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FIELD TRIALS OF *BACILLUS THURINGIENSIS* H-14 AND *BACILLUS SPHAERICUS* (STRAIN 2362) FORMULATIONS AGAINST *ANOPHELES ARABIENSIS* IN THE CENTRAL HIGHLANDS OF MADAGASCAR

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ABSTRACT. Malaria is highly endemic and unstable in the central Highlands plateau of Madagascar. The infection is seasonally transmitted by *Anopheles funestus* and *An. arabiensis.* The latter species is abundant especially in rice-growing areas. The field efficacies of commercial formulations of *Bacillus thuringiensis* H-14 and *B. sphaericus* (strain 2362) were assessed against *An. arabiensis* in 5 types of larval habitats. The granular formulation of *B. thuringiensis* (Vectobac® GR) provided very good control in small ponds and rainwater ditches. In ricefields complete larval control was achieved with Vectobac GR and the flowable concentrate (Vectobac® 12AS) at 2.5 kg/ha and 0.6 liter/ha, respectively. The granular formulation of *B. sphaericus* (ABG 6185) showed activity similar to that of Vectobac GR in small pools and ricefields. ABG 6185 was less effective in rainwater ditches where it gave satisfactory control at rates not lower than 6 kg/ha.

INTRODUCTION

Malaria is still the cause of greatest mortality in humans in Madagascar. The environmental factors which influence the disease transmission and its intensity vary according to the different geographical areas. The central region of the island, the Highlands plateau, is characterized by an altitude of over 1,100 m, a long dry season and a natural environment subject to deforestation. The Highlands are one of the 4 malaria epidemiological zones of the country. Locally this zone presents heterogeneous situations, with foci of high endemicity, and areas of unstable transmission, where epidemics occur from time to time. In the Highlands *Anopheles funestus* Giles and *An. arabiensis* Patton, the only member of the *An. gambiae* complex, are the malaria vectors present in high densities in various larval habitats (Ralisoa Randrianasolo and Coluzzi 1987, Fontenille and Rakotoarivony 1988, Fontenille et al. 1990, Severini et al. 1990). Ricelands represent the most important larval habitats for *An. arabiensis* in the Highlands, where they cover large areas. The asynchronous crop rotation, which comprises 2 planting cycles, ensures a production of *An. arabiensis* larvae during the whole year (Ravoniharimelina et al. 1992).

Since 1989, within the Italy–Madagascar cooperative program for malaria control, several studies and trials on biological and chemical control of vectors have been conducted. Objectives of these studies were to explore the possibilities for an integrated control of the vectors. This article reports the field efficacy of *Bacillus thuringiensis* H-14 (*B.t.i.*) and *B. sphaericus* (strain 2362) formulations against *An. arabiensis* larvae in the Alasora District, Highlands of Madagascar.

MATERIALS AND METHODS

The study was carried out in Mahitsy, Alasora District, a village 2.5 km southeast of Antananarivo, the capital of Madagascar. The village, at 1,277 m, is located on the west side of the Ikopa River, surrounded by irrigated ricefields. About 590 inhabitants live in straw-roofed houses built with mud bricks. The hot rainy season ranges from November to April (average temperature 25°C) and the cold and dry season from May to October (average temperature 15°C). The annual rainfall in 1991 was about 1,100 mm, with only 112 mm during the dry season. The village is located in an area of mesoendemic malaria. Surveys carried out from 1989 to 1990 showed a total parasite rate in schoolchildren (5–9 years old) from 27.9 to 41.6% (Rabenjarson et al. 1991). The indoor density of *An. arabiensis* during the rainy season reached a peak of 16.6 bites/man/night in Mahitsy village in January–February 1990 (Raveloarifera et al. 1991).

There are 2 types of *An. arabiensis* larval habitats in this area: 1) natural habitats—the temporary small pools partially or totally sun exposed, which originate during the rainy season between November and March (Grjebine 1956), and 2) artificial habitats—such as ricelands, borrow pits, ponds and ditches.

