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LABORATORY AND FIELD TRIALS WITH TWO BACILLUS THURINGIENSIS VAR. *ISRAELENSIS* PRODUCTS FOR *SIMULIUM* (DIPTERA: NEMATOCERA) CONTROL IN A SMALL POLLUTED RIVER IN SOUTH AFRICA

M. CAR, Veterinary Research Institute, Onderstepoort 0110[1]

ABSTRACT

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The effects on *Simulium adersi* and *S. hargreavesi* larvae of 2 *Bacillus thuringiensis* var. *israelensis* products, the liquid formulation "Teknar" (Sandoz) and a powder formulation produced by the Ben Gurion University, Israel, were compared in the laboratory and in the Pienaars River. This river was heavily polluted with effluent from a nearby sewage works and contained 77 mg/ℓ chloride.

In the laboratory *S. adersi* and *S. hargreavesi* larvae showed 26; 48; 95 and 100 % mortality 6 hours after a 10-minute application of 0,8; 1,6; 3,2 and 16 ppm "Teknar" in rain water. The powder formulation applied at 0,2; 1,0; 2,0 and 30 ppm resulted in a 7; 17; 35 and 100 % mortality. In polluted river-water the mortality was 85 % with 16 ppm "Teknar" and 80 % with 30 ppm *B. thuringiensis* powder.

In the field trials "Teknar" at 1,6 ppm and *B. thuringiensis* powder at 3 ppm did not cause any larval mortality at flow rates of 3 060 ℓ/min and 2 040 ℓ/min, respectively. However, 24 hours after application of the powder formulation, numbers of *S. hargreavesi* decreased significantly (P=0,05) 20 m below the application point. A further 24 hours later, after "Teknar" had been applied, the numbers of *S. adersi* decreased and those of Chironomidae increased significantly. There was a significant increase in *S. hargreavesi* 200 m downstream after treatment with "Teknar".

INTRODUCTION

Bacillus thuringiensis var. israelensis (B.t.i.) has been used successfully for blackfly control in the Ivory Coast (Lacey, Escaffre, Philippon, Sékétéli & Guillet, 1982) and recent trials by Car & Moor (1984) have shown its potential for blackfly control in South Africa. *B.t.i.* was used in Israel for mosquito control in a sewage ditch (Margalit, Lakhim-Tsror, Pascar-Gluzman, Bobroglo & Barak, 1981), but little was known about its performance against blackflies in heavily polluted rivers. This aspect was therefore studied in the Pienaars River, just east of Pretoria.

MATERIALS AND METHODS

Laboratory trials

To determine the most suitable concentration of *B.t.i.*, a set of laboratory trials was carried out before the field application. A 2nd set of laboratory trials was done afterwards to see the effect of sewage and chloride on the toxicity of *B.t.i.* to blackfly larvae. These experiments were carried out using the magnetic stirrer device described by Colbo & Thompson (1978) adapted for the use of 10 2,5 ℓ plastic jars (Fig. 1). Each jar was filled with 2 ℓ water which was changed 10 minutes after the treatment with the larvicide. The stirring speed was about 3 rev./2 sec. Approximately 100 simuliid larvae in instars 2–7, which had been collected from the nearby Pienaars River 45 minutes previously, were left for 1 hour in the jars to settle before the start of the experiments. Tap water used in the 1st set of trials was aged by leaving it outside in an asbestos container for 2 months. There it became mixed with rain water and was therefore regarded as "rain water". For the 2nd set of trials river water, brought in a closed plastic container together with the larvae, was used.

Percentage mortality was calculated by counting live and dead larvae 6 hours after the application of *B.t.i.* Because of the small number of larvae the mortality of the 2 *Simulium* species present was not calculated separately in the laboratory trials.

The products chosen were an emulsifiable concentrate[2] containing 0,8 % *B.t.i.* spores with a recommended field concentration of 1,6 ppm/10 min., and undesignated *B.t.i.* powder produced by the Ben Gurion University, Israel, with an LC_{50} of $6{,}3 \times 10^{-7}$ mg/mℓ for *Aedes aegypti*.

