EFFICACY OF *BACILLUS SPHAERICUS* AGAINST THE MALARIA VECTOR *ANOPHELES GAMBIAE* AND OTHER MOSQUITOES IN SWAMPS AND RICE FIELDS IN ZAIRE[1]

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ABSTRACT. The microbial control of *Anopheles gambiae* and other mosquitoes with a granular formulation of *Bacillus sphaericus* (Vectolex) was evaluated in rice fields and swamps, located around the suburban region of Kingabwa-village in Kinshasa, Zaïre. Ten treatment cycles with 15-day intervals were carried out with the same application rate, 10 kg/ha, during the dry season (May to September 1991). The treatments reduced larval populations of *An. gambiae* by 98% after 48 h, but repetitive applications were required every 15 days to maintain control. The persistence of *B. sphaericus* spores was more apparent in rice fields than in swamps. A significant reduction in nuisance biting by *Culex quinquefasciatus* and *Mansonia uniformis* was observed. For *An. gambiae*, a decrease of 13.6% in human biting was noted during the post-treatment period. The entomological inoculation rate was reduced from 0.238 to 0.143. The efficacy of *B. sphaericus* does not appear to offer outstanding potential for control of *An. gambiae* in rice fields and swamps and seems to be limited due to different factors tied to ecology and natural conditions in the fields.

INTRODUCTION

Few studies have been carried out on the experimental evaluation of *Bacillus sphaericus* as a microbiological control agent against malaria vectors in rice fields and swamps in Africa (Lacey and Lacey 1990). However, some researchers (Kramer 1984, Lacey et al. 1986, Lacey and Lacey 1990) have demonstrated that *B. sphaericus* is an effective mosquito control agent in rice fields. Lacey et al. (1988) evaluated *B. sphaericus* against *Anopheles* spp. and found that the control with both granular and flowable concentrate formulations was better than that obtained with *B. thuringiensis* H-14.

In the suburban area of Kingabwa, near Kinshasa, Zaïre, vast rice fields and swamps provide important breeding sites for *Anopheles gambiae* Giles, the principal vector of human malaria in Zaïre (Karch et al. 1992). This region located at approximately 40° 21′ longitude and 15° 19′ latitude, 15 km from the town center of Kinshasa. The climate is characterized by 2 seasons; the dry season lasts 4 months, from June to September; and the wet season lasts 8 months, from October to May.

This study investigated the impact of *B. sphaericus* on the entomological parameters affecting the transmission of malaria and on the nuisance of certain public health mosquitoes.

MATERIALS AND METHODS

Mosquito breeding sites: All mosquito breeding sites in rice fields and swamps situated around the Kingabwa-village (nearly 3.5 ha) were identified during the dry season in 1990 and treated during the dry season of 1991. In the center of Kingabwa, we also identified several types of breeding sites for *Anopheles* and *Culex* larvae which totaled 1959 m^2 (puddles of water on the road, in yards, gardens, open stone pits, ditches and breeding places alongside streams). These were also treated at the same period in 1991.

Bacterial treatments: Because of operational difficulties in the wet season, we treated only in the dry season. The granular formulation of Vectolex (ABG-6185, 20 BSITU Potency/mg) of *B. sphaericus* used in these studies was provided by Abbott Laboratories (North Chicago, USA). All breeding sites of *An. gambiae* and other mosquitoes in rice fields, in swamps and in Kingabwa-village were treated at the rate of 10 kg/ha. Application of the granules was carried out with a hand seeder. The treatment started on May 1, 1991, one month before the dry season. Ten application cycles were carried out with an interval of 15 days between treatments. Three to four days were required for the treatments during each cycle. The operation lasted 150 days.

Bacteriological counts: Ten samples of water were taken from the bottom of each field (rice and swamp) 2, 7 and 15 days following each application of *B. sphaericus* for spore counts. The samples were submitted to heat shock (80°C for 12 min) to kill vegetative cells and nonspore-forming bacteria. Then 0.1 ml of each subsample or a dilution of it was plated on MBS solid medium (Kalfon et al. 1983) in Petri dishes containing 100 mg/liter of streptomycin. The colony counts were recorded after a 24 h growth period at 35°C. Identification of *B. sphaericus* was confirmed by morphological and microscopic observations.

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Larval mortality evaluation: To evaluate the efficacy and the longevity of the granular formulation against larvae, 20 dips from each breeding site were taken in 47 places in rice fields, 58 in swamps and 87 in the village center using a standard mosquito dipper. This operation was repeated at 2, 7 and 15 days after treatment. The percent reduction was determined by comparing the number of 2nd to 4th instars of *An. gambiae* larvae in nontreated breeding sites left as a control which were located nearby, 5 km southeast of Kingabwa, in the Masina region.

