AN EVALUATION OF TWO FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* FOR LARVAL MOSQUITO CONTROL IN SOD-LINED SIMULATED POOLS

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ABSTRACT. Two wettable powder formulations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) manufactured by Abbott Laboratories and Biochem Products were tested for efficacy against 2 species of mosquito larvae in simulated pool conditions. Their effect on some aquatic insect predators of mosquito larvae was also investigated. Both formulations proved effective in controlling the test mosquito species. The Biochem Products material was formulated at a higher potency and gave 100% control of 4th instar *Culex restuans* after 12 hr at 0.8 mg/ℓ; the Abbott formulation gave 25% control after 12 hr. When applied at 3.2 mg/ℓ, the Abbott formulation gave 100% control of 4th instar *Aedes vexans* after 24 hr. Second instar larvae of both species were generally more susceptible than 4th instar larvae. Effective residual activity was less than 24 hr at the highest rates tested for both the Abbott and Biochem formulations. Mortality was negligable in non-target invertebrate predators following a 5 day exposure to 12 mg/l of both formulations of *B.t.i.*

INTRODUCTION

Laboratory studies with *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) have shown it to be toxic against several mosquito species (Goldberg and Margalit 1977; de Barjac 1978 a,b,c; de Barjac and Coz 1979; Garcia and Desrochers 1979). Recent field tests with *B.t.i.* have also demonstrated encouraging control potential against several pest mosquito species (Garcia et al. 1980b, Hembree et al. 1980). Tests conducted on non-target organisms have indicated that *B.t.i.* is considered quite safe to non-target vertebrates and invertebrates (Garcia et al. 1980a). The efficacy and safety characteristics of this pathogen have made it a suitable candidate for large scale production.

The objective of this study was to examine the efficacy of 2 commercial formulations of *B.t.i.* on 2nd and 4th instar larvae of *Aedes vexans* (Meigen) and *Culex restuans* Theobald under simulated pool conditions. The effect of these preparations on some selected aquatic insect predators of mosquito larvae was also investigated.

METHODS AND MATERIALS

Formulations of *B.t.i.* tested in this study were produced by Biochem Products (a division of Salisbury Laboratories) P.O. Box 264 Montchanin, Delaware 19710, and by Abbott Laboratories, North Chicago, Illinois 60064. The formulation supplied by Biochem Products, "Bactimos" was a wettable powder, batch no. 666 PM50, and had a potency of 3,000 International *Ae. aegypti* toxic units/mg. The Abbott Laboratories for-

mulation was also a wettable powder (ABG-6108D), lot 6478-194, and had a potency of 1,000 International *Ae. aegypti* toxic units/mg.

Larvae of *Ae. vexans* used in these experiments were obtained from eggs laid in the laboratory by field-collected females. Larvae were fed liver powder and maintained at 23 ± 2°C. *Culex restuans* larvae were hatched from egg rafts obtained from simulated pools outdoors, and cultured in the laboratory as described for *Ae. vexans*.

Tests were conducted in 1 m^2 plastic bottomed pools lined with landscaping sod, and filled with 72 ℓ of city water. The pools were filled 24 hr prior to initiation of the experiments, allowing for dechlorination. Cylindrical screen (Nitex) cages (10 cm dia. × 18 cm h) were used as bioassay testing units. Two cages, each containing 20 second instar larvae and 2 cages each containing 20 fourth instar larvae were placed in each pool prior to application of *B.t.i.*. The applications were quantitated as wt./volume. The Abbott formulation was tested against both species of mosquito larvae at concentrations of 0.8, 1.6, and 3.2 mg/ℓ. The Biochem formulation was tested at concentrations of 0.1, 0.4, and 0.8 mg/ℓ. Each test consisted of 3 test pools and one control pool.

Material to be applied to each pool was weighed, then thoroughly mixed in one liter of dechlorinated water. This suspension was applied to the pools using a one liter compressed air sprayer. Mortality counts were recorded at periods of 12, 24, and 48 hr after exposure. The pH of the water was taken in each pool at the beginning of the experiments and after 48 hr. Temperatures were also recorded at the start of each experiment, and during each mortality count.

Fourth instar larvae of *Cx. restuans* were also introduced in the 3.2 mg/ℓ treatment of the Abbott formulation 24 hr after the experiment was begun, and observations were made to note mortality after periods of 24 and 48 hr (days 2 and 3). A similar procedure was followed with 4th instar *Cx. restuans* larvae exposed to 0.8 mg/ℓ of the Biochem formulation. The purpose of these experiments was to examine residual activity of *B.t.i.*

The effect of *B.t.i.* on some selected non-target invertebrate predators of mosquito larvae was also examined. Three genera of Odonata (naiads of *Libellula, Anax* and *Gomphus*) and one genus of Hemiptera (adults of *Notonecta*) were exposed to a very high dose of each formulation (12 mg/ℓ). The tests were conducted in large plastic buckets filled with 15 ℓ of dechlorinated water. Mosquito larvae were introduced into the buckets at the start of the experiment and after periods of 24 and 48 hr to simulate a natural situation where predators would be feeding on treated prey. Mortalities were recorded after a period of 5 days.

