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EFFICACY OF *BACILLUS THURINGIENSIS ISRAELENSIS*, *BACILLUS SPHAERICUS* AND TEMEPHOS FOR MANAGING *ANOPHELES* LARVAE IN ERITREA

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ABSTRACT. We evaluated the larvicidal activity of the granular formulation of *Bacillus thuringiensis israelensis* (*Bti*) serotype H-14 (Vectobac® G, 200 ITU/mg) and *Bacillus sphaericus* (*Bsph*) serotype H5a5b (Vectolex® CG, 670 Bs ITU/mg) against *Anopheles arabiensis* and other mosquitoes in breeding habitats in 3 sites, Gash-Barka, Anseba, and Debub zones, in Eritrea. The primary objective was to determine the optimal application rate and duration of effect for *Bti* and *Bsph* in representative larval habitats as compared with the organophosphate temephos. The biolarvicides were tested at 100% (high) and 50% (low) of the maximum recommended application rate. Temephos was applied at a rate of 100 ml/ha. At least 4 replicate experiments with Vectobac G (5.6 and 11.2 kg/ha), Vectolex CG (11.2 and 22.4 kg/ha) were conducted in each study site. All 3 larvicides caused significant mortality of the main malaria vector species, *An. arabiensis,* and other mosquito species (*Anopheles cinereus, Anopheles pretoriensis, Culex quinquefasciatus*). The larvicidal activity for *Bti* and *Bsph* was variable depending upon breeding habitat, mosquito species, and general ecology of the area. Both biopesticides had a similar duration of activity (2–3 wk) and were generally as effective as temephos for these time periods. In some cases, the high and low application rates for *Bti* and *Bsph* produced equivalent control over 2–3 wk. The 2 *Bacillus* biopesticides were less effective in habitats with high algal content and in fast flowing streams primarily because of the inability to penetrate algal mats and dilution effect, respectively. The results show that application of the 2 biolarvicides bimonthly to streambed pools, rain pools, and similar habitats would maintain control of the anopheline mosquito population.

KEY WORDS Microbial insecticides, *Anopheles arabiensis,* larval control, malaria, Eritrea

INTRODUCTION

The National Malaria Control Program (NMCP) in Eritrea supplements adult vector mosquito control measures, such as the use of impregnated bednets and indoor DDT residual spraying, with larval control with temephos. As the NMCP intensifies its operations to meet expectations of the World Health Organization (WHO) Roll Back Malaria Program, it has become critical to develop and evaluate cost-effective and environmentally safe larvicidal alternatives to temephos in order to prevent the development of resistance yet provide effective control of malaria vectors in a variety of aquatic habitats. A strong association exists between the density and distribution of the pre-adult stages and that of the adult vectors (Grillet 2000). Therefore, vector control strategies aimed at suppressing larval production would subsequently affect adult population densities, thereby limiting malaria transmission.

One goal of the NMCP is to identify and characterize ecological settings in the country in which vector control, and more specifically larval control, can make a cost-effective contribution to malaria control. In redefining its long-term vector control strategies for the country, the NMCP has renewed interest in larval control as a critical component of the program's integrated vector management policy. The semiarid climatic conditions, the seasonal incidence of malaria, and the nature of human settlement in isolated towns and villages make Eritrea ideal for implementation of larval control as one of the principal interventions for reducing the burden of malaria in the country. In most regions of the country, the very short malaria transmission season coincides with the short rainy season from June to September in the central highlands and western lowlands and from December to February for the coastal strip. In some areas, however, mosquitoes and associated malaria transmission persist throughout the year, even during the long dry season. At key locations throughout the country, larval control with temephos has proved to be a feasible strategy under these dry or semiarid climatic conditions because mosquito larval habitats are discrete and easy to target. In Eritrea, *Anopheles arabiensis* Patton is a major vector of malaria (Carrara et al. 1994; Ministry of Health, Eritrea, unpublished data).

Two biolarvicides, *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*Bsph*), both ef-

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fectively control different mosquito species in a variety of breeding habitats and are environmentally safe (Mulla et al. 1984, Karch et al. 1992, Federici 1995). The biolarvicides and temephos have undetectable nontarget toxicity (Brown et al. 1999, Lawler et al. 1999). However, experimental evaluation of both agents specifically as control agents against malaria vectors in Africa is limited. Furthermore, because the performance of biolarvicides may be affected by habitat characteristics, it is important to evaluate the efficacy of these compounds in a variety of ecological settings.

