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LARVICIDAL PERSISTENCE OF FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* TO CONTROL LARVAL *AEDES AEGYPTI*

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ABSTRACT. After detection of resistance to the organophosphate temephos in populations of *Aedes aegypti* in Brazil, corncob granule (CG) and water-dispersible granule (WDG) formulations of *Bacillus thuringiensis* var. *israelensis* (*Bti*) were introduced in routine focal treatments. Larvicidal persistence and the influence of exposure to sunlight on VectoBac® formulations of *Bti* were compared in 250-liter fiberglass water containers. Production of pupal *Ae. aegypti* in containers was used to indicate control. In untreated containers, survival of larvae was always above 95%. A temephos sand granule formulation used as reference treatment maintained 100% control throughout the 12-wk period in all situations. Under sunlight exposure, control dropped below the 90% level in the 2nd week after treatment at both dosages of VectoBac CG (1 and 2 g/50 liters) and VectoBac tablet (T) formulation at 1 tablet/100 liters. VectoBac T at 1 tablet/50 liters provided 2 wk of 100% control. VectoBac WDG at dosages of 1 and 2 g/500 liters provided 100% control for 3 wk. Without sunlight exposure (covered containers), VectoBac CG provided 9 wk of continuous 100% control and 5 wk of continuous 100% control, respectively, at 1 and 2 g/50 liters. The VectoBac T formulation at both dosages initially provided 2 wk of 100% control. After this period, the control level fluctuated between 96 and 100%. VectoBac WDG provided continuous 100% control for 7 wk for the lower dosage and for 6 wk for the higher dosage. At both dosages of WDG, 100% control was achieved in 11 wk out of the 12-wk period.

KEY WORDS Biological control, mosquito, entomopathogenic bacteria, sunlight exposure, containers

INTRODUCTION

Among the activities of dengue control programs, larval source reduction is the key to sustaining low densities of *Aedes aegypti* (L.), to reduce the risk of dengue epidemics. In this context, larvicides are important for treating mosquito breeding sites when immediate physical elimination is not feasible, for instance in some water reservoirs (PAHO 1994). Water storage is essential for many communities in Brazil, mainly because of the absence of piped water in 25% of houses nationwide and 35% of houses in the northeastern region (IBGE 2004).

The organophosphate temephos has low mammalian toxicity and good larvicidal persistence in the sand granule formulation and has been widely used in dengue control programs (Rozendaal 1997). However, resistance to temephos has been detected in populations of *Ae. aegypti* (Rawlins and Wan 1995, Macoris et al. 1999), leading to the implementation of alternative larvicides to avoid failures in the field.

Such is the case in Brazil, where after the detection of resistance to temephos in populations of *Ae. aegypti,* corncob granule (CG) and water-dispersible granule (WDG) formulations of *Bacillus thuringiensis* var. *israelensis* (*Bti*) were introduced in routine focal treatments. For this purpose, the Brazilian Health Foundation (FUNASA), in the absence of national commercial *Bti* production, has imported hundreds of tons of *Bti* (Vilarinhos 2002).

The aim of this work was to compare the performance of 3 formulations of *Bti* in fiberglass water reservoirs, to investigate larvicidal persistence and the influence of exposure to sunlight.

MATERIALS AND METHODS

Fiberglass water containers (250-liter capacity) were placed in an open area in 2 groups, 1 group with lids covering just 50% of the container, allowing direct sunlight to penetrate through the water column, and another group with lids closed completely, blocking sunlight.

After filling the containers with chlorinated tap water, treatments and untreated controls were made in 3 replicates for both covered and uncovered containers. Products, formulations, and dosages are given in Table 1. The treatment with the temephos sand granule formulation routinely used by the National Dengue Control Program (Fersol 1G, 1 ppm) was used as a treated control reference. The lower *Bti* dosages of CG and WDG formulations tested are those currently used in the National Dengue Control Program. Water temperature in the containers was measured every 4 h with a 64-bit digital temperature recorder.

Once a week, 40 early 3rd-instar laboratory-reared *Ae. aegypti* were added to each container. Although the larvae were fed (dog chow) during initial stages at the laboratory, food was not added after they were transferred to the containers, to simulate the conditions observed in the field. A susceptible strain, originally from the U.S. Department of Agriculture laboratory at Gainesville, FL, was

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Table 1. Potency, formulations, and dosages of *Bacillus thuringiensis* var. *israelensis* and temephos tested for larvicidal persistence (from CENARGEN/EMBRAPA, Brasília, DF, Brazil, 2003).