Field trials were carried out from January to July 1991 and from January to March 1992. The formulations of *B.t.i.* evaluated in the field were one flowable concentrate, Vectobac® 12AS (1,200 ITU/mg), and one granular formulation, Vec-

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tobac® GR (200 ITU/mg), produced by Abbott Laboratories, North Chicago, IL, USA. The formulation of *B. sphaericus* was the granular ABG 6185, derived from isolate 2362 (5×10^{10} spores/g, Abbott Laboratories). Vectobac 12AS, Vectobac GR and ABG 6185 were evaluated against *An. arabiensis* larvae in field trials carried out in 5 types of larval habitats:

1) Eight small natural pools with water slightly muddy and exposed to the sunlight. On the average the water surface of a single pool was 0.7 m^2 (0.6–0.8 m^2) and the water depth was 14 cm. Only *An. arabiensis* was present with a mean larval density of 14.2 larvae/dip (mostly 3rd–4th instar larvae). The pools were treated with granular formulations of both agents. Four pools were treated with Vectobac GR and the others with *B. sphaericus* granules, ABG 6185. The rates of application ranged from 2 to 8 kg/ha. Three additional pools were used as controls. These experiments were conducted during February–March 1991.

2) Eleven ditches, made by the local population to produce bricks, had rainwater rich with organic matter and vegetation. On the average the water surface of a single ditch was 84.5 m^2 (78–128 m^2) and water depth was 114 cm. The mean larval density of *An. arabiensis* was low (5.6 larvae/dip), with mostly 2nd–4th instar larvae. Five of the ditches were treated with Vectobac GR at rates of application ranging from 3 to 10 kg/ha, and the other 6 with ABG 6185 at rates from 5 to 18 kg/ha. Two additional ditches were used as controls. These tests were conducted in April–May 1991.

3) Six small ricefields, where the young plants are initially grown before transplanting; water was clear without vegetation. On the average the water surface of a single field was 231 m^2 (160–410 m^2) and the water depth was 8 cm. The mean larval density was low (3.8 larvae/dip) with mostly 3rd–4th instar larvae. Of these ricefields, 2 were treated with Vectobac 12AS at rates of 0.6 and 1 liter/ha, 2 with Vectobac GR at rates of 5 and 10 kg/ha and the last 2 with ABG 6185 at 5 and 10 kg/ha rates. One additional ricefield was used as a control These tests were carried out in June–July 1991.

4) Five ricefields during early crop growth, where water was clear and plants reached about 60 cm high. On average the water surface of a single field was 646 m^2 (220–1,020 m^2) and the water depth was 9.5 cm. The mean larval density was very low (1.4 larvae/dip) with mostly 3rd–4th instar larvae. One of these ricefields was treated with a single dose of Vectobac 12AS (0.6 liter/ha) and the others with granular formulations, 2 with Vectobac GR and 2 with ABG 6185, at rates of 2.5 and 5 kg/ha, respectively. One additional ricefield was used as a control. The experiments were carried out in January–February 1992.

5) Two fallow ricefields where the crops were cut down; water was clear and exposed to sunlight. The water surfaces were 1,390 and 1,220 m^2, respectively, and the average water depth was 7.5 cm. The mean larval density was very low (1.1 larvae/dip) with mostly 3rd–4th instar larvae. Only *B.t.i.* formulations were applied to these paddy fields: Vectobac 12AS at a single dose of 0.3 liter/ha, and Vectobac GR at a single dose of 2.5 kg/ha. A 3rd ricefield was used as a control. The tests were carried out in February–March 1992.

In all the above mentioned ricefields, *An. arabiensis* larvae were mixed with those of *An. coustani* Laveran and *An. squamosus* Theobald. Larval samples from all treated and control areas were taken using the standard 250-ml dipper. In the pools, where the water surface did not exceed 1 m^2, only 2 dips/pool were taken. Samples along the entire length of the ditches and ricefields also were collected, from a minimum of 8 dip samples (ditches and small ricefields) to a maximum of 18 (fallow and in-growth ricefields), according to the size of the sites. Collected anopheline larvae were counted and identified. The dip samples were taken immediately prior to the treatment and then from one to 7 days after treatment. For treatment a 10-liter stainless steel pressurized hand sprayer was used to produce a uniform distribution of the flowable concentrate. Granular formulations were hand-dispersed. The percentage reduction of mosquito larvae due to the treatments was calculated and corrected by Abbott's formula.

