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EVALUATION OF GRANULAR CORNCOB FORMULATIONS OF *BACILLUS THURINGIENSIS* SEROVAR *ISRAELENSIS* AGAINST MOSQUITO LARVAE USING A SEMI-FIELD BIOASSAY METHOD

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ABSTRACT. A semi-field bioassay method using commonly available laundry tubs evaluated efficacy and residual activity of 6 granular corncob formulations of *Bacillus thuringiensis* serovar *israelensis* against laboratory-reared late 3rd- and early 4th-instar larvae of *Aedes taeniorhynchus* introduced to the tubs. These formulations produced appreciable larval mortalities (up to 75%) for 9 days posttreatment, with indications of having more prolonged activity. The consistent posttreatment larval mortality trends and the elucidated efficacy differences between formulations suggest that this bioassay method for the granular formulations is reliable and reproducible.

INTRODUCTION

In the USA, *Bacillus thuringiensis* serovar *israelensis* de Barjac (*B.t.i.*) has been registered for mosquito control since 1982 and continues to be one of the most effective and dependable microbial lavicides available for use in mosquito control programs. The rapid development and registration of *B.t.i.* was aided by the relative ease for its mass production, its high efficacy and selective action against some nematocerous Diptera, environmental safety, ease of handling, storage stability, cost effectiveness, lack of resistance, and suitability for integrated control programs based on possible community participation. There were also much lower costs for its development and registration (about U.S. $500,000) as compared to a conventional chemical insecticide costing nearly U.S. $20 million (Becker and Margalit 1993).

Due to the lack of alternative chemical and microbial agents, efforts are being made to improve the effectiveness of *B.t.i.* by prolonging its activity as well as targeting delivery of the active ingredient in the feeding zone of the larvae. These improvements are primarily based on development of a variety of formulations. To date, several fluid, powder, granular, pellet, and briquet formulations of *B.t.i.* are commercially available.

As *B.t.i.* is a microbial larvicide, it is necessary to routinely bioassay the different batches produced to elucidate the effectiveness and arbitrarily determine the potency of the material in comparison to known standards. Bioassay against mosquito larvae by serial dilution of liquid and powder formulations of *B.t.i.* can be readily performed in the laboratory in 100 ml of water in paper cups (Ali et al. 1981). However, solid formulations, such as granular, pellet, and briquet, due to their size limitation, pose dilution problems as they may not be divided below one granule, pellet, or briquet, and therefore require some suitable and reproducible bioassay system.

This paper describes a semi-field bioassay method using laundry tubs for evaluating granular corncob formulations of *B.t.i.* against laboratory-reared *Aedes taeniorhynchus* (Wied.) larvae introduced into the tubs. Such a method is a necessary and valuable tool for estimating residual activity of the granular formulations because the internationally accepted bioassay method for *B.t.i.* is only appropriate for determining product potency and not residual activity.

MATERIALS AND METHODS

Six *B.t.i.*-impregnated corncob formulations received from Abbott Laboratories, North Chicago, IL, were evaluated. According to the manufacturer, these experimental formulations provided different "release" characteristics, that is, the physical release of *B.t.i.* technical powder (active ingredient) from the corncob carrier. The formulations were differentiated by different code numbers, such as 16099-219-D, 16099-240, 16099-250, 79-976-BD, 79-977-BD, and 68-435-N8. The granule size and weight of these formulations ranged from 5 to 8 mesh to the inch (*ca.* 3–5 mm diam) and 59 to 72 granules/g. All formulations contained an arbitrarily defined 200 ITU (International Toxic Units) of *B.t.i.*/mg.

For the bioassays, polyvinylchloride laundry tubs, commonly available from hardware stores, were utilized. Each tub was 51 × 51 × 35 cm. Forty liters of tap water were left in each of 39 tubs for 24 h for the water to age. These tubs were maintained outdoors under a canopy of 40% shade cloth. On the following day, 100 late 3rd- and early 4th-instar larvae of *Ae. taeniorhynchus* were placed in each tub. The larvae were obtained from a colony established at the Florida

Table 1. Efficacy of 6 corncob formulations of *Bacillus thuringiensis* serovar *israelensis* applied at 1.25 and 2.5 kg/ha (3 replicates for each treatment) against laboratory-reared late 3rd- and early 4th-instar *Aedes taeniorhynchus* larvae introduced to laundry tubs placed outdoors, central Florida, August–October 1993. One hundred larvae were placed in each tub on days 0, 6, and 9. The experiment was repeated 3 times.

