FIELD TRIALS WITH VECTOLEX® (*BACILLUS SPHAERICUS*) AND VECTOBAC® (*BACILLUS THURINGIENSIS* (H-14)) AGAINST *ANOPHELES GAMBIAE* AND *CULEX QUINQUEFASCIATUS* BREEDING IN ZAIRE

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ABSTRACT. Under field conditions in Kinshasa, Zaire, an aqueous suspension of *Bacillus thuringiensis* (H-14), Vectobac® (12-AS), lost most of its larvicidal activity at all concentrations after 48 h against *Culex quinquefasciatus* breeding in polluted gutter water and *Anopheles gambiae* breeding in clear water irrigation ponds. However, good control of *Cx. quinquefasciatus* was obtained using a granular formulation of *B. sphaericus*, Vectolex-G® (ABG-6185), at concentrations of 10–30 kg/ha. High concentrations of Vectolex-G gave excellent control of *An. gambiae* breeding in irrigation ponds. The Vectobac-G was less active against *An. gambiae* than Vectolex-G, in spite of good dispersion of Vectobac-G particles.

INTRODUCTION

The urban mosquito, *Culex quinquefasciatus* Say, dominates the breeding area in polluted water sites in Kinshasa, Zaire, 500 km south of the equator in Central Africa. *Anopheles gambiae* Giles, the principal vector of malaria in the area (Coene et al. 1987), may be found in the breeding sites of *Cx. quinquefasciatus* but generally inhabits other water sources such as irrigated ponds at the periphery of the city. Both species pose an enormous nuisance problem as there are no good methods for draining or cleansing the breeding sites. Furthermore, there are serious problems of resistance of both species to chemical pesticides.

At present, the use of entomopathogenous bacteria such as *Bacillus thuringiensis* (H-14) and *B. sphaericus* seems to be effective against the larvae of vector and nuisance mosquitoes (Lacey and Undeen 1986). In addition, the 2 bacteria are safe for nontarget organisms (de Barjac et al. 1979). The following studies were conducted to assess whether it would be feasible and efficient to use these bacteriological control methods in highly polluted and other challenging situations in Zaire, Central Africa.

MATERIALS AND METHODS

The experimental formulations Vectobac® (12-AS) and Vectobac-G® (200 ITU) of *B. thuringiensis* (H-14), and Vectolex-G [ABG-6185 (200 BSITU/mg or 5 × 10^9 spores/g)] of *B. sphaericus* were supplied by Abbott Laboratories, No. Chicago, IL.

Field experiments were conducted during the rainy season, from October 1989 to January 1990. Three gutters that drain in the center of the Ndjili area of Kinshasa were colonized by very dense preimaginal populations of *Cx. quinquefasciatus*. These gutters were treated with 2, 4, and 6 liters/ha of an aqueous suspension formulation of Vectobac (12-AS). In breeding sites of *An. gambiae*, 3 irrigation ponds at Ndjili were treated with 10, 15 and 20 kg/ha rates of the Vectobac-G granule formulation of *B. thuringiensis* (H-14). Other mosquito breeding sites in Kinshasa including Ngafula, Barumbu and Ngiri Ngiri were treated with Vectolex-G granule formulation of *B. sphaericus*. Application rates were 2.5, 10, 15, 20 and 30 kg/ha against *Cx. quinquefasciatus* in gutters and in a sewage lagoon and 10, 20, and 30 kg/ha rates against *An. gambiae* in irrigation ponds at the Ndjili area. Application of both granular formulations were carried out directly by a hand seeder.

Sampling of each site consisted of 10 dips taken with a standard 250 ml capacity mosquito dipper. The post-treatment samples were taken on days 1, 2, 5, 7 and 14 or 21, depending on the length of residual effect. During each test, one breeding site was left untreated as a control.

The water temperature during these studies was between 25 and 28.5°C. The principal nontarget species found in the breeding site were: tadpoles, Daphnia, Isopoda and *Cx. tigripes* DeGrandpre and DeCharmoy with *Cx. quinquefasciatus* larvae, and fish, Isopoda and Dytiscidae with *An. gambiae* larvae.

