LABORATORY AND FIELD EFFICACY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND *BACILLUS SPHAERICUS* AGAINST *ANOPHELES GAMBIAE* S.L. AND *CULEX QUINQUEFASCIATUS* IN OUAGADOUGOU, BURKINA FASO

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ABSTRACT. Two wettable powders (Bactimos® and Vectobac®) and one flowable concentrate (Teknar®) of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and primary powders of *Bacillus sphaericus* isolates 1593 and 2362 were evaluated (laboratory) against field-collected larvae of *Anopheles gambiae* s.l. and *Culex quinquefasciatus* in Ouagadougou, Burkina Faso. Bactimos, Vectobac and a Corn-cob *B.t.i.* formulation (ABG-6138G) were field tested against *Cx. quinquefasciatus* and *An. gambiae* s.l. The isolates of *B. sphaericus* were also tested against *An. gambiae* s.l. in artificial ponds. Both wettable powders of *B.t.i.* showed superior activity than the flowable concentrate formulation against *An. gambiae* s.l. in the laboratory. *Culex quinquefasciatus* was more susceptible (3–4×) to *B.t.i.* (Bactimos) than *An. gambiae* s.l. The isolates of *B. sphaericus* were more effective (2–3×) against both mosquito species than Bactimos. In a ditch and two channels, Bactimos, Vectobac and ABG-6138G at 0.65, 1.5 and 5.6 kg/ha, respectively, gave 91–100% control of *Cx. quinquefasciatus* within 3 days of treatment. The same formulations at rates ranging from 0.25 to 5.6 kg/ha, produced 82–97% control of *An. gambiae* s.l. in rainwater pools 24 h after treatment. Isolates 1593 and 2362 at 0.12 and 0.24 kg/ha gave excellent control of *An. gambiae* s.l. in artificial ponds.

INTRODUCTION

Ouagadougou, the capital of Burkina Faso, is located in a typical Sudan savanna region where malaria transmission occurs at high levels during the rainy season. Recent entomological studies to assess the malaria vectorial system in the Ouagadougou area have shown that *An. gambiae* s.l. is the most common vector in Ouagadougou as well as in the surrounding rural areas (Rossi et al. 1984). Another mosquito species, *Culex quinquefasciatus* Say, is widespread year-round in urban areas of Burkina Faso and is a source of great discomfort.

Although malaria vector control projects in Burkina Faso through the use of organochlorine insecticides have been conducted in the past (Hamon et al. 1957, 1958), no measures for vector control have been taken in that country with any regularity. Local health authorities sporadically used some chlorinated hydrocarbons (particularly DDT) until 10 years ago. Organophosphorus (OP) insecticides (malathion and temephos) have also been employed for mosquito control, but information on the dosage and frequency of use of these insecticides is not available in the literature.

In 1983, a cooperative program on malaria and vector control was established between the governments of Italy (Ministry of Foreign Affairs) and Burkina Faso. The objectives of this program are to generate information on the entomological and epidemiological aspects of the mosquito vectors and malaria, and to explore the possibilities for biological and chemical control of the vector. Majori et al. (1986) have recently reported on the laboratory susceptibility of larval and adult *An. gambiae* s.l. and *Cx. quinquefasciatus* collected from Ouagadougou area to several organic insecticides. This paper reports the effectiveness of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and *Bacillus sphaericus* (isolates 1593 and 2362), tested in the laboratory and field against *An. gambiae* s.l. and *Cx. quinquefasciatus* in Ouagadougou.

MATERIALS AND METHODS

The formulations of *B.t.i.* evaluated in the laboratory and/or in the field were two wettable powders (WP), Bactimos® (Biochem Products, Montchanin, DE, USA) and Vectobac® (Abbott Laboratories, North Chicago, IL, USA), one flowable concentrate (FC), Teknar® (Sandoz, San Diego, CA, USA), and one granular corncob (ABG-6138G) (Abbott Laboratories, North Chicago, IL, USA). The potencies of Bactimos, Vectobac, Teknar and ABG-6138G, respectively, were 3500, 2000, 1500 and 200 *Aedes aegypti* International Toxic Units (ITU)/mg. The isolates of *B. sphaericus* evaluated in the laboratory and field were 1593 (IF 119, spray dried) and 2362 (IF 118, spray dried).