MATERIALS AND METHODS

Study sites: The efficacy studies were conducted in Tessenei, Elabered, and Korbaria in the Gash-Barka, Anseba, and Debub zones, respectively. All 3 sites lie at different altitudes and their climatic conditions are variable. Tessenei is at 500 m above sea level (asl), Elabered is at 1,500 m asl, and Korbaria is at 1,800 m asl. Rainfall seasonality in the 3 sites is similar, with a rainy season from June to September. The larval habitat types selected included 1) stream and stream bed habitats at Elabered in the Anseba zone, 2) temporary ponds and associated seepage areas at Korbaria in the Debub zone, and 3) roadside ditches and rain pools at Tessenei in the Gash-Barka zone. These sites represented areas of relatively high adult mosquito abundance and larval productivity as determined by earlier countrywide vector distribution surveys conducted in the country (Shililu et al. in press).

Experimental design: The efficacy studies were conducted with natural larval breeding sites. The larval habitats (experimental test plots) were mapped and marked with numbered flags and a survey tape to secure them against human interference. Villagers were instructed to keep their animals away from the test plots. Prior to the start of the experiments, up to 10 plots were sampled to assess the densities of different larval instars, and out of these, 6 plots with the highest densities of 1st (L_1) and 2nd (L_2) instars were selected. Plots were separated by no less than a 5-m buffer to prevent any cross contamination.

Each replicate experiment involved 6 plots so that the 3 test products, *Bti, Bsph,* and temephos, were tested simultaneously relative to an untreated control plot. The 6 plots were randomly assigned as follows: 2 plots to *Bti* (high and low doses), 2 plots to *Bsph* (high and low doses), 1 plot to temephos, and 1 plot kept as a control per replicate experiment. In total, 4, 5, and 6 replicate experiments were conducted in Tessenei, Korbaria, and Elabered, respectively. To achieve the desired number of replicate experiments, the same test plots were in some instances used when high densities of larvae were collected. In this case, the larvicide type and dose rate were maintained for each plot in subsequent replicate experiments. In cases where the plots dried up, new plots were selected.

Plot sizes and water depth at the deepest point were recorded. The plot sizes ranged from 21.0–60.0 m^2 with a mean water depth of 9.7 cm in Elabered (Anseba zone) to 8.4–30.4 m^2 with a mean depth of 49.2 cm in Korbaria (Debub zone). In Tessenei, Gash-Barka zone, the plots ranged from 6.0 m^2 to 16.3 m^2 and had an average depth of 19.7 cm.

Larvicidal application rates: Granular formulations of *Bti* serotype H-14 (VectoBac® G, 200 ITU/mg) and *Bsph* serotype H5a5b, strain 2362 (VectoLex® CG, 670 Bs ITU/mg) were tested at the maximum application rate and at 50% of the maximum application rate. Both larvicides were supplied by Valent BioSciences™ Corporation (Chicago, IL). A single plot was treated with temephos (Abate® 47.4% w/w a.i., 500 g a.i. per liter), and an untreated plot was kept as a control in each replicate. The larvicides were applied at 100% and 50% label rates to the treated plots as follows: 100% VectoBac G, 11.2 kg/ha (high); 50% VectoBac G, 5.6 kg/ha (low); 100% VectoLex CG, 22.4 kg/ha (high); 50% VectoLex CG, 11.2 kg/ha (low); and Abate® at 100 ml/ha. Calibration of the Maruyama® granular spreader was made for 5/8 mesh VectoBac granules and the 10/14 mesh VectoLex CG granules at the high and low application rates and used for the application of the granular formulations. Clean Hudson liquid sprayers were used for the application of temephos.

Larval sampling: Prior to treatment, mosquito larvae in each plot were sampled by standard dipping methods. A minimum of 5 and a maximum of 10 dips were taken randomly depending on the size of the test plots. The number of mosquito immature developmental stages (L_1, L_2, L_3, L_4, and pupae) per dip was recorded. An estimate of larval density was then made by calculating the number of larvae per dip. One sample each of 3rd- and 4th-stage larvae was preserved in 70% alcohol and identified by standard larval taxonomy on the basis of external morphology. During the course of the experiment, each plot was sampled for larvae at 24 and 48 h and then once per week posttreatment. In Tessenei, however, because of the high rate of dying in larval plots, sampling was conducted daily. The experiment was terminated when high densities of larvae were detected in all of the treated test plots or when the plots dried up. Mulla's formula was used to determine efficacy of the larvicides and application rates relative to untreated control plots (Mulla et al. 1971).