RESULTS

The efficacy of *B.t.i.* and *B. sphaericus* formulations against *A. arabiensis* larvae in natural pools and artificial ditches is shown in Table 1. Both granular formulations, Vectobac GR and ABG 6185, gave complete larval control in small pools. Vectobac GR provided 100% mortality within 24 h at all rates of application. ABG 6185 yielded 100% larval control within 24 h at rates of 6 and 8 kg/ha and within 48 h at rates of 2 and 4 kg/ha. Vectobac GR in rainwater ditches gave complete larval control after 24 h at rates of 4, 6 and 10 kg/ha. The ditch treated at the rate of 5 kg/ha gave only 87% mortality, probably because of the presence of thick vegetation. The rate of 3 kg/ha gave 93% reduction within 24 h. ABG 6185 at rates of 12 and 18 kg/ha gave 100% mortality within 48 h in rainwater ditches, while at rates of application from 6 to 10 kg/ha it gave

Table 1. Field evaluation of *Bacillus thuringiensis* H-14 and *B. sphaericus* formulations against *Anopheles arabiensis* in natural and artificial pools, Alasora District, Madagascar.

Formulation	Rate (kg/ha)	Mean no. larvae/dip Pre- and posttreatment (days) Pre-	1	2	Posttreatment % reduction 24 h	48 h
Small pools[1]						
Vectobac GR	8	12	0	0	100	—
	6	14	0	0	100	—
	4	15	0	0	100	—
	2	16	0	0	100	—
ABG 6185	8	16	0	0	100	—
	6	15	0	0	100	—
	4	13	2	0	85	100
	2	15	6	0	60	100
Controls	—	14	16	18	0	0
	—	16	16	14	0	13
	—	16	18	15	0	6
Ditches[2]						
Vectobac GR	10	6.3	0	0	100	—
	6	3.2	0	0	100	—
	5	4.2	0.9	1.0	87	75
	4	4.5	0	0	100	—
	3	3.8	0.2	0.2	93	93
ABG 6185	18	4.5	0.3	0	93	100
	12	4.8	0.3	0	94	100
	10	11.6	0.4	0.3	97	97
	8	10.6	0.6	0.5	94	96
	6	5.4	0.6	0.5	88	91
	5	2.4	1.5	0.8	37	77
Controls	—	5.5	5.8	5.5	0	0
	—	5.4	5.6	5.6	0	0

[1] Surface area 0.6–0.8 m^2 containing 12–15 cm of water (pH 6.9). Mean pretreatment larval density 14.2 ± 1.2 larvae/dip, mostly 3rd–4th instar. Water temp. 26 ± 1°C.

[2] Surface area 60–112 m^2 containing 80–150 cm of water (pH 6.8). Mean pretreatment larval density 5.6 ± 2.8 larvae/dip, mostly 2nd–4th instar. Water temp. 21 ± 2°C.

more than 90% mortality. Only the rate of 5 kg/ha yielded 77% mortality after 48 h.

The results of ricefield trials are reported in Table 2. *Anopheles arabiensis* larvae were controlled by both granular formulations of *B.t.i.* and *B. sphaericus.* Vectobac GR provided excellent larval control in the 3 different types of ricefields. In every case 100% mortality after 24 h was recorded at all rates of application (2.5, 5 and 10 kg/ha). Also, ABG 6185 gave complete control after 48 h in the same types of paddy fields and at the same rates of application. Vectobac 12AS, the *B.t.i.* flowable concentrate, provided complete control in 24 h at the rate of 0.6 liter/ha, while the higher dosage, 1 liter/ha, yielded 100% mortality only after 48 h. The lower dosage, 0.3 liter/ha, yielded 89% mortality within 24 and 48 h.

The residual activity of all formulations seemed to be very short. Dip samples taken between 5 and 7 days after treatment indicated a quick and continual recolonization of all treated sites by 1st and 2nd larval instars of anophelines.

DISCUSSION

The 2 formulations of *B.t.i.* provided effective control of *An. arabiensis* larvae in all habitats. Complete control was achieved by the granular formulation Vectobac GR at all rates of application used in small pools and in all types of ricefields. In rainwater ditches, the rate of 3 kg/ha gave highly satisfactory control. The flowable concentrate gave similar results in ricefields.

The field efficacy of *B.t.i.* formulations on *Anopheles* spp. larvae was previously reported in several publications (Lacey and Lacey 1990), although data on *An. gambiae s.l.* control in Africa are relatively limited. The present data on efficacy of Vectobac formulations agree with those

Table 2. Field evaluation of 3 formulations of *Bacillus thuringiensis* H-14 and *B. sphaericus* against *Anopheles arabiensis* in ricefields, Alasora District, Madagascar.