Night-biting evaluation on human bait: Pre- and posttreatment surveys were conducted during the dry season in the treated zone of Kingabwa (1991), in the nontreated zone of Masina (1991) and the nontreated zone of Kingabwa (1990). This operation was carried out monthly by 12 collectors, during a 4-day period (at the end of the second treatment), from 1900 until 0600 h. Two collectors were positioned indoors and 2 outdoors in different regions, the rice fields, swamps and village center (Fig. 1). The mosquitoes were captured in small individual glass tubes collected every hour. After identification, only the *Anopheles* spp. were dissected to look for sporozoites and to determine their physiological age using Detinova's method (Detinova 1963). Captures of house-resting mosquitoes were carried out to compile the information necessary to make the epidemiological analysis and to know the intensity of transmission of malaria in the region. These collections were carried out by a team of 2 using the pyrethrum spray technique; the number of rooms examined per house was variable. All calculations were based on the data of the dry seasons during 1990 and 1991 in Kingabwa.

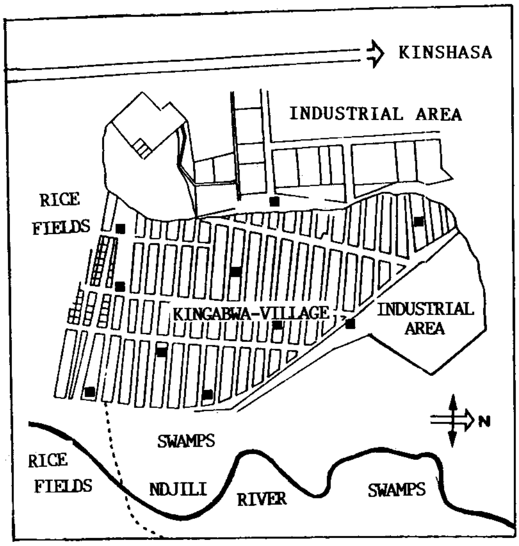

Fig. 1. Map of Kingabwa with boundary areas indicated. ■ Location of collections on human bait.

Table 1. Efficacy of *Bacillus sphaericus* against *Anopheles gambiae* larvae, in the dry season, 1991 (June, July, August and September). Results of the last treatment cycle.

Location	Mean no. larvae/dip pretreatment[1]	(%) Reduction post-treatment (days) 2	7	14
Rice fields	6.1 + 3.4	98	65	43
Swamps	2.7 + 1.8	96	52	29
Village[2]	1.6 + 0.9	99	76	66

[1] Average number larvae/dip during the dry season in Masina region (as a control).

[2] Breeding sites in different places in the village center.

RESULTS

Larvicidal effects against An. gambiae: The reduction of *Anopheles gambiae* Giles larval populations in rice fields, swamps and the village center after application of granular Vectolex is presented in Table 1. According to the control in the Masina region, the reduction in larval numbers was more important in rice fields than in swamps. Despite the repetition of treatments with 15-day intervals during 150 days, the reduction of the mortality rate was moderate (29% in swamps and 43% in rice fields).

Fate of B. sphaericus in fields: The decrease of *B. sphaericus* spore counts was more rapid in the swamps than in rice fields. Spore counts ranged from 400 to 1,100 and 120 to 285 spores/ml immediately after treatment, and the last treatment cycle in September, in rice fields and swamps, respectively (Figs. 2 and 3).

Results of man-biting mosquitoes: A reduction in the nocturnal human biting of *An. gambiae* was observed on and after the second month of treatment (Fig. 4). Results of the captures in Kingabwa were compared with captures during the dry season in Kingabwa (1990) and in Masina (1991). The total number of adult Culicidae collected in Kingabwa (1990 and 1991) and in Masina (1991) in the dry season was 46,674, 24,979 and 32,412, respectively, among which there were 1,689, 1,459 and 890 *An. gambiae*. Table 2 shows the percent reduction of *Culex antennatus* (Becker), *Cx. quinquefasciatus* Say, *Mansonia africana* Theobald) and *Ma. uniformis* (Theobald) as compared with the control villages, while Fig. 5 shows the mean number of bites/night/man during dry season months in control and treated villages.

