SMALL-SCALE FIELD TRIALS OF *BACILLUS SPHAERICUS* (STRAIN 2362) FORMULATIONS AGAINST *MANSONIA* MOSQUITOES IN MALAYSIA[1]

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ABSTRACT. Five formulations of *Bacillus sphaericus* (strain 2362) including aqueous suspension BSP 1, BSP 2, technical powder ABG 6184, corncob granules ABG 6185 (potencies 2×10^{10}, 2×10^{7}, 9.5×10^{10}, 5×10^{10} spore/g, respectively) and wettable powder ABG 6232 (1,000 BS ITU/mg) were tested against laboratory-cultured late third/early fourth instar larvae of *Mansonia uniformis* in floating screened cages in small plots at swampy ditches on Penang Island, Malaysia. Mean dosage/response values at 90% mortality levels were 6.93, 95.32, 1.45, 11.92 and 2.86 liters or kg per ha, respectively, for the formulations tested. There were practically no residual effects for the formulations tested with larvae introduced at 48, 96, and 168 h post-treatment. In trials of BSP 1, ABG 6184 and ABG 6185 (1 liter or 1 kg per ha) against immature *Mansonia* spp. in impounded paddy field ditches, improved efficacy and residual effects were obtained with mean reductions of 93.1, 91.9 and 80.4% at days 3, 7 and 14 post-treatment, respectively.

INTRODUCTION

Mansonia mosquitoes are the main vectors of Brugian filariasis caused by *Brugia malayi* in the broader Southeast Asian regions including the Indian subcontinent, Indochina and the Western Pacific islands. A recent survey (World Health Organization 1984) indicated that there were more than 900 million people living in endemic areas with approximately 8.6 million cases of infection in these regions. To date, the control of Brugian filariasis has been through mass drug treatment using diethylcarbamazine citrate. There have been no operational vector control approaches against *Mansonia* mosquitoes (Yap 1985).

In comparison with other genera of vector mosquitoes, there has been a lack of information concerning field larvicidal tests using either conventional insecticides or biological control agents against *Mansonia* species (Yap 1985). Laboratory tests on the susceptibility of *Mansonia* larvae using conventional insecticides were conducted earlier (Yap et al. 1968, Yap and Sulaiman 1976). Among the biological control agents and microbial insecticides, *Bacillus thuringiensis* H-14 (Foo and Yap 1982, Foo 1986[2]), *B. sphaericus* (Cheong and Yap 1985, Yap et al. 1988) and *Tolypocladium cylindrosporum* (Serit and Yap 1984) have been tested in the laboratory against *Mansonia* in comparison with *Aedes*, *Anopheles* and *Culex* larvae. Laboratory studies on 3 strains of *B. sphaericus* indicated that *Mansonia uniformis* (Theobald) larvae were susceptible to strains 1593 and 2362 but not to strain 2297 (Yap et al. 1988). More recently, laboratory efficacy of *Bacillus thuringiensis israelensis* (U.S. *B.t.i.* standard) and *B. sphaericus* 2362 (primary powder) have also been determined against *Mansonia titillans* (Walker) and *Mansonia dyari* Belkin, Heinemann and Page (Lord and Fukuda 1990). Preliminary field trials of *B. thuringiensis* H-14 against *Mansonia* have been reported earlier (Foo and Yap 1983). Field trials of *B. sphaericus* against various genera of vector mosquitoes, in particular *Aedes*, *Anopheles* and *Culex*, have also been reviewed (Singer 1985, Lacey and Undeen 1986, Yap 1990). We are reporting the results of the small-scale field trials of *B. sphaericus* against *Mansonia* larvae in northern Malaysia. The potential use of *Bacillus* species for *Mansonia* control is discussed.

MATERIALS AND METHODS

Two types of small-scale trials were conducted for the studies. The first included the efficacy assessments of formulations of *B. sphaericus* (strain 2362) against introduced laboratory-cultured late third/early fourth instar larvae of *Ma. uniformis*, originally collected from Penang Island, in floating screened cages in small plots (15–18 m^2) covered with natural host plants of *Mansonia* mosquitoes, i.e., water hyacinth (*Eichhornia crassipes* Solm). The field plots

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[2] Foo, A. E. S. 1986. Laboratory and field evaluations on the efficacy of *Bacillus thuringiensis* H-14 for the control of *Mansonia* and other vector mosquitoes (Diptera: Culicidae), including some comparative studies with *B. sphaericus*, Ph.D. thesis, Universiti Sains Malaysia, Penang.