Formulation	Rate (kg/ha)	Surface area (m²)	Mean no. larvae/dip Pre- and posttreatment (days)			Posttreatment % reduction (corrected)	
			Pre-	1	2	24 h	48 h
Small ricefields[1]							
Vectobac 12AS	1[4]	180	2.2	0.2	0	92 (89)	100
	0.6[4]	260	5.3	0	0	100	—
Vectobac GR	10	220	2.3	0	0	100	—
	5	410	2.3	0	0	100	—
ABG 6185	10	160	8.6	1	0	88 (84)	100
	5	160	2.3	1	0	57 (42)	100
Control	—	230	2.4	1.7	2.1	26	11
Fields in growth[2]							
Vectobac 12AS	0.6[4]	1,020	1.3	0	0	100	—
	5	850	2.3	0	0	100	—
Vectobac GR	2.5	600	1.1	0	0	100	—
	5	460	1.3	0.4	0	69 (67)	100
ABG 6185	2.5	300	1.1	0.5	0	45 (41)	100
Control	—	220	1.4	1.3	1.6	7	0
Fallow ricefields[3]							
Vectobac 12AS	0.3[4]	1,390	1.3	0.1	0.1	89	89
Vectobac GR	2.5	1,220	1.0	0	0	100	—
Control	—	850	1.4	1.6	1.6	0	0

[1] Surface area 180–410 m² containing 7–9 cm of water (pH 7.7). Mean pretreatment larval density 3.8 ± 2.3 larvae/dip, mostly late instars. Water temperature 20 ± 1°C.
[2] Surface area 600–1,020 m² containing 8–14 cm of water (pH 6.8). Mean pretreatment larval density 1.4 ± 0.4 larvae/dip, mostly late instars. Water temperature 23 ± 2°C.
[3] Surface area 1,200–1,440 m² containing 5–10 cm of water (pH 6.7). Mean pretreatment larval density 1.1 ± 0.2 larvae/dip, mostly late instars. Water temperature 29 ± 1°C.
[4] Flowable concentrate, rate liter/ha.

reported by other authors. Vectobac GR provided 92–96% larval reduction in Louisiana ricefields when applied at a rate from 5.6 to 22.4 kg/ha (Lacey and Inman 1985). In California ricefields, Vectobac GR provided 72–95% *An. freeborni* Aitken larval reduction at a 6-kg/ha application rate (Kramer et al. 1988). Walker and Lacey (*in* Lacey and Lacey 1900) achieved satisfactory control of *An. albimanus* larvae in Honduras ricefields (94–97% reduction) using Vectobac 12AS at 2.5 liter/ha and Vectobac GR at 10 kg/ha. In Burkina Faso a corncob formulation (ABG 6138), comparable to Vectobac GR, gave approximately 81 and 97% *An. gambiae s.l.* larval reduction at 2.8 and 5.6 kg/ha, respectively, in rainwater pools (Majori et al. 1987).

Our study has shown that *An. arabiensis* larvae were completely reduced in all types of ricefields by applying 2.5 kg/ha of Vectobac GR and 0.6 liter/ha of Vectobac 12AS. The granular ABG 6185 *B. sphaericus* formulation showed activity comparable to that of Vectobac GR in small pools as well as in the different types of ricefields. It appears to be less effective in rainwater ditches where it gave satisfactory control at rates not lower than 6 kg/ha. There was no evidence of residual larvicide activity of this bacterium in treated sites after 48 h, as reported by other authors in culicine larval habitats (Des Rochers and Garcia 1984, Mulla et al. 1988, Matanmi et al. 1990). The dip samples taken in all treated sites 5, 6 and 7 days after treatment revealed the presence of early instar larvae, showing that newly hatched larvae were not affected by ABG 6185. These data agree with those reported by other authors that describe short-term control of anopheline larvae due to rapid settling of spores out of the larval feeding zones (Davidson et al. 1984, Nicolas et al. 1987).

It is evident from our study that Vectobac and ABG 6185 were highly effective larvicides against *An. arabiensis* in the Alasora District in small-scale trials. In particular the Vectobac granular formulation was more practical to apply than the flowable concentrate. The presence of vegetation such as rice plants may adversely affect the efficacy of a flowable concentrate by trapping the applied material, while granular formulations afford greater penetration of vegetation. Moreover, Vectobac GR appears to be more efficient than the *B. sphaericus* granular formulation when used at the same rates of application. A *B.t.i.* granular formulation, applied at low rates, can produce an effective control of *An. arabiensis* larvae, especially in the ricefields that represent the most important anopheline larval habitats in the Alasora District. Large-scale trials in the same area should be carried out to assess the impact of both larvicides on malaria transmission.

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