For laboratory assays, field-collected late 3rd and early 4th instars of *An. gambiae* s.l. and *Cx. quinquefasciatus* were used. The larvae of *An. gambiae* s.l. were collected from the area of Nongremassm, while those of *Cx. quinquefasciatus* were collected from Bilibambili and Zone du Bois areas of Ouagadougou. The former species

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was exposed to Bactimos, Vectobac, Teknar and the 1593 and 2362 isolates. *Culex quinquefasciatus* was exposed to Bactimos and the two isolates of *B. sphaericus*. Methods utilized for bioassays were generally the same as described by Mulla et al. (1982). Twenty larvae were placed in 120-ml disposable paper cups containing 100 ml tap water (pH 7.4 ± 0.2). For treatments, each formulation of *B.t.i.* and primary powder of *B. sphaericus* was suspended in tap water by using a magnetic stirrer to make a 1% stock suspension (w/v) and as needed, serial dilutions were made to obtain the appropriate range of concentrations for testing. Each material was tested on at least three different occasions. Each time, 5–6 different concentrations of a material were each applied to three cups (replicates) while three cups were left untreated as controls. The stock suspensions and serial dilutions were freshly prepared on each occasion. Due to the lack of temperature and light control facilities in the laboratory, the test cups were placed under natural light near a closed glass-window where the room temperatures varied from 25 to 27°C during the tests in January–February 1984 and 27 to 30°C in July–August 1984. Larval mortality in the treated and control cups was assessed after 24 and 48 h for *B.t.i.* and *B. sphaericus* treatments, respectively. The mortality in the treated cups in a test was adjusted against any mortality in the controls (Abbott 1925). The corrected mortality was subjected to log-probit regression analysis.

In field studies, Bactimos, Vectobac and ABG-6138G were evaluated against *Cx. quinquefasciatus* in three different habitats during the dry season in Ouagadougou in January–February 1984. These habitats were a roadside ditch in Bilibambili, a concrete-lined channel in Gounghin Nord and a similar channel in Zone du Bois. The ditch was 40 cm wide and contained 15 cm deep turbid and stagnant water bordered by dense-rooted vegetation. A 60 m length of the ditch was treated with Bactimos at 0.65 kg/ha. The Gounghin Nord channel was treated (0.16 km length) with Vectobac at 1.5 kg/ha. This channel was 50 cm wide and contained 10 cm of very slow moving (<10 cm/min) sullage water with a high content of organic matter. The channel in Zone du Bois was of the same width and depth and contained almost stagnant (current <5 cm/min) sullage water rich in organic matter. The water was covered with sparse patches of floating vegetation. This channel received ABG-6138G applied at 5.6 kg/ha to 0.22 km of the channel. An untreated length of 10–15 m immediately upstream of each treated habitat served as control during these trials. For larval sampling, 12 to 22 dip samples from various locations along the entire length of the treated areas and five dip samples from the control area in each habitat were randomly taken. The number of dip samples to be collected from each habitat was determined by the size of the treated area. The dip samples were taken immediately before and 1, 3 and 5–7 days after the treatments.

In the 1984 rainy season (July–August), Bactimos, Vectobac and ABG-6138G were tested against *An. gambiae* s.l. inhabiting rainwater pools located on the outskirts of Ouagadougou. These pools ranged from 4 to 10 m^2 in surface area and 5 to 15 cm in depth. Bactimos was applied to these pools at 0.25 and 0.5 kg/ha and Vectobac at 0.5 and 1.0 kg/ha. Each rate was applied to three pools, thus utilizing 12 pools for the treatments while three untreated pools were monitored as controls. ABG-6138G was also tested in additional nearby pools in the rainy season. The granules were applied at 2.8 and 5.6 kg/ha to each of three separate pools (replicates) while three untreated pools served as controls. Due to the temporary nature of these pools, pretreatment and only 24-h posttreatment dip samples were taken. The number of dips taken from each pool varied between 5 and 10, depending upon the pool size.

The primary powders of *B. sphaericus* isolates 1593 and 2362 were tested against *An. gambiae* s.l. in outdoor artificial ponds (60 cm diam. and 20 cm deep) maintained in Ouagadougou. The water source of these ponds was a nearby reservoir. Water to each pond was added daily (as needed) to maintain the initial level. The ponds were rapidly colonized by *An. gambiae* s.l. after filling. The primary powders of isolates 1593 and 2362 were both applied at 0.12 and 0.24 kg/ha to each of three ponds thus utilizing 12 ponds for the treatments, while three ponds were left untreated as controls. Immediately before and 48 h posttreatment, mosquito larvae were sampled by collecting four dips from each treatment and control pond. The percent reductions of mosquito larvae in each field test were calculated as in Mulla et al. (1971). All WP (*B.t.i.*) and primary powder (*B. sphaericus*) treatments in these studies were made by using a 2-liter hand sprayer to disperse a material evenly over the entire treatment area. Separate sprayers were utilized for the *B.t.i.* and *B. sphaericus* applications. ABG-6138G was dispersed by utilizing a granular applicator. Water pH was measured in each habitat studied. A maximum and minimum mercury thermometer was left in a treated habitat to gather the water temperature data during each field trial.