RESULTS

Larvicidal effects of *Bti, Bsph,* and temephos against *An. arabiensis*

Preliminary larval studies undertaken have shown that *An. arabiensis* is the major vector of malaria in Eritrea and breeds predominantly in streambed pools, roadside ditches, ponds, and run-offs at domestic water collection points (Table 1). Over 80% of the larval samples identified were *An. arabiensis.*

All 3 larvicides caused significant mortality of immature stages of *An. arabiensis* and other minor anopheline species (e.g., *Anopheles cinereus* Theobald, *Anopheles pretoriensis* Gough). The variation in mean densities of the anopheline pre-adult stages in the 3 habitat types (i.e. streambed pools, ponds, and roadside ditches) are shown in Tables 2–4. Overall, the densities of the larvae (L_1–L_4 instars) and pupae decreased after application of the 3 insecticides, with a greater effect being recorded in the first 48 h. Reduction in larval populations was generally pronounced in the first 14 days posttreatment.

Roadside ditches and streambed habitats

At 24 and 48 h posttreatment, temephos, VectoBac G, and VectoLex CG at the high and low application rates provided over 90% larval control for *An. arabiensis* and other mosquito larvae in roadside ditches and streambed pools. From 7 days posttreatment onward, the activity of both *Bti* and *Bsph* tended to vary depending on the breeding habitat. For example, in streambed habitats in the Anseba zone, 84.4% and 97.8% control were recorded with *Bsph* at the high and low dose rates, respectively. *Bti* at both rates and temephos, on the other hand, produced at least 75% control at 7 days posttreatment (Table 5). In the streambed habitats, a 14-day control against anophelines was achieved by the 3 larvicides at both application rates. In the roadside pools in Gash-Barka, 100% control was recorded for *Bti, Bsph,* and temephos. This result showed that both the low and the high application rates were effective against the anopheline species at 7 days posttreatment. The efficacy trials in the roadside ditches could not be sustained beyond 7 days because of the fast rate of drying of the test plots.

Ponds

In Korbaria (1,800 m asl), the performance of both *Bsph* and *Bti* at both application rates in the ponds was generally similar, giving over 85% larval control mortality. Temephos produced 100% control in these habitats at 7 days postapplication. Overall, the data show that all 3 larvicides provided effective control against *An. arabiensis* and *Culex quinquefasciatus* Say up to 7 days posttreatment. *Bti* at the high and low rates produced appreciable control over 21 days posttreatment (Table 5). A mean control percentage of 89% was recorded at 28 days posttreatment. *Bsph,* on the other hand, was effective only at the maximum rate at 28 days, with control by the low application rate breaking down

Table 1. Anopheline larval species composition and distribution among different aquatic habitats in Eritrea.[1]

Zone	Habitat type	*Anopheles* species					
		arabiensis	*funestus*	*pretoriensis*	*cinereus*	*squamosus*	*demeilloni*
Anseba	Pond	56	0	0	0	0	0
	Ditches	78	0	0	0	0	0
	Streambed pool	387	17	24	136	15	0
	Swamp	12	0	0	0	0	0
Debub	Dam	9	0	0	0	0	0
	Stream	142	7	6	20	74	3
Gash-Barka	Pond	42	0	0	0	0	0
	Ditches	191	0	0	0	0	0
	Streambed pool	345	25	0	0	0	0
	Swamp	74	0	0	0	0	0
	Water supply	192	0	0	0	0	0
Total		1528	49	30	156	89	3

[1] The values represent the numbers of each species positively identified by morphological criteria for each larval habitat. We identified 23% of the total *Anopheles* larvae (3rd and 4th instars) to species.

Table 2. Mean densities of pre-adult stages of anopheline larvae after treatment with *Bacillus thuringiensis israelensis (Bti)*, *Bacillus sphaericus* (*Bsph*), and temephos among streambed pools in Elabered, Anseba zone, Eritrea.

