Journal of the American Mosquito Control Association, 18(4):341–343, 2002

LABORATORY EVALUATION OF VECTOBAC® AS AGAINST *AEDES AEGYPTI* IN MONTERREY, NUEVO LEÓN, MEXICO

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ABSTRACT: Intensive use of the organophosphate insecticide malathion against adults and temephos against larvae of *Aedes aegypti* in Mexico over the past 30 years has led to problems requiring the use of new larvicides. Toward this objective, *Bacillus thuringiensis* var. *israelensis* (*Bti*), a target-specific and environmentally safer control agent, was evaluated. Laboratory bioassays were done to determine the susceptibility of 2nd- and 3rd-stage larvae of *Ae. aegypti* to Vectobac® 12 AS (aqueous suspension, 600 ITU/mg). A median lethal concentration of 0.0104 ppm and a 95% lethal concentration of 0.18 ppm were determined after 24 h of exposure to the agent. The values obtained were adjusted for field application and were further tested in the field by the State of Nuevo León, Mexico Vector Control Program. Suspensions of *Bti* were poured into pipe-water trucks and transferred to domestic 200-gal metal water drums. Larval populations were reduced during a 2-week study period. However, residents complained about a fine dusty film on the water surface. Nevertheless, these results are promising for future *Bti* field applications.

KEY WORDS Dengue, insecticide resistance, mosquito control, *Bacillus thuringiensis* var. *israelensis*, Vectobac®

INTRODUCTION

Classical dengue, along with its more serious forms dengue hemorrhagic fever and dengue shock syndrome, is a serious health problem in many parts of the Americas, and may affect national economies in the region (PAHO 1994). Dengue reappeared in Mexico during the late 1970s, and since then, yearly outbreaks have occurred consistently throughout temperate and tropical areas (Gomez-Dantes 1992). Dramatically, the northeastern region covering the states Tamaulipas, Nuevo León, and Coahuila reported nearly 70% of the 14,000 confirmed cases registered nationwide (Secretaría de Salud, Mexico 1999). The increasing trend of dengue outbreaks is blamed on several factors. Notably, traditional, centralized, and vertically structured programs are largely ineffective, principally because they are neither affordable nor manageable. In addition, the explosive growth of urban areas, limited government resources, and inadequate field personnel account for the epidemic dispersal in Latin America (Gubler and Kuno 1997). On the other hand, the excessive reliance on and improper application of insecticides, plus reports of insecticide resistance, have all brought attention to the need for alternative control methods. Insecticides have exerted a selection pressure that has favored the survival of strains with increased resistance. In Mexico, as in most countries, many different insecticides have been used for vector control. In the 1950s, DDT was used for malaria control. Since then, organophosphate insecticides such as malathion (adulticide) and temephos (larvicide), have been employed intensively. Malathion was used as a space spray or applied by ultra-low volume cold fogging, whereas temephos granules were applied to water containing domestic and peridomestic infestations of *Aedes aegypti* (L.). In Monterrey City, both 200-liter metal drums and discarded tires have become the most important breeding sites for *Ae. aegypti* larvae. Therefore, since 1981, larval control has been conducted intensely annually with 1% temephos granules. Presently, strong indications exist that resistance to this organophosphate is occurring, and temephos resistance has been confirmed elsewhere in the Caribbean area. Wirth and Georghiou (1999) reported resistance in the Virgin Islands, where temephos has been used intensively in the *Ae. aegypti* Dengue Control Program.

Development of resistance to *Bacillus thuringiensis* var. *israelensis* (*Bti*) by mosquitoes has been much less pronounced (Georghiou and Wirth 1977), and this insecticide has proven to be highly efficient for the control of many mosquito species and is relatively nondisruptive to aquatic environments. After a dengue outbreak in 1999, the Nuevo León State *Ae. aegypti* Control Program requested tests on the use of Vectobac® 12 AS, the *Bti* product available in the Mexican market. The aims of the study were the determination of *Ae. aegypti* susceptibility to *Bti* under laboratory conditions and the establishment of field application rates for local mosquito breeding sites.

MATERIALS AND METHODS

Aedes aegypti *susceptibility:* Vectobac 12 AS (aqueous suspension, 600 ITU/mg) of *Bti* (H-14) manufactured by Abbott Laboratories, North Chicago, IL, USA, was used to determine mortality–concentration lines. The bioassays utilized 2nd- and 3rd-stage larval *Ae. aegypti*. Laboratory ambient conditions were 70% relative humidity and 28–

Table. 1 Description of *Bacillus thuringiensis* var. *israelensis* (*Bti*) concentrations in bioassays to establish mortality–concentration line of VectoBac 12 AS[a] on 2nd- and 3rd-stage larvae of *Aedes aegypti* in Monterrey, Mexico.