RESULTS

The data on susceptibility of larval *An. gambiae* s.l. to three *B.t.i.* formulations and two isolates of *B. sphaericus* in the laboratory are

presented in Table 1. *Anopheles gambiae* s.l. was susceptible to Bactimos and Vectobac with LC_{90} values of 0.231 and 0.375 ppm, respectively. Teknar showed much lower level of activity against *An. gambiae* s.l. (LC_{90} = 1.743 ppm). The isolates of *B. sphaericus* showed superior activity against *An. gambiae* s.l. (LC_{90} = 0.107 ppm 1593 and 0.13 ppm 2362). Larvae of *Cx. quinquefasciatus* were highly susceptible to *B.t.i.* (LC_{90} = 0.065 ppm Bactimos) as well as to the two isolates of *B. sphaericus* (LC_{90} = 0.02 ppm 1593 and 0.031 ppm 2362) (data not included in Table 1). In general, the primary powders of *B. sphaericus* were more toxic to *An. gambiae* s.l. as well as to *Cx. quinquefasciatus* than the *B.t.i.* formulations although the larvae were exposed to *B. sphaericus* for a longer period (48 h) as compared to *B.t.i.* (24 h).

Under field conditions, Bactimos at 0.65 kg/ha caused 91 to 97% larval reduction of *Cx. quinquefasciatus* in a ditch during the one-week observation period, while Vectobac at 1.5 kg/ha gave complete control of *Cx. quinquefasciatus* in a channel after 3 days of treatment (Table 2). In this channel, the larval populations remained reduced by at least 90% for up to 7 days posttreatment. ABG-6138G at 5.6 kg/ha in another channel provided 96% control of *Cx. quinquefasciatus*; the larval reduction ranged from 69 to 96% during seven posttreatment days.

Bactimos at 0.25 and 0.5 kg/ha yielded 82 and 95% larval control of *An. gambiae* s.l., respectively, in rainwater pools within 24 h of treatment (Table 3). Vectobac in similar pools gave 86% control at 0.5 kg/ha and 95% control at 1 kg/ha. Treatments of additional water pools in the same area with ABG-6138G at 2.8 and 5.6 kg/ha resulted in 97% larval reduction of *An. gambiae* s.l. at the higher rate. At the lower rate, 82% larval reduction occurred after 24 h of treatment.

Larvae of *An. gambiae* s.l. in the artificial

Table 1. Laboratory bioassay of three commercial formulations of *Bacillus thuringiensis* var. *israelensis* and two isolates of *B. sphaericus* against 3rd and 4th instars of *Anopheles gambiae* s.l. collected from Nongremassm area of Ouagadougou, Burkina Faso.

Formulation or isolate	Lethal concentration (ppm)			
	LC_{50}	95% CL	LC_{90}	95% CL
	B. thuringiensis israelensis (24 h exposure)			
Bactimos (WP)	0.081	0.070–0.094	0.231	0.180–0.297
Vectobac (WP)	0.110	0.096–0.126	0.375	0.296–0.476
Teknar (FC)	0.662	0.445–0.984	1.743	1.737–2.015
	B. sphaericus (48 h exposure)			
1593 (IF-119, spray dried)	0.043	0.034–0.054	0.107	0.073–0.155
2362 (IF-118, spray dried)	0.022	0.014–0.035	0.130	0.095–0.172

Table 2. Field evaluation of three commercial formulations of *Bacillus thuringiensis* var. *israelensis* against *Culex quinquefasciatus* in three habitats in Ouagadougou, Burkina Faso (Jan.–Feb. 1984).

Materials and formulations	Rate (kg/ha)	Habitat/ location	Mean no. larvae/dip[a] pre-, and posttreatment (days)			
			Pretreatment	1	3	5–7
Bactimos, WP (3500 ITU/mg)	0.65	Roadside ditch,[b] Bilibambili	423	27	10	22
			(0)	(91)[e]	(97)	(92)
Control			242	167	212	160
Vectobac, WP (2000 ITU/mg)	1.5	Concrete channel,[c] Gounghin Nord	191	7	0	14
			(0)	(97)	(100)	90)
Control			221	270	143	156
ABG-6138G (Corn-cob, 200 ITU/mg)	5.6	Concrete channel,[d] Zone du Bois	19	5	4	20
			(0)	(91)	96)	(69)
Control			62	187	324	213

[a] 250 ml water.