Treatment	Days posttreatment	Mean density[1,2]		
		$L_1 + L_2$	$L_3 + L_4$	Pupae
UTC[3]	0[4]	14.97	4.82	0.68
	1	8.17	2.87	0.93
	2	4.25	1.30	0.68
	7	9.48	4.63	1.37
	14	9.90	4.23	1.23
	21	17.95	9.25	3.85
Bsph 100%[5]	0	26.07	4.53	1.40
22.4 kg/ha	1	2.45	2.02	0.12
	2	0.80	0.35	0.10
	7	3.02	0.60	0.12
	14	12.17	4.17	0.70
	21	15.80	6.85	0.30
Bsph 50%	0	5.08	2.18	0.25
11.2 kg/ha	1	0.48	0.10	0.07
	2	0.10	0.05	0.00
	7	0.65	0.23	0.12
	14	3.70	1.32	0.13
	21	6.20	0.30	0.30
Bti 100%	0	13.28	2.15	0.45
11.2 kg/ha	1	0.20	0.23	0.18
	2	0.00	0.00	0.00
	7	2.13	0.55	0.05
	14	6.73	1.15	0.25
	21	6.35	1.60	1.05
Bti 50%	0	12.00	1.64	0.44
5.6 kg/ha	1	1.17	0.43	0.69
	2	0.03	0.10	0.26
	7	2.49	0.36	0.16
	14	11.81	1.64	0.40
	21	8.10	3.00	1.00
Temephos	0	14.28	3.80	0.45
100 ml/ha	1	0.07	0.65	0.37
	2	0.07	0.07	0.13
	7	0.88	0.17	0.03
	14	9.75	2.95	0.30
	21	17.40	4.90	0.90

[1] Density is expressed as number of larvae/pupae per dip.
[2] L_1, 1st instar; L_2, 2nd instar; L_3, 3rd instar; L_4, 4th instar.
[3] UTC, untreated control.
[4] Days zero (0) denotes pretreatment sampling.
[5] 100%, 100% of the maximum application rate; 50%, 50% of the maximum application rate.

after 21 days posttreatment. Temephos produced 100% control at 21 days posttreatment but only 50% control at 28 days posttreatment.

DISCUSSION

The corncob granular formulation of *Bti* produced significant mortality of anopheline larvae 48 h postapplication. The control activity observed was consistent up to the first 7 days posttreatment in all 3 types of larval habitat (i.e., roadside pools, streambed pools, and ponds). In the roadside pools, however, the experiment could not be extended beyond 7 days because the experimental plots dried up. Use of artificial experimental plots where water would be managed would remedy this problem in future investigations. In the streambed habitats, VectoBac G applied at half (5.6 kg/ha) and maximum (11.2 kg/ha) label rates suppressed larval production of *An. arabiensis* and other anopheline species for up to 2 wk. Both larval habitats are sunlit and shallow and are the main breeding sites for malaria vectors in the country (Shililu et al. in press). Anopheline larvae tend to prefer open sunlit waters (Gillies and Coetzee 1987). Although biolarvicides have the potential of being used in the Afro-tropical situation to control the most efficient vectors of malaria of the *Anopheles gambiae* complex, only limited studies have been carried out to evaluate their efficacy (Karch et al. 1991). Because of the wide range of larval habitats and ecological diversity available in most malaria endemic countries in sub-Saharan Africa, it is imperative that experimental evaluation of *Bti* and other microbial

Table 3. Mean densities of pre-adult stages of anopheline larvae after treatment with *Bacillus thuringiensis israelensis* (*Bti*), *Bacillus sphaericus* (*Bsph*), and temephos in ponds in Korbaria, Debub zone, Eritrea.

Treatment	Days posttreatment	Mean density[1,2]		
		$L_1 + L_2$	$L_3 + L_4$	Pupae
UTC[3]	0[4]	13.73	1.13	0.00
	1	21.13	2.93	0.13
	4	12.60	4.33	0
	7	6.27	1.87	0
	14	7.80	0.40	0
	21	7.40	4.40	0
	28	5.89	3.97	0.09
Bsph 100%[5]	0	12.73	1.40	0
22.4 kg/ha	1	3.13	0	0
	4	8.93	0.07	0
	7	1.67	0	0
	14	3.30	0.10	0
	21	8.80	0	0
	28	2.40	0	0
Bsph 50%	0	4.27	0.07	0
11.2 kg/ha	1	0.67	0	0
	4	2.33	0	0
	7	3.40	0	0
	14	4.27	0.27	0
	21	7.13	0.53	0
	28	2.15	0.95	0
Bti 100%	0	11.60	1.07	0
11.2 kg/ha	1	0.13	0.20	0
	4	0.13	0.070	0
	7	3.53	0	0
	14	6.70	0	0
	21	8.30	3.00	0
	28	0	0	0
Bti 50%	0	8.60	0.47	0
5.6 kg/ha	1	3.27	0	0
	4	1.47	0	0
	7	0.33	0	0
	14	2.60	0.20	0
	21	2.80	1.10	0
	28	2.50	0.40	0
Temephos	0	1.30	0.20	0
100 ml/ha	1	0.40	0	0
	4	0.25	0	0
	7	0.00	0	0
	14	2.20	0.05	0
	21	0.02	0.00	0
	28	1.87	0.13	0