RESULTS AND DISCUSSION

The results of the tests with the Abbott formulation of *B.t.i.* are shown in Table 1. All 2nd instar larvae of *Cx. restuans* were killed after 48 hr at the 3 concentrations tested (0.8, 1.6, and 3.2 mg/ℓ). Mortality in 4th instar larvae was 85% at 0.8 mg/ℓ and 95% at 3.2 mg/ℓ after 48 hr. *Ae. vexans* 2nd instar larvae were all killed after 48 hr at all 3 concentrations. Mortality of 4th instar larvae was 45% at 0.8 mg/ℓ and 98% at 3.2 mg/ℓ. Generally, mortality increased with dose and duration of exposure for both species of mosquito larvae. Second instar larvae were more susceptible to the pathogen than 4th instar larvae at all dosages tested.

The results of the tests involving the Biochem formulation are listed in Table 2. Mortality of *Cx. restuans* 2nd instar larvae was 38% at 0.1 mg/ℓ and 78% at 0.8 mg/ℓ after 48 hr. Fourth instar larvae showed 63% mortality at 0.1 mg/ℓ and 100% mortality at both the 0.4 and 0.8 mg/ℓ levels. *Ae. vexans* 2nd instar larvae exposed to 0.1 mg/ℓ showed 75% mortality after 48 hr; this was 100% at both the 0.4 and 0.8 mg/ℓ dosages. Forty-eight hr

Table 1. Efficacy of *B. thuringiensis* var. *israelensis* (Abbott) on 2nd and 4th instar larvae of *Culex restuans* and *Aedes vexans*. Mean water temperature 18.5°C.

Species	Water pH	Percent Mortality									Control
		0.8 mg/ℓ			1.6 mg/ℓ			3.2 mg/ℓ			
		12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h	48 h
Culex restuans											
2nd instar	7.1	86	98	100	85	98	100	70	93	100	8
4th instar	7.1	25	65	85	40	70	85	53	88	95	3
Aedes vexans											
2nd instar	7.1	28	73	100	93	100	100	93	100	100	23
4th instar	7.1	8	38	45	48	93	100	63	95	98	10

mortality was 43% at a dose of 0.1 mg/ℓ and 100% at 0.8 mg/ℓ when 4th instar larvae were exposed. Ninety-three percent mortality occurred at 0.4 mg/ℓ. Again, in this test, mortality generally increased with dose and duration of exposure for both species of mosquito larvae. Second instar larvae were more susceptible to the pathogen than 4th instar larvae at the 3 dosages tested for *Ae. vexans*. However, 4th instar larvae showed higher mortality than 2nd instar larvae at each dosage tested against *Cx. restuans*. This is contradictory to the other results obtained in this study, and those of previous studies. Control mortality for the 4th instar larvae was more than twice as high as recorded for the 2nd instar larvae, indicating that this test group was probably less viable.

Larvae of *Cx. restuans* (4th instar) introduced 24 hr after the initial 3.2 mg/ℓ exposure of the Abbott formulation showed only a 5% mortality after a period of 24 hr, and 15% mortality after 48 hr. The Biochem formulation exposed at 0.8 mg/ℓ produced 20% mortality after 24 hr, and 25% mortality after 48 hr in 4th instar larvae of *Cx. restuans* introduced 24 hr after the initial exposure. These results indicate that effective residual activity of both formulations is restricted to less than 24 hr.

The results of *B.t.i.* exposure on some selected invertebrate predators of mosquito larvae are shown in Table 3. Both formulations applied at 12 mg/ℓ produced no mortality or adverse effects on the 3 genera of dragonfly naiads after a test period of 5 days. A 20% mortality was recorded in *Notonecta* sp. exposed to the Abbott formulation. However, there was also a 10% mortality in the control group, suggesting that the mortality may have been due to confinement in the plastic containers, rather than a direct effect of

Table 2. Efficacy of *B. thuringiensis* var. *israelensis* (Biochem) on 2nd and 4th instar larvae of *Culex restuans* and *Aedes vexans*. Mean water temperature 19.0°C.

Species	Water pH	Percent Mortality									Control
		0.1 mg/ℓ			0.4 mg/ℓ			0.8 mg/ℓ			
		12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h	48 h
Culex restuans											
2nd instar	7.1	23	38	38	50	75	80	55	68	78	8
4th instar	7.1	25	38	63	95	98	100	100	100	100	15
Aedes vexans											
2nd instar	6.9	33	53	75	90	200	100	100	100	100	18
4th instar	6.9	8	25	43	60	83	93	88	95	100	8

Table 3. Effects of an exposure concentration of 12 mg/ℓ of *B. thuringiensis* var. *israelensis* on selected non-target invertebrate predators of mosquito larvae.

Organism	Number tested	Formulation	% mortality after 5 days	
			Test	Control
Odonata				
Libellula sp.	20	Abbott	0	0
	20	Biochem	0	0
Anax sp.	5	Abbott	0	0
	7	Biochem	0	0
Gomphus sp.	5	Abbott	0	0
Hemiptera				
Notonecta sp.	20	Abbott	20	10
	20	Biochem	5	0

the pathogen. *Notonecta* sp. exposed to the Biochem formulation showed only a 5% mortality after a test period of 5 days.

These data indicate that both the Abbott and Biochem formulations of *B.t.i.* provide effective mosquito control. The Biochem formulation was 2–4 times as potent as the Abbott formulation against both species of mosquitoes. This would be expected as the Biochem Products formulation had a potency of 3,000 International *Ae. aegypti* toxic units/mg. compared to a potency of 1,000 International *Ae. aegypti* toxic units/mg. for the Abbott formulation.

Research to develop formulations that extend residual activity could make *B.t.i.* a very important agent in the management of mosquitoes in the future.

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