Bioassay	*Bti* concentrations (ppm)	Number of exposed larvae
1	0, 0.0002, 0.0004, 0.0008, 0.015, 0.030, 0.06	360
2	0, 0.0015, 0.0030, 0.0060, 0.0090, 0.012, 0.02	360
3	0, 0.006, 0.009, 0.012, 0.020, 0.025, 0.030, 0.035, 0.040	240
4	0, 0.006, 0.009, 0.012, 0.020, 0.025, 0.030, 0.035, 0.040	540
5	0, 0.006, 0.009, 0.012, 0.020, 0.025, 0.030, 0.035, 0.040	420
6	0, 0.006, 0.009, 0.012, 0.020, 0.025, 0.030, 0.035, 0.040	420
7	0, 0.0015, 0.0030, 0.0090, 0.020, 0.035, 0.040	420
8	0, 0.0015, 0.0030, 0.0090, 0.020, 0.035, 0.040	540
9	0, 0.0015, 0.0030, 0.0090, 0.020, 0.035, 0.040	780

29°C. The photoperiod was maintained at 12:12 h light : dark.

Larvae were collected from the city cemetery Camp Saint "El Roble." Larvae thriving in cemetery vases were taken to the Medical Entomology Laboratory at the University of Nuevo León. Larvae were identified and reared in 30 × 30-in. plastic trays. Larvae were fed ground dog food. Adults were fed 10% sugar water. Females were also allowed to take blood meals from mice. Field-collected larvae were added every 2 months. The larvae hatched from eggs obtained in the laboratory corresponding to the F_1 progeny were utilized for bioassays.

Bioassays were conducted as recommended by the World Health Organization (1981); a total of 9 bioassays was conducted. Each of the bioassays included 8 different *Bti* concentrations and 3 replicates for individual concentrations. A control was run concurrently in every bioassay series. Variation in bioassay concentration was modulated by variation in larval susceptibility response; thereby, concentrations were adjusted to a mortality range of 10–90%. To obtain each of the different *Bti* concentrations, Vectobac 12 AS was dissolved in dechlorinated water with the aid of a magnetic stirrer, to provide a stock suspension of 1%. A series of 1% stock dilutions was prepared (Table 1) to provide the appropriate concentration ranges to determine mortality–concentration curves. Bioassays were conducted on 2nd and 3rd larval instars of *Ae. aegypti*. Larvae were not fed during the tests. Mortality readings were taken after 24 h.

Mortality data were analyzed by probit analysis by using the maximum likelihood method (Finney 1977). Data were corrected by using the Abbott (1925) mortality correction formula when necessary.

RESULTS AND DISCUSSION

Figure 1 and Table 1 indicate the concentration–mortality relationship for *Ae. aegypti* after 24 h of exposure to Vectobac 12 AS. According to the maximum likelihood method, the median lethal concentration (LC_{50}) for 24 h exposure was 0.0104 ppm with a confidence interval of 0.0089–0.0120 ppm and the 95% lethal concentration was 0.1843 ppm with a confidence interval of 0.1312–0.2856 ppm ($P < 0.05$).

The results showed a higher LC_{50} value compared to that observed by Vega (unpublished data), who evaluated a Vectobac AS formulation against larval *Ae. aegypti* collected in several localit.

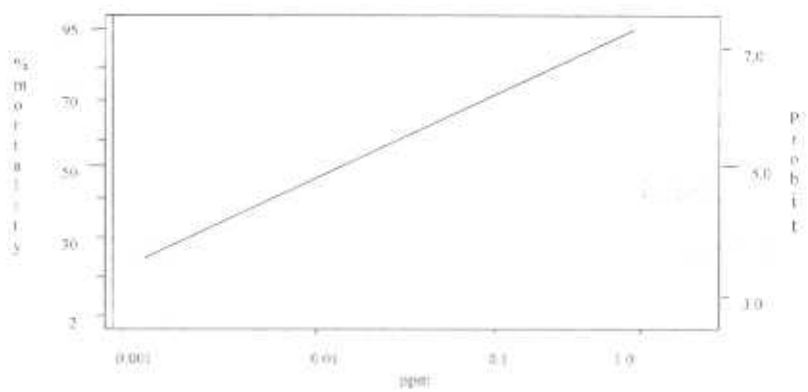

Fig. 1. Response to Vectobac AS[b] (24 h) in 2nd and 3rd larval instars of *Aedes aegypti* based on the maximum likelihood method.

from south and northeastern Mexico. In this latter study, LC_{50} values fluctuated between 0.0012 and 0.0026 ppm. As explained by Skovmand et al. (1998), variation may be associated with such factors as age, stage, and strain of larvae used, as well as amount and type of food provided to larvae. These authors also demonstrated variation in the LC_{50} between laboratories in a quality control study of different laboratories. They tested and compared similar *Bti* products formulated by 6 different companies from 4 different countries against the international standard powder IPS-82. They found significant differences among companies related to the potency of their products.