[1] Density is expressed as number of larvae/pupae per dip.
[2] L_1, 1st instar; L_2, 2nd instar; L_3, 3rd instar; L_4, 4th instar.
[3] UTC, untreated control.
[4] Day zero (0) denotes pretreatment sampling.
[5] 100%, 100% of the maximum application rate; 50%, 50% of the maximum application rate.

larvicides be conducted with a view of establishing their efficacy against malaria vector species in different ecological settings. Studies have demonstrated that the efficacy of microbial larvicides is notably affected by environmental and habitat related factors (Mulla et al. 1990), which provides reason for testing the biolarvicides in different ecological situations so that decisions to implement their use are evidence driven.

The larvicidal activity for *Bti* and *Bsph* varied depending on breeding habitat, mosquito species, and general ecology. Interspecific differences in susceptibility of mosquito species to microbial larvicides have been demonstrated (Mulla et al. 1982, Mahmood 1998). These differences mainly are associated with the surface-feeding behavior of different species and their overall feeding rates. The variation in the percentage of control by the larvicides among sites would also be attributed to differences in the type of breeding habitats and associated water qualities and availability of the lethal toxin in appreciable concentration in the feeding zone (Mulla et al. 1990, Becker et al. 1992). In Elabered, the streambed breeding sites experienced

Table 4. Mean densities of pre-adult stages of anopheline larvae after treatment with *Bacillus thuringiensis israelensis* (*Bti*), *Bacillus sphaericus* (*Bsph*), and temephos among roadside ditches in Tessenei, Gash-Barka zone, Eritrea.

Treatment	Days posttreatment	Mean density[1,2]		
		$L_1 + L_2$	$L_3 + L_4$	Pupae
UTC[3]	0[4]	4.53	1.19	0.11
	1	4.67	1.73	0.25
	2	7.73	1.52	0.28
	3	5.33	0.60	0.03
	4	0.50	0.30	0
	5	1.20	1.10	0.10
	6	1.00	0.80	0
	7	1.67	0.37	0
Bsph 100%[5] 22.4 kg/ha	0	5.26	2.50	0.36
	1	0.02	0	0
	2	0.33	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	6	2.70	0	0
	7	5.05	1.5	0
Bsph 50% 11.2 kg/ha	0	3.98	3.03	0.05
	1	0	0	0.03
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	6	0	0	0
	7	0	0	0
Bti 100% 11.2 kg/ha	0	2.52	1.72	0.02
	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	4.70	0	0
	6	2.20	0	0
	7	2.40	1.05	0
Bti 50% 5.6 kg/ha	0	3.08	2.22	0.10
	1	0	0	0
	2	0.13	0	0
	3	0	0	0
	4	0	0	0
	5	1.70	0	0
	6	1.80	0	0
	7	2.90	1.1	0
Temephos 100 ml/ha	0	1.33	1.43	0.05
	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	6	0	0	0
	7	0	0	0

[1] Density is expressed as number of larvae/pupae per dip.
[2] L_1, 1st instar; L_2, 2nd instar; L_3, 3rd instar; L_4, 4th instar.
[3] UTC, untreated control.
[4] Day zero (0) denotes pretreatment sampling.
[5] 100%, 100% of the maximum application rate; 50%, 50% of the maximum application rate.

some water seepage through the sandy substratum, thereby leading to dilution effect. Furthermore, we found evidence of drifting of the light granular material to one end of the plot or downstream, meaning that the full dose may not have been achieved in instances where there was some limited water outflow. A 3rd factor that could have contributed to the relatively low activity in this site was the presence of algal growth that may have compromised feeding activity of larvae on the larvicidal

Table 5. Percentage of reduction of early and late-stage larvae of *Anopheles arabiensis* and other anophelines after treatment with Vectobac® G (11.2 and 5.6 kg/ha) (*Bti*), Vectolex® CG (22.4 and 11.2 kg/ha) (*Bsph*), and temephos (100 ml/ha).