were situated at a swampy ditch of an abandoned coconut plantation in Permatang Damar Laut on the southern coastal alluvial plain of Penang Island, Malaysia. The second type of experiment involved the assessment of *B. sphaericus* formulations against natural populations of *Mansonia* mosquitoes in sections of impounded paddy ditches at Bagan Serai, a coastal town in the state of Perak, northern Peninsular Malaysia. The detailed protocols for conducting small-plot trials as well as efficacy assessment of natural *Mansonia* populations followed essentially those of Foo and Yap (1983). Modifications included the following: 1) Fifty instead of 100 laboratory-cultured *Ma. uniformis* of late third/early fourth instar larvae were used per floating cage in the small plot trials. 2) Residual effects of the small plot trials were determined by introduction of new batches of larvae into exposed cages at 48, 96 and 168 h post-treatment. 3) Assessment of efficacy and residual activity of *B. sphaericus* formulations against natural populations of *Mansonia* in paddy fields were carried out at days 1, 3, 5, 6, 14 and 21 post-treatment.

The formulations of *B. sphaericus* (strain 2362) tested included: 1) aqueous suspension BSP 1 (potency 2×10^{10} spore/g), 2) aqueous suspension BSP 2 (2×10^{7} spore/g), 3) technical powder ABG 6184 (9.5×10^{10} spore/g), 4) corncob granules ABG 6185 (5×10^{10} pore/g or 200 BS ITU/mg), and 5) wettable powder ABG 6232 (1,000 BS ITU/mg). All 5 formulations were tested against introduced *Ma. uniformis* larvae in small-plot trials, but only BSP 1, ABG 6184 and ABG 6185 were assessed against natural *Mansonia* populations in paddy field ditches. For the small-plot trials, dose-response studies with appropriate dosages for respective formulations ranging from 0.1 to 40 liters or kg per ha were used depending on the potency of the formulations (see Table 2). For the assessment against natural populations, a single dose of 1 kg (ABG 6184 and ABG 6185) or 1 liter (BSP 1) per ha was used. These were tested against *Mansonia* immatures attached to water hyacinth plants in 4 separate impounded paddy field ditches.

All formulations except corncob granules (ABG 6185) were diluted with seasoned tap water from the laboratory. All aqueous-based formulations were applied using a Geizhal ES10 pressurized knapsack sprayer (Dr Stahl & Sohn GmbH & Co., Uberlingen, Germany), and the corncob formulation was mixed with fine sand and dispersed by hand. Due to the different potencies of the bacterial formulations, preliminary efficacy trials were carried out to determine the suitable ranges for the dose-response studies of these formulations.

The meteorological and water quality parameters of rainfall, temperature, pH, dissolved oxygen and water conductivity at the field sites were recorded daily. Measurements were made using portable meters including membrane pH meter (Hanna HI 8314, Italy), dissolved oxygen meter (Yellow Spring Instruments, YS IM57, USA) and conductivity meter (WTW LF 91 with probe KLE I/T, USA). A minimum of 6 readings were recorded for each of the parameters each time at the respective test locations. Statistical analyses, including Duncan's multiple range test and probit analysis (Finney 1962) assisted by the use of a computer program (Daum 1970), were used when appropriate.

RESULTS

The meteorological and water quality conditions of the 2 test locations are given in Table 1. There were no major differences in values in the environmental parameters measured, except for that of conductivity, between the small plots in swampy ditches at Permatang Damar Laut, Penang Island and those of impounded paddy field ditches at Bagan Serai, Perak, northern Peninsular Malaysia. The water temperature measured in the late mornings was around 27 ± 2° C. The water was weakly acidic. The values of dissolved oxygen and conductivity indicated that the impounded paddy field ditches were less polluted in terms of organic matter but had

Table 1. Physicochemical conditions of the small plots in swampy ditches on Penang Island and impounded paddy field ditches at Bagan Serai, Peninsular Malaysia.

Location	Total rainfall in mm (range)	Water condition (mean ± SE)			
		Temperature (°C)	pH	Dissolved oxygen content (mg/liter)	Conductivity (μmho/cm)
Small plots in swampy ditch	74.3 (12.5–147.3)	26.8 ± 0.28	6.8 ± 0.04	1.1 ± 0.02	11.1 ± 2.20
Impounded paddy field ditches	119.4 (69.0–150.9)	27.3 ± 0.30	5.8 ± 0.12	1.6 ± 0.30	64.7 ± 11.20

Table 2. Larvicidal effects of formulations of *Bacillus sphaericus* (strain 2362) against *Mansonia uniformis* larvae in floating cages in small plots.