The number of sporulated cells/mg was determined as $1{,}6 \times 10^7$ for liquid *B.t.i.* by viable colony counts and haemacytometer, and as $1{,}5 \times 10^8$ for the *B.t.i.* powder by haemacytometer. The application temperature was 20 °C in the laboratory and 17 °C in the river.

For the 1st laboratory trial the emulsifiable, liquid *B.t.i.* formulation was stirred in 100 mℓ water on a magnetic stirrer and then applied to rain water at concentrations of 0,8; 1,6 and 3,2 ppm. The powder was applied in the same way for concentrations of 0,2; 1,0 and 2,0 μg/mℓ (=ppm).

In the 2nd laboratory trial the effect of 16 ppm/10 min liquid, and 30 ppm powdered *B.t.i.* was compared in rain water and polluted river water.

Field trials

These were carried out between 9 and 12 May 1983 at a gauging weir of the Pienaars River close to the Roodeplaat Dam about 2,5 km below the inflow from the Baviaanspoort Sewage Works. The stones of the riverbed were covered with sludge and simuliid larvae could only be found on trailing vegetation.

In the 1st field trial 60 g of *B.t.i.* powder was blended with 1,5 ℓ distilled water for 20 minutes and then added to a container with 6,5 ℓ distilled water. At the application site, 1 hour later, the mixture was topped up to 20 ℓ with river water and applied with a hose-pipe back and forth 15 times per minute for 10 minutes across the 3 m wide overflow of the weir (Fig. 2). At a flow rate of 2 040 ℓ/min, read from a gauge plate, the concentration of the product was 3 ppm.

For the 2nd field trial 50 mℓ of liquid *B.t.i.* was dissolved in 1 ℓ distilled water, mixed with 19 ℓ river water at the application site and applied at the same site in the same way, 30 hours after the 1st field trial. This time the river flow was 3 060 ℓ/min and the concentration of the product 1,6 ppm.

Sampling points were selected 20; 80 and 200 m below the application site. Fauna was collected before each application and again 24 hours afterwards on 5 totally submerged leaves of reeds (Fig. 3). At the 20 m and 200 m points each leaf was preserved separately in formalin. At the 80 m point the 5 leaf samples were pooled since only small areas on them were colonized by simuliid larvae; these samples were therefore used solely for reference. In the laboratory the leaf surfaces were measured, the insects on each leaf counted separately and calculated as numbers per 1000 cm^2. The significance of the results was evaluated by the Mann-Whitney

[1] Present address: A. Hruzastr. 3, A-2345 Brunn am Gebirge, Austria

[2] Sandoz 4021 SC ("Teknar"), Hoechst Pharmaceuticals (Pty) Ltd

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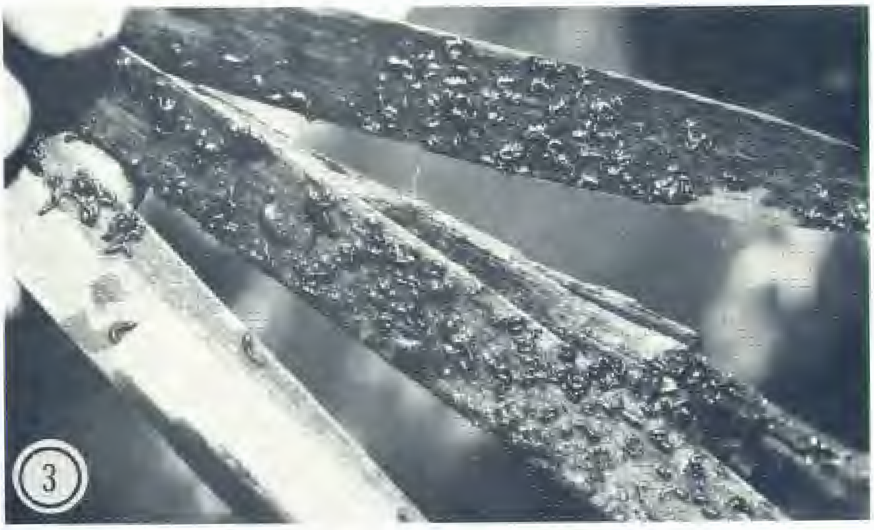