[b] Forty cm wide containing 15 cm stagnant sewage water (pH 7.2) with dense rooted vegetation along the edges. Minimum and maximum water temperatures ranged from 22 to 28°C during the test.

[c] Open drainage 50 cm wide containing 10 cm slow moving (<10 cm/min) sullage water (pH 7.5) rich in organic matter. Minimum and maximum water temperatures ranged from 22 to 30°C during the test.

[d] Open drainage 50 cm wide containing 10 cm slow moving (<5 cm/min) sullage water (pH 7.5) rich in organic matter and sparse patches of floating vegetation. Minimum and maximum water temperatures ranged from 22 to 31°C during the test.

[e] Posttreatment percent reductions in parentheses.

Table 3. Field evaluation of three commercial formulations of *Bacillus thuringiensis* var. *israelensis* against *Anopheles gambiae* s.l. in natural pools,[a] Ouagadougou, Burkina Faso (July–August 1984).

Materials and formulations	Rate (kg/ha)	Mean no. larvae/4 dips[b] Pre-treatment	24-h Post-treatment	% Reduction
Bactimos, WP (3500 ITU/mg)	0.25	52	11	82.4
	0.5	31	2	94.6
Vectobac, WP (2000 ITU/mg)	0.5	36	6	86.1
	1.0	48	3	94.8
Control		40	48	—
ABG-6138G (Corncob, 200 ITU/mg)	2.8	32	8	81.5
	5.6	45	2	96.7
Control		68	92	—

[a] Rainwater pools, 4–10 m^2 in surface area containing 5–15 cm water (pH 7.6–7.8) with suspended clay particles. Minimum and maximum water temperatures ranged from 26 to 30°C during the tests.
[b] 250 ml water/dip.

Table 4. Field evaluation of primary powders of two isolates of *Bacillus sphaericus* against *Anopheles gambiae* s.l. in small uniform artificial ponds,[a] Ouagadougou, Burkina Faso (August 1984).

Isolate (formulation)	Rate (kg/ha)	Larval instar	Mean no. larvae/4 dips[b] Pre-treatment	48-h Post-treatment	% Reduction
1593 (IF-119, spray dried)	0.12	1–2	12	5	72.2
		3–4	8	1	95.8
	0.24	1–2	48	14	80.6
		3–4	4	0	100
2362 (IF-118, spray dried)	0.12	1–2	42	20	68.3
		3–4	8	0	100
	0.24	1–2	28	2	95.2
		3–4	12	0	100
Control		1–2	16	24	—
		3–4	4	12	—

[a] Each pond 60 cm in diameter at the surface and containing 20 cm clean, fresh water (pH 7.9–8.1). Minimum and maximum water temperatures ranged from 27 to 31°C during the test.
[b] 250 ml water/dip.

ponds were significantly reduced by *B. sphaericus* (Table 4). Isolate 1593 at 0.12 kg/ha produced 72% reduction of 1st and 2nd instars and 96% reduction of 3rd and 4th instars of *An. gambiae* s.l. in the ponds. At 0.24 kg/ha, 1593 gave 81% control of the 1st and 2nd instars and 100% control of the 3rd and 4th instars. Isolate 2362 showed a similar or slightly superior activity than 1593, giving complete control of 3rd and 4th instars of *An. gambiae* s.l. at both rates, while the younger instars were reduced 68 and 95% by the two rates, respectively.

DISCUSSION

Data on laboratory and field evaluations of *B.t.i.* against mosquitoes in Africa are relatively limited. However, 2nd instar field-collected *An. gambiae* (in Nigeria) exposed to 1 ppm of IPS-78 (the *B.t.i.* international standard) were reduced by 89% (Prasertphon and Rishikesh 1979), and 2nd instar *An. arabiensis* Patton were highly susceptible to three *B.t.i.* formulations in the laboratory (Nugud and White 1982). The present data on *B.t.i.* activity against *An. gambiae* s.l. are generally compatible with those reported by Prasertphon and Rishikesh (1979) and Nugud and White (1982). However, the difference of potencies of various test formulations of *B.t.i.* should be considered while making their activity comparisons. The differential in levels of susceptibility of various instars of a mosquito species to *B.t.i.* (Ali et al. 1981) should also be considered.

In field situations, *An. gambiae* were completely controlled in a wet season (July–August) in northern Nigeria when exposed to 10 ppm of a WP formulation of *B.t.i.* comparable to Bactimos (Prasertphon and Knudsen 1980). The present study has shown that larval *An. gambiae* s.l. in the field were reduced by 82 to 95% by applying only 1 ppm or less of Bactimos and Vectobac while ABG-6138G at 2.8 and 5.6 kg/ha or ca. 3 and 6 ppm gave similar levels of control of this species.