	Days post-treatment	*Bsph* 100%[1] 22.4 kg/ha	*Bsph* 50% 11.2 kg/ha	*Bti* 100% 11.2 kg/ha	*Bti* 50% 5.6 kg/ha	Temephos 100 ml/ha
Streambed pools[2] (Elabered, Anseba zone)	1	90.2 ± 11.2	93.8 ± 11.9	96.2 ± 4.2	90.6 ± 15.1	97.0 ± 4.4
	2	86.9 ± 32.1	98.3 ± 4.2	100 ± 0.0	98.5 ± 2.6	97.0 ± 5.1
	7	84.4 ± 11.9	97.8 ± 4.9	77.5 ± 25.3	75.7 ± 10.7	74.9 ± 34.9
	14	83.8 ± 5.9	73.8 ± 24.9	54.0 ± 22.5	57.0 ± 12.5	73.7 ± 25.7
	21	ND[3]	ND	23.6 ± 1.15	19.7 ± 10.4	ND
Roadside ditches[2,4] (Tessenei, Gash-Barka zone)	1	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
	2	99.9 ± 0.3	100 ± 0.0	100 ± 0.0	99.2 ± 1.8	100 ± 0.0
	3	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
	4	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
	5	100 ± 0.0	100 ± 0.0	ND	73.9 ± 6.4	100 ± 0.0
	6	ND	100 ± 0.0	ND	47.6 ± 13.2	100 ± 0.0
	7	ND	100 ± 0.0	ND	ND	100 ± 0.0
Ponds[5] (Korbaria, Debub zone)	1	95.5 ± 10.2	94.3 ± 9.2	99.5 ± 0.76	96.8 ± 7.8	92.7 ± 16.3
	2	73.2 ± 35.6	87.3 ± 28.3	89.1 ± 20.9	97.7 ± 3.1	97.3 ± 6.08
	7	91.9 ± 13.5	97.3 ± 7.1	94.0 ± 8.19	94.6 ± 12.1	100 ± 0.0
	14	75.8 ± 41.9	84.2 ± 22.6	73.4 ± 35.3	78.7 ± 20.5	84.7 ± 21.7
	21	82.5 ± 24.8	93.2 ± 9.69	83.1 ± 22.1	91.7 ± 14.4	100 ± 0.0
	28	67.8 ± 10.2	23.9 ± 11.2	78.8 ± 30.1	100 ± 0.0	53.2 ± 9.2

[1] 100%, 100% of the maximum application rate; 50%, 50% of the maximum application rate.
[2] *Anopheles* were the predominant species (>95%) in streambed pools and ditches.
[3] ND, Test plots were dry and/or high densities of larvae were sampled (% control = zero).
[4] Experiments not done beyond 7 days because of drying of test plots.
[5] Culicine larvae were predominant (>65%) compared with anophelines in ponds.

agent. Studies have indicated that anopheline larvae are highly associated with algae (Gimnig et al. 2001), and formulations that would penetrate such algal carpets would be a good option in specific situations. Previous entomological studies showed that over 90% of anopheline breeding activity in the country takes place on stream edges and streambed pools that are associated with algal growth (Shililu et al. in press). This habitat becomes the critical larval habitat to be targeted for larval control interventions with microbial larvicides, and therefore the efficacy of different formulations needs to be evaluated.

Temephos and both *Bti* and *Bsph* provided control of mosquito larvae on stream-edge and streambed habitats for a 2-wk period. Application of the 2 larvicides once every 2 wk would maintain control. A similar application cycle would be appropriate for rain pools and roadside ditches. The high and low application rates for *Bti* and *Bsph* produced equivalent control over the 2-wk period. A 3-wk larvicidal activity was recorded for *Bsph* at 100% and only a 2-wk activity at the low rate in ponds at 1,800 m asl. Application with *Bsph* at the maximum label rates on a 3-wk cycle would be an appropriate operational regime for ponds and similar breeding sites within this ecological stratum. *Bti* applied at the low rate (50% of the maximum recommended application rate) every 3 wk would also be appropriate under these specific conditions.

The data show that both microbial larvicides, *Bti* and *Bsph,* would form viable options for control of pre-adult stages of *An. arabiensis* and other mosquito species in Eritrea, especially because larval control is an important component of the integrated vector management strategy in the country.

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