FIG. 1 Magnetic stirring apparatus for rearing blackfly larvae and testing larvicides

FIG. 2 Application of *B.t.i.* at a 3 m wide overflow of a gauging weir in the Pienaars River

FIG. 3 *Simulium adersi* and *S. hargreavesi* larvae on leaves collected at a sampling point 200 m below the application point

U-test (Elliott, 1977), comparing the ranking of simuliid densities on each leaf before and after application of *B.t.i.*

RESULTS

Laboratory trials

The results of both trials are shown in Table 1. Liquid *B.t.i.* at the recommended field concentration of 1,6 ppm (which gives a calculated spore concentration of 2,1 × 10^4 spores/mℓ water) caused 48 % mortality. At 2,0 ppm and a calculated spore concentration of 3 × 10^5 spores/mℓ water, the *B.t.i.* powder caused 35 % mortality. In the 2nd trial, concentrations of 16 ppm (liquid *B.t.i.*) and 30 ppm (powdered *B.t.i.*) gave 100 % mortality in rain water, but resulted in only 85 % and 80 % mortality respectively in river water.

TABLE 1 Percentage mortality of *Simulium adersi* and *S. hargreavesi* larvae in the laboratory 6 hours after a 10 min application of different concentrations of a liquid and a powder formulation of *Bacillus thuringiensis* var. *israelensis* in rain water and in river water with a high chloride content

	Concentration	Mortality in:	
		Rain water	River water
B.t.i. Liquid (Teknar)	0,8 ppm	26%	—
	1,6 ppm	48%	—
	3,2 ppm	95%	—
	16,0 ppm	100%	85%
B.t.i. Powder (Ben Gurion University)	0,2 ppm	7%	—
	1,0 ppm	17%	—
	2,0 ppm	35%	—
	30,0 ppm	100%	80%
Control	0,0 ppm	3%	5%

Field trials

In the field trial *B.t.i.* powder reduced the numbers of *S. hargreavesi* significantly (P = 0,05) 20 m downstream, but did not show significant effects on *S. adersi* and Chironomidae (Table 2).

After the application of liquid *B.t.i.*, the numbers of *S. adersi* decreased significantly and those of chironomid larvae increased significantly 20 m below the application point. Two hundred metres downstream the only significant effect was an increase of *S. hargreavesi* larvae (Table 2).

DISCUSSION

A field experiment in the Vaal River resulted in 100 and 85 % mortality in *S. adersi* and *S. hargreavesi* respectively 9 hours after the application of 1,6 ppm/10 min liquid *B.t.i.* 35 m upstream (Car & Moor, 1984). The comparatively low mortality of the same 2 species in the present laboratory studies can be attributed to the short observation period of 6 hours and a lower feeding rate than under natural conditions. Frommer, Hembree, Nelson, Remington & Gibbs (1980) left simuliid larvae in the breeding jars for 3 hours and Undeen & Nagel (1978) for 24 hours to adapt to the test environment before *B.t.i.* was added.

As simuliid larvae in the rearing jars had already begun to feed within an hour, and because the mortality in the control jars was low (Table 1), the test facilities were considered adequate for the purpose of screening the products.

In the field trial the concentration of the *B.t.i.* powder was increased to 3 ppm to obtain a mortality comparable to that expected from the application of 1,6 ppm of the liquid formulation. After enquiries from the Department of Environment Affairs (Jan Schutte, personal communication, 1983) the surprisingly low efficacy of both products in the Pienaars River could be attributed to its high sewage level and a chloride content of 77 mg/ℓ during the application period. Walmsley & Toerin (1978) found that, in August 1973, at the application point of this study, 83 % of the river flow consisted of sewage outflow of the Baviaanspoort Sewage Works. The average chloride content during the winter months of 1973 was 80 mg/ℓ. They also found that the lower the river flow, the higher the sewage content will be.