Formulation	Rate (kg/ha)	Cumulative[1] mean % larval mortality posttreatment (days) 1	2	3	7	8	9	10
16099-219-D	1.25	27	54	77	42	53	61	7
	2.50	37	78	85	38	54	69	4
16099-240	1.25	43	56	59	28	40	51	4
	2.50	78	86	88	23	38	48	6
16099-250	1.25	41	65	76	42	50	55	4
	2.50	89	94	94	32	43	50	6
79-976-BD	1.25	79	90	93	25	43	57	4
	2.50	97	99	99	29	53	63	11
79-977-BD	1.25	77	88	89	33	44	50	5
	2.50	95	98	99	34	50	60	12
68-435-N8	1.25	56	75	82	25	43	54	4
	2.50	80	92	99	38	57	67	8
Control	—	2	4	4	4	8	15	4

[1] Average of 3 tests.

Medical Entomology Laboratory, Vero Beach, FL, since 1981, and were reared using standard procedures. Within 1–2 h of the placement of larvae in each tube, 1 g of powder of beef liver and yeast (1:1) mixture was added to each tub for larval food and the *B.t.i.* granules needed for each treatment were randomly applied to the tubs by hand. Each formulation was evaluated at 1.25 and 2.5 kg/ha. For the lower rate, 3 granules with a total weight of 32.5 mg, and for the higher rate, 6 granules with a total weight of 65 mg, of each formulation were selected for the treatments to maintain the same weight as well as number of granules for each formulation. Each rate of a formulation was replicated 3 times and 3 untreated controls were maintained. The posttreatment larval mortality in each tub was monitored daily for 2–4 days. All dead and living larvae from each tub were removed with a 10-cm-deep fish net mounted on a 15 × 12-cm metal (wire) frame, and the net contents transferred to a small tray and examined. The treatment granules from each tub were gently removed with forceps prior to the removal of larvae and transferred to a paper cup. This practice prevented loss of granules as well as any release of *B.t.i.* toxin due to turbulence and water currents generated by operating the fish net. The living larvae and the treatment granules were returned to the appropriate tub after scoring larval mortality. After 2–4 days of daily mortality assessments, all remaining living and dead larvae and pupae were removed from each tub and discarded. On the same day or 2–3 days later, another batch of 100 laboratory-reared late 3rd and early 4th instars of *Ae. taeniorhynchus* were introduced to each tub and the larval mortality checked daily for 3–4 days post-introduction of larvae. Multiple (3 or 4 times) posttreatment introductions of larvae to the tubs were made to estimate residual activity of the test formulations over time. A maximum and minimum thermometer was placed in one tub to record daily temperatures. Rainfall in the area was also noted. The test was repeated on 3 different occasions between August and October 1993. Data were analyzed by using ANOVA and Student's *t*-test.

RESULTS AND DISCUSSION

All corncob formulations of *B.t.i.* at 1.25 kg/ha and 2.5 kg/ha produced significant ($P < 0.001$) larval mortality of *Ae. taeniorhynchus* as compared to the controls (Table 1). The low rate of each formulation gave a lower level of control than the high rate during the first 3–6 days of posttreatment (Fig. 1). However, the larval mortality differences produced by the low and the high rates were not significantly different (*t*-test, $P > 0.05$) for formulations 16099-219-D, 16099-240, and 16099-250, whereas formulations 79-976-BD, 79-977-BD, and 68-435-N8 did produce significantly higher mortality (*t*-test, $P < 0.01$) at the high rate.