Epidemiological analysis: Karch et al. (1991) and our dissections indicate that only *An. gam-*

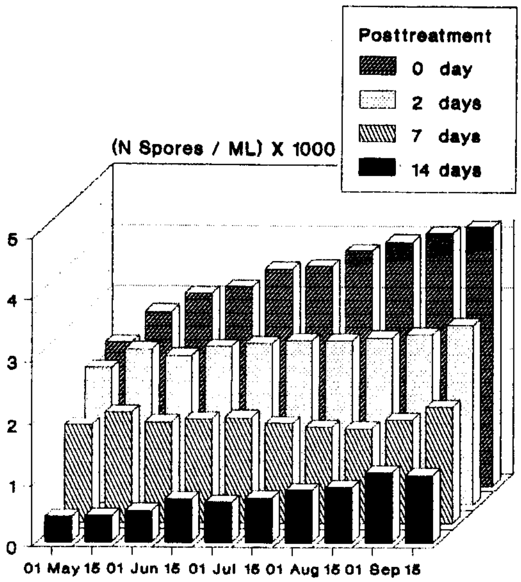

Fig. 2. Average number of *Bacillus sphaericus* spores present in rice fields during the dry season, 1991. The evaluation number was carried out in 0, 2, 7 and 14 days for each cycle.

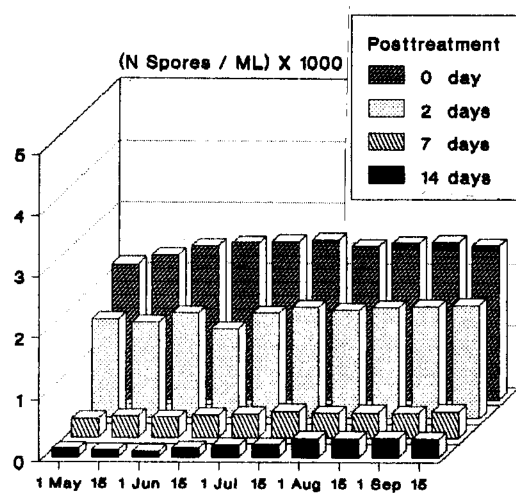

Fig. 3. Average number of *Bacillus sphaericus* spores present in swamps during the dry season, 1991. The evaluation number was carried out in 0, 2, 7 and 14 days for each cycle.

biae, which represents 93.7% of anopheline fauna in this region, is involved in the transmission of human malaria in the area under study. The entomological inoculation rate (h) is calculated using the formula (h = ma × s) where ma = man-biting rate/night and s = the proportion of mosquitoes with sporozoites in their salivary glands). For Kingabwa-village, in the dry season (1990) we obtained the following values: ma = 8.8 and s = 2.7%, the entomological inoculation rate h = 0.238, which corresponds to one infective bite per person every 4 nights, or 30 infective bites per season before treatment. In the 1991 dry season, the values were h = 7.6 × 1.8% = 0.143, which correspond to one infective bite per person every 7 nights, or 17 infective bites in the post-treatment season.

The daily survival rate (P) of *An. gambiae* was based on the physiological age according to De-

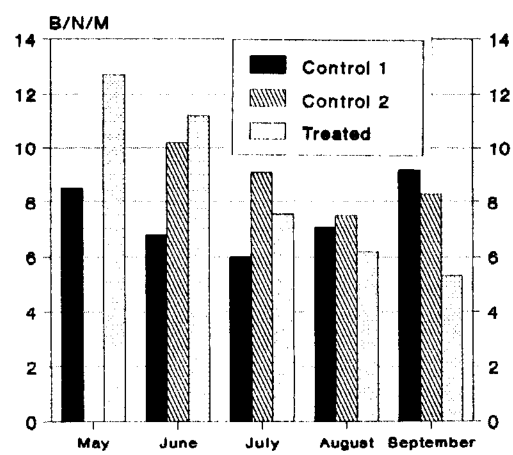

Fig. 4. Number of bites/night/man (B/N/M) of *Anopheles gambiae*, during the dry season, in Masina region (Control 1, in 1991), in Kingabwa region (Control 2, in 1990, except May) and in treated region of Kingabwa in 1991.

Table 2. Comparison of average number of preponderant mosquito bites/night/man before (in 1990) and after treatment (in 1991) in the dry season (June, July, August and September).

Species	Control 1 Masina (1991)	Control 2 Kingabwa (1990)	Posttreatment (1991)	% reduction[a]	% reduction[b]
Cx. antennatus	80.9	120.9	64.6	20.2	46.6
Cx. quinquefasciatus	50.1	55.7	18.3	63.5	67.2
M. africana	40.9	46	29.2	28.6	36.5
M. uniformis	22.3	11.7	10.4	53.4	11.1
An. gambiae	7.5	8.8	7.6	—	13.6

[a] According to control 1.
[b] According to control 2.

