuringiensis* serovar. *israelensis* formulations, VectoBac® 12AS and VectoBac® TP, stored at 5 and 25°C for various time periods prior to the bioassays.

Species and storage temp	24-h LC_{50} (ppm) values for various storage periods				
	0 wk	8 wk	16 wk	24 wk	32 wk
VectoBac 12AS (1,200 ITU/mg)					
Cx. nigripalpus					
5°C	0.053 (0.038–0.066)[1]	0.058 (0.051–0.064)	0.056 (0.048–0.066)	0.049 (0.044–0.053)	0.053 (0.048–0.058)
25°C	0.048 (0.038–0.058)	0.044 (0.003–0.089)	0.048 (0.040–0.058)	0.058 (0.051–0.065)	0.065 (0.061–0.069)
Ae. taeniorhynchus					
5°C	0.090 (0.080–0.103)	0.155 (0.103–0.130)	0.112 (0.090–0.120)	0.116 (0.108–0.122)	0.110 (0.088–0.142)
25°C	0.090 (0.080–0.103)	0.154 (0.128–0.196)	0.152 (0.152–0.159)	0.159 (0.142–0.165)	0.147 (0.134–0.162)
VectoBac TP (5,000 ITU/mg)					
Cx. nigripalpus					
5°C	0.005 (0.005–0.007)	0.007 (0.006–0.008)	0.007 (0.002–0.014)	0.007 (0.004–0.012)	0.007 (0.003–0.012)
25°C	0.005 (0.005–0.007)	0.007 (0.005–0.008)	0.011 (0.004–0.021)	0.010 (0.004–0.021)	0.021 (0.019–0.022)
Ae. taeniorhynchus					
5°C	0.012 (0.009–0.016)	0.014 (0.021–0.016)	0.024 (0.021–0.031)	0.026 (0.021–0.028)	0.026 (0.005–0.037)
25°C	0.012 (0.001–0.069)	0.039 (0.030–0.047)	0.038 (0.029–0.048)	0.033 (0.028–0.041)	0.025 (0.019–0.032)

[1] 95% fiducial limits in parentheses.

Table 9. Efficacy of *Bacillus thuringiensis* serovar. *israelensis* formulation VectoBac® CG (200 ITU/mg) stored for various lengths of time (weeks) under 5°C, 25°C, and variable temperature (13–38°C) and humidity (40–80%) conditions and evaluated at 2.5 kg/ha against 3rd- and 4th-instar larvae of *Aedes taeniorhynchus* in pans maintained at 25°C in the laboratory.

Storage temp/RH	Weeks	Mean % larval mortality posttreatment						
		1 day	2 days	3 days	4 days	8 days	9 days	10 days
5°C/60%	0	100	100	96	95	94	90	75
	8	100	100	97	86	84	68	60
	16	100	100	99	98	96	82	62
	24	100	97	86	86	85	84	72
	32	98	96	88	86	85	78	74
25°C/60%	0	100	100	96	95	94	90	75
	8	99	98	95	94	90	86	76
	16	98	97	93	78	76	65	58
	24	100	100	91	90	88	76	61
	32	97	96	94	87	86	86	47
13–38°C/40–80%	0	100	100	96	95	94	90	75
	8	100	99	97	73	73	57	51
	16	100	100	98	85	85	82	75
	24	99	98	96	86	83	60	48
	32	94	92	83	80	80	46	45
13–38°C/40–80% test granules obtained from a new bag opened at 8-wk intervals)	0	100	100	96	95	94	90	75
	8	100	100	93	84	77	64	45
	16	100	99	98	95	93	89	85
	24	98	98	98	90	85	82	76
	32	99	97	97	66	65	55	50

(35°C) accelerate development of larvae, with the result that the larvae consume more nutrients and become more susceptible. This is why the efficacy of VectoBac 12AS and VectoBac TP was higher at higher temperatures (32–35°C) than at lower temperatures (15–20°C). Similar observations were reported on other mosquitoes species by Mulla et al. (1990) and Becker et al. (1992). Thus, higher field application rates of *B.t.i.* may be necessary during fall and spring months than during summer months to achieve the same level of control.

Aedes taeniorhynchus larvae breed in waters ranging in salinity from slightly brackish to nearly 50% seawater (Nayar 1967). These larvae can adapt to the prevailing salinity concentration and can either retard their development in high salinity or accelerate development in low salinity conditions. Thus, when the salinity of water in *Ae. taeniorhynchus* habitats increases because of high temperature and evaporation of water, the larvae become less susceptible to *B.t.i.* formulations than when it rains and salinity levels are diluted. Therefore, higher treatment rates of *B.t.i.* formulations may be required during summer months, when there is little rain and the water levels in the mosquito habitats are relatively low or when high salinity tides stimulate egg hatch.

Increased sunlight has been shown to lower the efficacy of *B.t.i.* (Morris 1983, Becker et al. 1992). However, in the tropics and subtropics, where the intensity of sunlight as well as the water temperature are high, especially during the summer months, the combined effect of higher intensity of sunlight and high temperature can reduce the potency of *B.t.i.* formulations substantially even when aqueous suspension and technical powder formulations of *B.t.i.* are exposed only for 6 h of a day. This suggests that, ideally, field applications of *B.t.i.* should be made during the later part of the day (after 4:00 p.m.) rather than in the morning hours, particularly during the summer months.

Our laboratory results showed that both VectoBac 12AS and VectoBac TP remained suspended in solution for up to 24 h. VectoBac 12AS showed a 2-fold decrease in activity from 0 h to 24 h, whereas VectoBac TP showed no difference in activity. Therefore, these formulations were available to larvae for ingestion and caused significant larval mortalities. However, when Becker and Rettich (1994) tested Teknar® TC powder (13,500 *Aedes aegypti* units [AAU]/mg), less than 50% of the δ-endotoxin remained in the top 1 cm of the water after 25 min, less than 20% remained after 2 h, and less than 2% was available after 24 h. On the other hand, when Teknar® HP-D FC (flowable concentrate) (3,000 AAU/mg) was tested (Becker and Rettich 1994), more than 80% of the toxin remained available in the upper 1 cm layer of water after 25 min, more than 60% after 2 h, and less than 10% after 24 h. In our bioassay study with VectoBac CG-treated water, the granules settled rather rapidly after application in test pans and did not release the active ingredient at or near the surface of the 9-cm-deep water to produce any larval mortalities. However, releasing *Ae. taeniorhynchus* larvae directly into VectoBac CG-treated tubs containing 15-cm-deep water resulted in excellent short-term as well as sustained control for 7–10 days (Ali et al. 1994). Our results with VectoBac CG in the pans were compatible with the observations of Ali et al. (1994).

Our results also showed that storage of all 3 formulations of *B.t.i.* under normal environmental conditions (temperature = 20–35°C and RH 60–80%) for up to 8 months did not significantly decrease their potency. However, storage of these products at temperatures >40°C and high humidity conditions (80–90%) for any long period should be avoided. Consideration of the above discussed biotic and abiotic factors should maximize larval control of mosquitoes in the field through efficient use of the *B.t.i.* products.

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