Becker et al. (1992) considered the possibility that field application factors such as water temperature and larval density may affect bacterial activity on mosquito larvae. These investigators showed that temperatures below 5°C produce 10-fold lower toxicity compared to higher temperatures (>25°C) against *Aedes vexans* (Meigen). Becker and his group also demonstrated a reduction in bacterial effectiveness with an increase in larval density. Lee and Seleena (unpublished data), in a separate study, determined an LC_{50} of 0.0027 mg/liter when utilizing Vectobac (an aqueous suspension of *Bti* 1200 ITU/mg) against *Ae. aegypti*.

Amalraj et al. (2000) determined the efficacy of aqueous suspension and granular formulations of *Bti* (Vectobac). These laboratory tests showed that aqueous suspensions were relatively more effective against *Culex quinquefasciatus* Say than against *Ae. aegypti* and *Anopheles stephensi* Liston. The LC_{50} value for *Ae. aegypti* was 0.060 mg/liter.

The field dose was calculated at 4 times the LC_{50} values resulting form the laboratory bioassays. This concentration was adopted for use by the *Ae. aegypti* Control Program. Vectobac 12 AS was poured into 10,000-liter pipe-tank trucks. Trucks dispensed water treated with *Bti* into 200-gal metal water drums in the backyards of dwellings. Larval indices for this specific container were reduced to zero during the 2-wk treatment period. Dispensing of treated water was stopped when residents complained of a fine dusty film appearing on the water surface (Chiú et al., unpublished data).

Finally, this study demonstrated the potential of using *Bti* formulations against *Ae. aegypti* in Latin America as an alternate larvicide when temephos resistance is suspected or demonstrated. We agree with local residents that appropriate formulations should be made to prevent undesirable effects on domestic water sources. Indeed, community acceptance is of paramount importance to the introduction of any new product.

REFERENCES CITED

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econom Entomol* 18:265–267.

Amalraj DD, Sahu SS, Jambulingam P, Doss PS, Kalyanasundaram M, Das PK. 2000. Efficacy of aqueous suspension and granular formulation of *Bacillus thuringiensis* (Vectobac) against mosquito vectors. *Acta Tropica* 75:243–246.

Becker N, Zgomba M, Ludwig M, Petric D, Rettich F. 1992. Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. *J Am Mosq Control Assoc* 8:285–289.

Finney DJ. 1971. *Probit analysis* 3rd ed. Cambridge, MA: Cambridge Univ. Press.

Gomez-Dantes H. 1992. Monografía sobre la epidemiología del dengue. México, Distrito Federal, México: Secretaría de Salud.

Gubler DJ, Kuno G. 1997. *Dengue and dengue hemorrhagic fever* New York: CAB International.

Knepper RG, Wagner SA, Walker ED. 1991. Aerially applied liquid *Bacillus thuringiensis* var. *israelensis* (H-14) for control of spring *Aedes* mosquitoes in Michigan. *J Am Mosq Control Assoc* 7:307–312.

Lee HL, Gregorio ER Jr, Khadri MS, Seleena P. 1996. Ultralow volume application of *Bacillus thuringiensis* var. *israelensis* for the control of mosquitoes. *J Am Mosq Control Assoc* 12:651–655.

PAHO [Pan American Health Organization]. 1994. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. *PAHO Plant Protection Bull* 27:29–32.

Secretaría de Salud, México. 1999. Sistema nacional de vigilancia epidemiológica. *Epidemiologia* 52(16):10.

Skovmand O, Thiery I, Benzon, GL, Sinegre G, Monteny N, Becker N. 1998. Potency of products based on *Bacillus thuringiensis* var. *israelensis*: interlaboratory variations. *J Am Mosq Control Assoc* 14:298–304.

Sulaiman S, Pawanchee ZA, Wahab A, Jamal J, Sohadi AR. 1997. Field evaluation of Vectobac G, and Vectobac 12AS and Bactimos WP against the dengue vector *Aedes albopictus* in tires. *J Vector Ecol* 22:122–124.

Wilmot TR, Allen DW, Harkanson BA. 1993. Field trial of two *Bacillus thuringiensis* var. *israelensis* formulations for control of *Aedes* species mosquitoes in Michigan woodlands. *J Am Mosq Control Assoc* 9:344–345.

Wirth MC, Georghiou GP. 1999. Selection and characterization of temephos resistance in a population of *Aedes aegypti* from Tortola, British Virgin Islands. *J Am Mosq Control Assoc* 15:315–320.

WHO [World Health Organization]. 1981. *Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides* WHO/VBC/81.807. Geneva, Switzerland: World Health Organization.