RESULTS AND DISCUSSION

Vectobac (12-AS) aqueous suspension of *B. thuringiensis* (H-14) has a mediocre efficacy against *Cx. quinquefasciatus* larvae in polluted gutter water. Although, an initial larvicidal activity was obtained after 48 h post-treatment, the level of *Cx. quinquefasciatus* larvae was the same 5 days post-treatment at any concentration applied (Table 1). The other formulation of *B. thuringiensis* (H-14) (Vectobac-G) produced

Table 1. Efficacy of Vectobac (12-AS) aqueous suspension of *Bacillus thuringiensis* (H-14) against *Culex quinquefasciatus* larvae in 3 gutters (polluted water) at Ndjili area.

Rate (liters/ha)	Area W × L (m^2)	Mean water depth (cm)	Mean no. larvae/dip. pret.[a]	Mean (%) reduction after treatment (days) 1	2	5	7
2	1.2 × 20 (24)	19.5	182	97	35	6	0
4	1.2 × 40 (48)	74.0	225	95	42	9	6
8	1.8 × 130 (234)	19.5	152	98	38	28	6
Control	1.8 × 40 (72)	27.5	123	0	4	0	0

[a] Mean pretreatment larval density; water pH 6.3–7.1, polluted water in full sunlight; optical density during study, 0.0900–0.0800 at 493 wavelength.

Table 2. Evaluation of Vectobac-G granules of *Bacillus thuringiensis* (H-14) against *Anopheles gambiae* complex in 3 irrigation ponds at Ndjili area.

Rate (liters/ha)	Area W × L (m^2)	Mean water depth (cm)	Mean no. larvae/ dip. pret.[a]	Repetition[b]	Mean (%) reduction after treatment (days) 1	2	5	7
10	25	25.5	16	(1)	100	98	26	6
			19	(2)	86	99	6	15
15	22	28	21	(1)	99	98	12	0
			12	(2)	100	100	13	40
20	20	21	14	(1)	89	97	0	17
			18	(2)	100	100	19	0
			14	(3)	100	100	0	28
Control	15	18	12	—	0	11	7	0
			21	—	9	23	5	15
			21	—	0	8	11	0

[a] Mean pretreatment larval density; water pH 7.2–7.5, clear water with sparse vegetation in border; optical density during study, 0.0900–0.4740 at 493 wavelength.
[b] (1) first, (2) second and (3) third treatment; 1 wk between each treatment.

a good initial control against *An. gambiae* larvae 48 h post-treatment in the irrigation ponds, but the population began to recover 5 and 7 days after each treatment (Table 2).

The granular formulation of *B. sphaericus* (Vectolex-G) also gave a good initial control against *Cx. quinquefasciatus* in all gutter breeding sites after 24 and 48 h post-treatment. However, the larvicide lost almost all of its potency 5–7 days post-treatment in gutters treated with 2.5, 15 and 20 kg/ha. Whereas, in the sewage lagoon treated with 10 kg/ha and in gutters treated with 30 kg/ha, good control was observed 21 days post-treatment (Table 3). In addition, in the sewage lagoon, 85% reduction of larval population of *Cx. quinquefasciatus* was obtained up to 35 days post-treatment.

Despite the high efficacy of Vectolex-G against *Cx. quinquefasciatus*, its larvicidal activity was limited to a few days, especially in the gutters. In these breeding sites, a variety of factors associated with the hydrodynamics of the larval habitat have a marked influence upon the larvicidal activity. One of the mechanisms is that fluctuations of water from domestic human activity is apparently responsible for the decreased residual activity of *B. sphaericus* in gutters. This phenomenon was frequently observed in the city.

Vectolex-G was applied against *An. gambiae* in irrigation ponds at the periphery of Kinshasa City. Excellent control was obtained 48 h post-treatment. The reduction of *An. gambiae* larvae was significant after 5 and 7 days, especially after the second treatment. However, we observed a positive relation between the duration of persistence of larvicidal activity and the repetition of treatment. Also, higher rates of application gave better control (Table 4).

The Vectolex-G granular formulation appears to be more active in both polluted water breeding sites of *Cx. quinquefasciatus* (gutters and sewage lagoon) than in the clear water irrigation ponds of *An. gambiae*. However, *B. sphaericus* persists

Table 3. Evaluation of *Bacillus sphaericus* Vectolex-G (ABG-6185) granules against *Culex quinquefasciatus* larvae in different areas of Kinshasa.