Product (potency)	Formulation	Dosage (ITU/liter)[1]
VectoBac T™ (2,200 ITU/mg)	Tablet	T1 = 1 tablet/100 liters (7,700) T2 = 1 tablet/50 liters (15,400)
VectoBac WDG (3,000 ITU/mg)	Water-dispersible granule	WDG1[2] = 1 g/500 liters (6,000) WDG2 = 2 g/500 liters (12,000)
VectoBac CG (200 ITU/mg)	Corn cob granule	CG1[2] = 1 g/50 liters (4,000) CG2[2] = 2 g/50 liters (8,000)
Fersol 1 G	Sand granule	1 g/10 liters

[1] ITU, international toxic units of *Aedes aegypti*.
[2] Current field dosage used by the Brazilian Health Foundation (FUNASA).

used because the influence of resistance to temephos on persistence was not investigated in this work.

Containers were checked daily for the production of pupae. Pupae produced in the control and treated containers were removed and counted, and the total was recorded on a weekly basis. The percentage of pupal production was used to evaluate the larvicidal activity and the persistence of formulations. The treatments were considered failed when the rate of production of pupae was 10% or higher.

The results were compared by using Kruskal–Wallis 1-way analysis of variance (ANOVA) on ranks and Dunnett's method to compare with procedures from the Sigmastat computer program (Kuo et al. 1992).

RESULTS AND DISCUSSION

Under sunlight exposure, none of the biological products provided more than 4 wk of control (Fig. 1). VectoBac® CG (Valent Biosciences Corporation, Libertyville, IL) at both dosages and the lower-dosage VectoBac tablet (T) formulation T1 maintained the population under total control (without pupae) for 1 wk. The control level dropped during the 2nd week after treatment, resulting in more than 10% larval survival and pupal production. Statistical analysis of these 3 products in the 3nd week did not show differences among the data (Fig. 1). VectoBac T2 (higher dosage) provided 2 wk of 100% control, although its effect began to decrease in the 3rd week (4% of larvae survived to produce pupae) until the 4th wk, where 25% of the exposed population reached the pupal stage. The WDG at both dosages provided 100% control for 3 wk. Persistence failed in the 4th week, allowing more than 10% of the larvae to become pupae. The statistical analysis done with the number of pupae in the 4th week showed no differences between the 2 dosages of VectoBac WDG. However, a difference was found between the 2 WDG treatments and VectoBac T2 (Kruskal–Wallis 1-way ANOVA, $H = 5.793$; df = 2; $P = 0.05$). According to these results, Vectobac CG seems to be the most affected by sunlight even though the potency of the CG2 formulation was higher than that of the lowest WDG formulation (8,000 and 6,000 ITU/liter, respectively). Examination of these data suggests that features other than the active ingredient concentration play important roles in the larvicidal persistence of *Bti* formulations. The longest larvicidal persistence with sunlight that was obtained with VectoBac WDG in both dosages sustains this hypothesis. The results observed by Melo-Santos et al. (2001) with an experimental tablet formulation

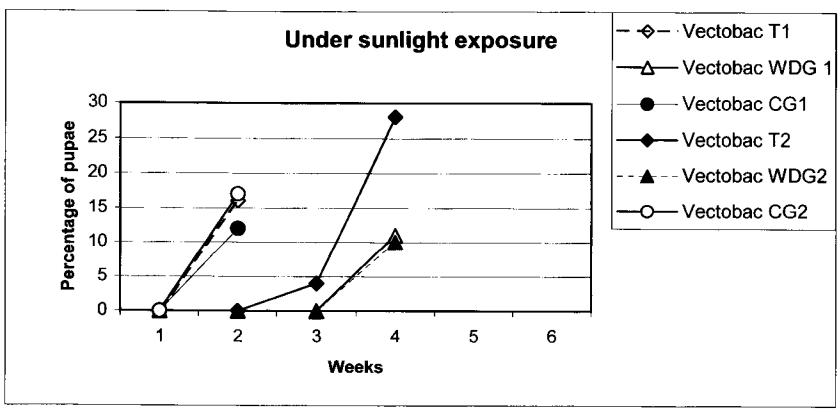

Fig. 1. Percentage of pupae produced during 4 wk of treatment with various formulations of biological products under sunlight exposure (from EMBRAPA Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil, 2003).