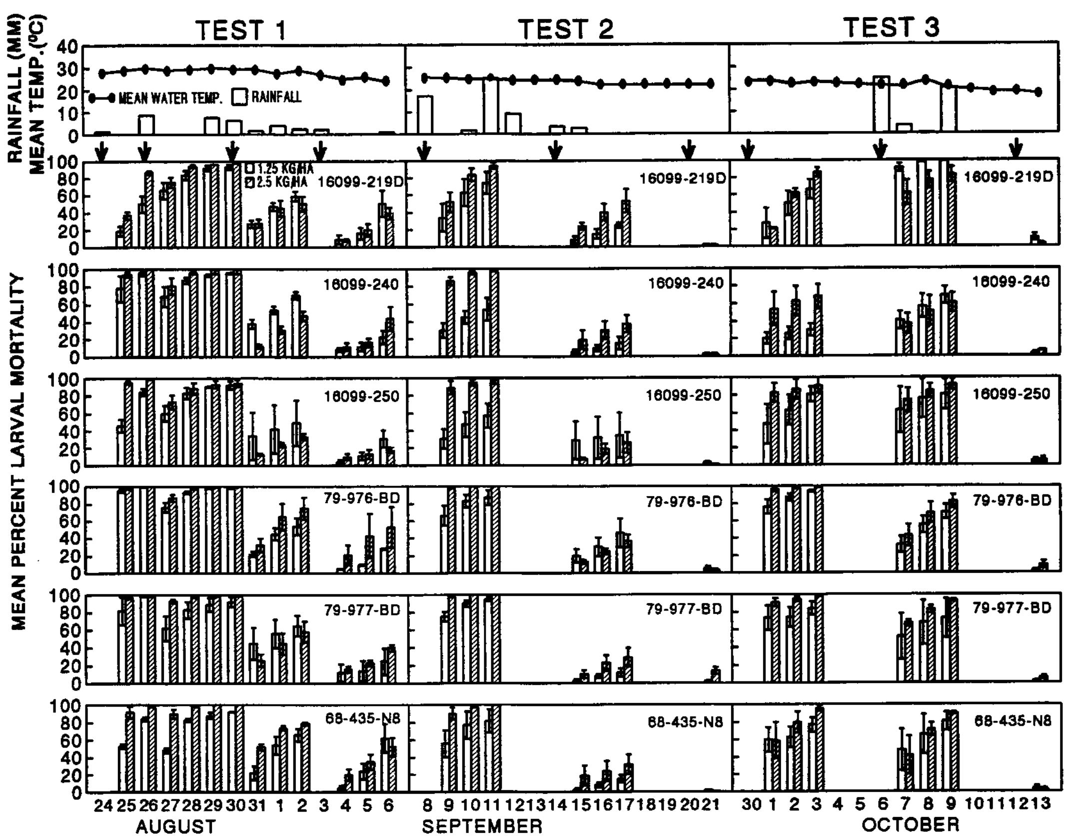

Fig. 1. Mean percent larval mortality of late 3rd- and early 4th-instar larvae of *Aedes taeniorhynchus* in laundry tubs placed outdoors and treated with 6 different corncob granular formulations of *Bacillus thuringiensis* serovar *israelensis* at 1.25 (open bars) and 2.5 kg/ha (cross-hatched bars). Arrows indicate introductions of mosquito larvae to the tubs. Tubs were treated with the granules on August 24, September 8, and September 30, 1993 (tests 1, 2, and 3). Water temperature and rainfall during the study period are also shown.

In test 1, all formulations at the low rate produced at least 92% larval mortality during 6 days posttreatment. In the same period, the high rate of these formulations produced 98–100% larval mortality. Among the test formulations, 79-976-BD was slightly superior in residual activity, causing 75% larval mortality at 9 days posttreatment in test 1. In test 2, 79-976-BD, 79-977-BD, and 68-435-N8 produced 100% larval mortality at 3 days posttreatment. During test 2, unusually heavy rainfall of *ca.* 5 cm during 7 days posttreatment significantly increased the water level in many tubs. This diluted the *B.t.i.* toxin, affecting larval mortality, the maxima for which ranged only from 12 to 53% at both rates for the various formulations at 9 days posttreatment. All formulations in test 2 were almost completely ineffective at 13 days posttreatment. In test 3, all formulations even at the low rate maintained >69% reduction of larvae at 9 days posttreatment despite *ca.* 2.5 cm of rainfall recorded until 8 days posttreatment. At 13 days posttreatment in test 3, all formulations became ineffective because again nearly 5 cm of rainfall was recorded by day 13, causing a significant dilution of the *B.t.i.* toxin in the tubs.

Statistical analysis to elucidate efficacy differences between formulations revealed significantly lower larval mortality caused by 16099-240 than formulations 16099-250, 79-976-BD, 79-977-BD, and 68-435-N8 (t-test, df = 6; low rate, $P < 0.05$; high rate, $P < 0.01$). Also, there was significantly lower larval mortality caused by 16099-250 than formulations 79-976-BD and 79-977-BD (t-test, df = 6, $P < 0.01$) at 2.5 kg/ha. The tested formulations in order of decreasing efficacy were 79-976-BD followed by 79-977-BD, 68-435-N8, 16099-219-D, 16099-250, and 16099-240.

Previous studies (e.g., Margalit et al. 1983, Silapanuntakul et al. 1983), as well as commercial and operational experience with liquid or powder formulations have shown that the high mosquito larvicidal activity of *B.t.i.* disappears within 2–3 days. Applications of excessively high rates (5–10 × the minimum effective rate) of a liquid *B.t.i.* formulation (Vectobac 12 AS) against *Culex* spp. did not significantly extend the duration of effectiveness (Mulla et al. 1993). The present study has revealed that the corncob formulations being developed for slow or sustained release of the *B.t.i.* toxin remain effective at least 9 days posttreatment. The residual effectiveness of the formulations tested may have lasted beyond 9 days posttreatment, had there not been an unusually excessive rainfall during the study period. The prolonged residual activity of *B.t.i.* through formulation improvements is highly desirable to reduce the number of treatments for achieving effective larval control in a given habitat because the cost of treatment, monitoring, and surveillance of mosquito larval populations is much greater than the cost of material. The corncob formulations can be an important addition to briquets and pellets for achieving prolonged control of mosquito larvae.

The somewhat similar and consistent posttreatment larval mortality trends noted in this study indicate that the method of bioassay developed for the corncob formulations of *B.t.i.* is reproducible and reliable for the preliminary screening for effective formulations and their residual efficacy.

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