The susceptibility of *Cx. quinquefasciatus* to *B.t.i.* is documented in several laboratory studies (Ali et al. 1981, Lacey and Lacey 1981, Mulla et al. 1982). In polluted waters (a ditch and channels) in Ouagadougou, *B.t.i.* caused 91–100% larval reductions of *Cx. quinquefasciatus* for three days after treatment at rates ranging from 0.65 to 5.6 kg/ha (ca. 0.5 to 5.6 ppm) for the various formulations. Previously, Prasertphon and Knudsen (1980) had reported 65 to 99% reduction of *Cx. quinquefasciatus* larvae in a roadside ditch in Nigeria but at a rather high rate of 10 ppm of a *B.t.i.* formulation comparable to Bactimos. Mulla et al. (1982) reported 0, 81 and 99% reductions of *Cx. quinquefasciatus* with Bactimos applied at 0.56, 1.12, and 2.24 kg/ha, respectively, to dairy lagoons in California. In Florida, Bactimos applied at rates of 0.1 and 1 ppm against *Cx. quinquefasciatus* in sewage treatment tanks produced 30 to 96% and 97 to 100% larval reductions, respectively (Stewart et al. 1981).

The laboratory and field activity of some potent isolates of *B. sphaericus* (including 1593 and 2362) against a large number of mosquito species in different parts of the world were previously documented (World Health Organization 1985). In Africa (Nigeria), isolate 1593 was

shown to be toxic to larvae of *An. gambiae* and *Cx. quinquefasciatus* (Obeta and Okafor 1983). However, the present laboratory data on the activity of isolates 1593 and 2362 against *An. gambiae* s.l. indicated these isolates to be 5 to 7× more toxic than previously reported on this species (World Health Organization 1985). Similarly, field applications of preparations of 1593 and 2362 at 7.5 and 8.2 ppm against *An. gambiae* in polluted water caused 94 and 96% larval reductions, respectively (World Health Organization 1985). Whereas, in the presently studied artificial ponds containing clean water, relatively low rates of 0.12 and 0.24 kg/ha (or 0.06 and 0.12 ppm) of the primary powders of isolates 1593 and 2362 produced 96 to 100% control of 3rd and 4th instars and 68 to 95% control of 1st and 2nd instars of *An. gambiae* s.l. 48 h after treatment. The lower level of control of the younger instars was probably due to the addition of newly hatched larvae to the ponds. These larvae were not exposed to the pathogen for sufficient length of time to suffer mortality. A similar field observation on asynchronously growing stagnant water mosquitoes exposed to *B. sphaericus* was reported by Mulla et al. (1984a).

The present laboratory data on the susceptibility of *Cx. quinquefasciatus* to isolates 1593 and 2362 of *B. sphaericus* are compatible with those of Mulla et al. (1984a) for the same species, showing LC_{90} range of 0.017 to 0.04 ppm for four different preparations of 2362 and a lyophilized preparation of 1593 (RB-80, Pasteur Institute, International Standard). Although no field applications of *B. sphaericus* against *Cx. quinquefasciatus* were made in Ouagadougou, previous field studies on isolates 1593 and 2362 applied at 0.25 kg/ha against *Cx. quinquefasciatus* produced 90% larval reduction in polluted waters in Ivory Coast (World Health Organization 1985). Also, a flowable concentrate (BSP-1, containing 12% primary powder of isolate 2362) applied at 20 g/m^2 provided effective control of *Cx. quinquefasciatus* for 6 to 10 weeks in cesspits and latrines in the United Republic of Tanzania (World Health Organization 1985).

It is evident from this study that *B.t.i.* and *B. sphaericus* (isolates 1593 and 2362) are highly toxic larvicides of *An. gambiae* s.l. and *Cx. quinquefasciatus* in the Ouagadougou area. These bacteria applied at low and cost-effective rates would produce a quick reduction (particularly *B.t.i.*) of the larvae in a manner similar to the use of chemical larvicides. *Bacillus sphaericus* may provide larval control for a longer period of time than *B.t.i.*, particularly in polluted water situations (Hornby et al. 1984). Both bacteria are safe to the nontarget organisms coexisting with mosquito larvae (Ali 1981, Mulla et al. 1984b). Thus, these bacteria are useful biological control agents in the overall integrated control program of *An. gambiae* s.l. and *Cx. quinquefasciatus* in Africa. They should be considered very valuable because of the increasing problems of vector (larval and adult) resistance to chemical insecticides in several malarious regions of Africa (Goriup and van der Kay 1984).

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