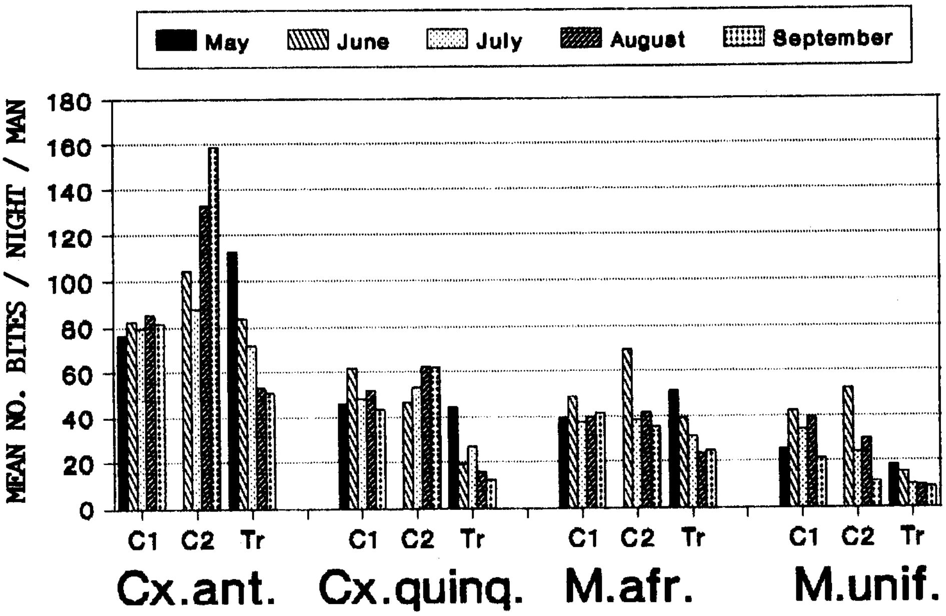

Fig. 5. Number of bites/night/man of preponderant mosquitoes, during the dry season, in Masina region (C1: Control 1, in 1991), in Kingabwa region (C2: Control 2, in 1990) and in treated region (Tr) of Kingabwa in 1991.

Table 3. Epidemiological analysis of some parameters before (in 1990) and after treatment (in 1991) in the dry season (June, July, August and September), in Kingabwa region. The calculations are based upon the average numbers of entomological data.

Parameter	Pretreatment (1990 dry season)	Post-treatment (1991 dry season)
Sporozoite rate	2.70%	1.88%
Entomological inoculation rate	0.238	0.143
Daily survival rate	0.943	0.871
Expectation of life (days)	17.260	7.260
Expectation of infective life (days)	8.430	1.390
Stability index	6.500	2.760

tinova's method and Davidson's formula, $p = \sqrt[x]{A}$, where A is the proportion of parous females and x the number of days separating 2 blood meals (in this study x = 2.5 days). The daily survival was P = 0.943 in the pre-treatment season and P = 0.871 in the post-treatment season. The result of other parameters is present in Table 3.

DISCUSSION AND CONCLUSIONS

A significant reduction of *An. gambiae* larvae in rice fields and swamps with a granular formulation of *B. sphaericus* was obtained at 2 days post-treatment. The granular formulation enabled good penetration of dense rice and permitted even distribution of the bacterium in fields (Lacey and Lacey 1990, Karch et al. 1991).

Bacillus sphaericus does not appear to offer an outstanding potential for control of *An. gambiae* in swamps and in rice fields. It may provide an option for control of other more susceptible culicines, especially *Culex*. However, *B. sphaericus* spores persisted in rice fields and in swamps. The accumulation of spores after a treatment cycle was more important in rice fields than in swamps. This phenomenon may explain the difference in mortality rates in rice fields and in swamps. It seems that the efficacy of the treatment with *B. sphaericus* depends not only on the formulation and on the type of breeding sites (Yap 1986, Service 1990), but also on the persistance of the bacteria in relation with the aquatic fauna and feeding strategy of the target species (Karch et al. 1990).

The ultimate effect of 10 treatment cycles with *B. sphaericus* in the dry season on the dynamics of mosquito populations is based on human bait collections. In comparison with the control, the population of *Cx. quinquefasciatus* and *Ma. uniformis* decreased respectively by 63.5% and 53.4%. Yap et al. (1991) also obtained good control of *Mansonia* populations with an aqueous suspension and granular formulations of *B. sphaericus*. Nevertheless, only a relative decrease of 13.6% of *An. gambiae* biting was observed in our study. It is possible that the duration of treatment was not sufficient to obtain sustained reduction in the malaria vector population. However, we noted the appearance of young larvae of *An. gambiae* at the end of treatment periods.

The entomological parameters affecting the transmission of malaria and certain characteristics of the parasitology were evaluated. The reduction of the number of infectives bites of *An. gambiae* in the post-treatment dry season may be due to the sporozoite rate and in part to the decrease of night-biting mosquitoes. However, an important reduction was observed in the stability index due to the decrease of the daily survival rate.

It seems that application of *B. sphaericus* can reduce the natural population of *An. gambiae* larvae in different habitats in the field, but the degree of reduction is limited due to different factors (Yap 1986, Mulla et al. 1988) which intervene in the field. More satisfactory results may be obtained by improving the method of application or the formulation.

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