Rate (liters/ha)	Breeding sites	Area W × L (m²)[a]	Location	Mean no. larvae/ dip. pret.[b]	Repetition[c]	Mean (%) reduction after treatment (days)					
						1	2	5	7	14	21
2.5	Gutter	0.8 × 30	Ngafula	121	(1)	84	84	35	0	0	0
		(24)		136	(2)	91	76	42	0	0	0
2.5	Gutter	1.1 × 50	Ngafula	97	(1)	82	78	45	0	12	0
		(55)		113	(2)	100	95	56	26	0	6
10	Sewage lagoon	2.5 × 6	Barumbu	212	(1)	100	100	100	97	100	85
		(15)									
15	Gutter	0.6 × 280	Barumbu	276	(1)	100	100	95	54	0	0
		(168)		176	(2)	100	98	100	68	4	0
20	Gutter	0.6 × 60	Ngiri-Ngiri	202	(1)	100	96	31	4		
		(36)		186	(2)	100	89	0	10		
30	Gutter	1.6 × 80	Ndjili	127	(1)	100	100	100	95	66	26
		(128)									
Control	Sewage lagoon	3.5 × 3	Barumbu	152	—	0	0	5	0	25	6
		(10.5)		176	—	11	9	0	0	19	3
Control	Gutter	1.1 × 20	Ngiri-Ngiri	165	—	0	15	13	0		
		(22)		203	—	0	9	8	0		

[a] Mean water depth of gutters and sewage lagoon, 17–26 cm.
[b] Mean pretreatment larval density; in gutter water pH = 6.3–6.9 and optical density 0.0914–0.2064 at 493 wavelength. In sewage lagoon pH = 7.1–7.4 and optical density 0.1086–0.2650 with vegetation in border.
[c] (1) first, (2) second and (3) third treatment; 1 week between each treatment.

Table 4. Efficacy of *Bacillus sphaericus* Vectolex-G (ABG-6185) granules against *Anopheles gambiae* larvae in irrigation ponds at Ndjili area.

Rate (liters/ha)	Area (m²)	Mean water depth (cm)	Mean no. larvae/ dip. pret.[a]	Repetition[b]	Mean (%) reduction after treatment (days)			
					1	2	5	7
10	15	18	32	(1)	98	98	59	4
			27	(2)	100	100	77	55
20	28	29	16	(1)	95	100	48	11
			26	(2)	100	100	80	85
30	20	22	22	(1)	99	100	64	44
			19	(2)	100	100	88	90
Control	9	26	14	—	0	9	16	4
			16	—	0	12	23	0

[a] Mean pretreatment larval density; water pH 7.0–7.4, clear water with sparse density of vegetation in border; optical density of water during study, 0.0740–0.3200 wavelength.
[b] (1) first and (2) second treatment; 1 wk each treatment.

longer in the sewage lagoon than in other sites. This phenomenon could be attributable to the vegetation covers in the sewage lagoons that protect the crystal/spore from sunlight photodegradation (Lacey and Smittle 1985, Karch et al. 1986). Mulla et al. (1988) evaluated the same formulation against *Aedes nigromaculis* (Ludlow) larvae in irrigated pastures and obtained complete control at the rate of 11.2 kg/ha after 48 h post-treatment.

In comparing the dispersion of the 2 granular formulations at the water surface, the particles of Vectobac-G float better than Vectolex-G. This phenomenon may be due to the volume and the floating properties of the granules.

Culex tigripes, the predatory mosquito larva occurring in *Cx. quinquefasciatus* breeding sites, was not susceptible to *B. sphaericus*. We observed that the density of this larval predator increased after application of Vectolex-G in gutters at Ngafula and Ndjili. In addition, other aquatic arthropods found in the breeding sites may play a role in the persistence or recycling of bacteria (Karch et al. 1987, 1990).

From our studies, it appears that both Vectobac formulations (aqueous suspension and granular) of *B. thuringiensis* (H-14) were less active than the Vectolex-G granule formulation of *B. sphaericus*. Under our conditions it is suitable to use the Vectolex-G against the urban mosquito *Cx. quinquefasciatus* and against the principal malaria vector, *An. gambiae*, in Kinshasa.

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