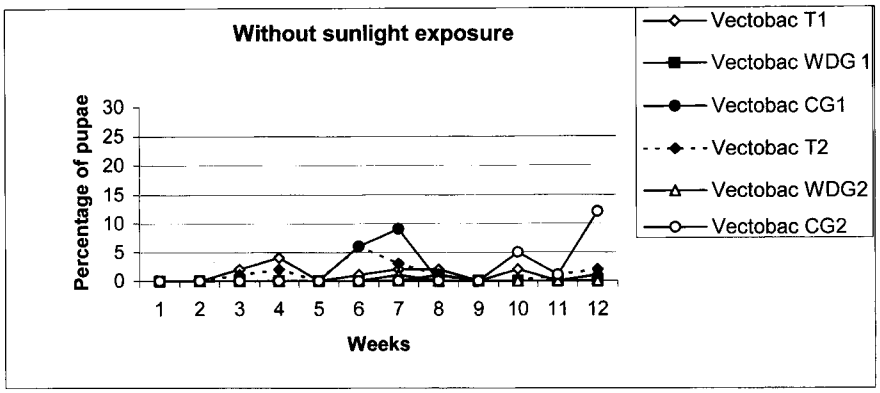

Fig. 2. Percentage of pupae produced during 12 wk of treatment with various formulations of biological products without sunlight exposure (from EMBRAPA Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil, 2003).

from Far-Manguinhos (CP41) (Rio de Janeiro, RJ, Brazil) in shaded and sunlight-exposed containers are somewhat similar to the results obtained in the present study. Melo-Santos et al. (2001) obtained 5 wk of 90% control in sunlight-exposed containers, which is equal to our observations for the WDG formulation at the high dosage. The persistence of the tablet formulation VectoBac T under sunlight was 3 wk shorter than that observed for CP41. However, the potency of CP41 and data for duration of 100% control in their work were not given.

All biological treatments maintained the population under 90% of control during 11 wk (Fig. 2) without sunlight exposure (covered containers). The data analysis did not show differences among all treatments. VectoBac CG2 provided 9 wk of continuous 100% control at the higher dosage, whereas the lower dosage provided 5 wk of continuous 100% control. After the 10th week, the results were equal at both dosages. VectoBac T initially provided 2 wk of 100% control. After this period, the control level fluctuated between 96 and 100% and both dosages achieved 100% controls for 5 wk out of the 12-wk period. VectoBac WDG provided continuous control of 100% for 7 wk for the lower dosage and for 6 wk for the higher dosage. In both dosages of WDG, 100% control was achieved for 11 wk out of the 12-wk period.

In the covered containers, the persistence of all *Bti* formulations was equivalent in this study and longer than that observed for CP41, because in all situations more than 90% control was obtained during 11 wk. However, results with WDG were better, because all treatments provided 11 wk of 100% control; the closest result to that level of control was obtained with CG at the highest dosage for 9 wk.

Susceptibility of *Bti* to sunlight and ultraviolet radiation is well documented (Ignoffo et al. 1982, Becker et al. 1992, Liu et al. 1993, Myasnik et al. 2001). The results obtained in this study are in agreement with those observations, because a noticeable difference was found in the overall persistence with exposure to sunlight, when compared to treatments in covered containers. Similar with the results of the present study, Chui et al. (1995), who used higher concentrations of Vectobac CG and 4th-instar *Ae. aegypti,* observed a 16% decrease in larval mortality after 8 days of exposure to a 14:10 h light:dark photoperiod.

Production of pupal *Ae. aegypti* in each control container was never below 95% (data not shown). Water temperature in the containers varied between 18.5 and 31.0°C in the uncovered containers (average 23.4°C) and 19.0 and 31.0°C in the covered containers (average 22.5°C). The organophosphate temephos sand granule formulation (Fersol 1G) maintained 100% control throughout the 12-wk period in both situations (data not shown).

Considering the 60-day cycle of visits employed by the National Dengue Control Program in Brazil, examination of the results of this study suggests that the WDG formulation of VectoBac is more suitable than the other formulations for routine treatments in drinking water containers.

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