The author observed that the average flow of the Pienaars River during the first 2 weeks of May 1983 was below 4200 ℓ/min, which is less than ⅓ of the flow in August 1973. Presumably, therefore, the sewage outflow made up more than 90 % of the river flow during the present study period.

Sinégre, Gaven & Vigo (1980, cited by Gaugler & Finney, 1982) noted the inhibiting influence of chlorine in tap water on the endotoxin of *B.t.i.*, leading to an eight-ninefold decrease in the larvicidal activity of *B.t.i.* powder in the laboratory. The chloride content in tap water can vary but, according to L. M. Siebert (personal communication, 1983), the chloride content of the Pienaars River during the study period was 4 times higher than the yearly mean of Pretoria tap water.

Gaugler & Finney (1982) suggested that neither the pH nor the level of salinity affect the toxicity of *B.t.i.*, but turbidity can have a negative influence on its efficacy. Margalit *et al.* (1981) found that in mosquitoes the LC_{50} was 100 times higher in sewage than in distilled water. According to the technical bulletin supplied with

TABLE 2 Pre- and post treatment comparisons of the fauna in the Pienaars River (expressed as numbers per 1000 cm^2) collected from 5 submerged reeds at each of 3 sampling points 20; 80 and 200 m below the point at which 2 formulations of *Bacillus thuringiensis* var. *israelensis* were applied in May 1983. Statistical comparisons were made using the Mann-Whitney U-test

Sampling point	Invertebrates	Total number in samples			Significance (P = 0,05) comparing samples (U-value in parenthesis)*	
		Before treatment (I)	24 h after application of 3 ppm *B.t.i.* powder (II)	24 h after application of 1,6 ppm *B.t.i.* liquid (III)	I and II	II and III
1 (20 m downstream)	*S. adersi*	4 410	13 043	1 012	NS (8,0)	S− (0,0)
	S. hargreavesi	6 027	2 757	882	S− (2,0)	NS (5,0)
	Chironomidae	703	336	983	NS (7,5)	S+ (2,0)
2 (80 m downstream)	*S. adersi*	310	1 460	320	Not calculated	
	S. hargreavesi	1 317	672	320	Not calculated	
	Chironomidae	78	63	80	Not calculated	
3 (200 m downstream)	*S. adersi*	5 720	6 732	6 359	NS (12,0)	NS (11,0)
	S. hargreavesi	1 560	1 483	6 421	NS (11,0)	S+ (0,0)
	Chironomidae	140	103	519	NS (10,0)	NS (3,0)

* NS = not significantly different (U > 2); S− = significantly less (U < 2); S+ = significantly more (U< 2)

the liquid formulation, the recommended concentration for mosquito control in sewage-polluted water is 2–4 times higher than that prescribed for floodwaters. No recommendations, however, are made for the use of higher concentrations for blackfly control in polluted rivers.

The results of the 2nd trial, in which the liquid *B.t.i.* formulation was used, could have been influenced by the 1st application of *B.t.i.* powder only 30 hours before. This, however, is unlikely as in previous trials in the Vaal River (Car & Moor, 1984) *B.t.i.* killed *S. adersi* and *S. hargreavesi* within 18 hours after application and no significant decrease of simuliid larval numbers could be observed afterwards.

The increase of Chironomidae after the application of the liquid *B.t.i.* formulation at the 20 m sampling point could be due to recolonization with *B.t.i.*-resistant Chironomidae after simuliid larvae had drifted off. A significant increase of *S. hargreavesi* larvae at the 200 m sampling point could indicate the resettling of larvae which had been disturbed further upstream.

CONCLUSION

The efficacy of *B.t.i.* products for blackfly control decreases about 10 times in a river with a high sewage content and a chloride concentration of 77 mg/ℓ.

Liquid *B.t.i.* formulations generally have the advantage over powder formulations in that they mix more easily with water. In the field trials, the *B.t.i.* powder, though applied at nearly double the concentration and 20 times higher in spore numbers, showed the same low efficacy as the liquid formulation. In the laboratory the powder was also less effective than the liquid *B.t.i.*

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