Formulation	Treatment received (kg or liter/ha)	Recovery of live larvae of 50 introduced[1] 48 h	72 h	96 h	Combined % reduction
Aqueous suspension (BSP I)	Untreated	39 ± 3.5	41 ± 1.0	37 ± 5.0	—
	0.1	47 ± 2.1	43 ± 0.0	33 ± 6.4	—
	0.5	27 ± 4.0	23 ± 1.7	28 ± 0.6	33
	1.0	19 ± 5.5	21 ± 2.9	11 ± 4.0	56
	2.5	13 ± 5.4	13 ± 3.8	7 ± 0.6	72
	5.0	8 ± 2.1	1 ± 0.6	3 ± 0.6	90
	10.0	6 ± 3.5	2 ± 0.6	5 ± 2.6	90
Aqueous suspension (BSP 2)	Untreated	37 ± 4.0	36 ± 1.5	36 ± 0.3	—
	1.0	28 ± 4.6	37 ± 1.5	33 ± 2.6	8
	2.5	39 ± 0.6	29 ± 2.1	32 ± 3.5	11
	5.0	25 ± 2.1	19 ± 2.1	21 ± 0.7	42
	10.0	23 ± 4.9	24 ± 2.1	21 ± 2.2	42
	20.0	12 ± 4.0	14 ± 1.0	13 ± 2.3	64
	40.0	13 ± 2.9	10 ± 2.1	8 ± 3.9	78
Technical powder (ABG 6184)	Untreated	42 ± 1.2	40 ± 1.0	40 ± 1.5	—
	0.1	40 ± 2.3	35 ± 0.6	24 ± 1.0	20
	0.5	6 ± 0.6	2 ± 0.6	4 ± 1.5	90
	1.0	7 ± 2.9	3 ± 1.2	9 ± 4.4	85
	2.5	2 ± 0.6	2 ± 0.6	4 ± 0.6	93
	5.0	1 ± 0.6	1 ± 0.0	1 ± 0.0	98
	10.0	0 ± 0.0	1 ± 0.6	3 ± 1.5	98
Corncob granules (ABG 6185)	Untreated	40 ± 1.5	40 ± 1.2	38 ± 0.6	—
	1.0	40 ± 2.1	27 ± 2.9	35 ± 0.6	13
	2.5	28 ± 4.9	26 ± 1.2	18 ± 5.2	39
	5.0	15 ± 7.5	11 ± 3.8	6 ± 3.2	72
	10.0	7 ± 4.04	3 ± 1.53	0 ± 0.0	92
	20.0	4 ± 0.58	0 ± 0.0	1 ± 0.6	95
	40.0	1 ± 0.58	0 ± 0.0	1 ± 0.6	97
Wettable powder (ABG 6232)	Untreated	42 ± 1.9	37 ± 3.1	34 ± 1.7	—
	0.1	34 ± 3.0	33 ± 1.2	33 ± 1.9	13
	0.25	38 ± 2.6	28 ± 5.5	32 ± 2.6	13
	0.5	27 ± 3.4	17 ± 5.1	12 ± 7.4	50
	1.0	17 ± 8.4	11 ± 4.8	11 ± 6.0	66
	2.5	5 ± 1.1	6 ± 3.1	2 ± 1.2	90
	5.0	3 ± 1.9	3 ± 1.0	1 ± 0.5	95

[1] Means ± SE of 3 experiments.

greater ion content when compared with small plots in swampy ditches (Table 1).

A survey of the natural larval populations conducted at the impounded paddy field ditches at Bagan Serai indicated the presence of both *Ma. uniformis* and *Mansonia indiana* (Edwards), with the latter constituting about 80% of the late third/early fourth instar larvae sampled.

The larvicidal effects of the 5 formulations of *B. sphaericus* (strain 2362) tested against the sentinel *Ma. uniformis* in small plots are presented in Table 2. All formulations tested provided a minimum 90% mortality of the *Mansonia* larvae at 48 h post-treatment at the higher dosages used except that of aqueous suspension BSP 2 (Table 2). The LD90s calculated from the data are given in Table 3, representing the dose response for each formulation evaluated.

When new *Ma. uniformis* larvae were introduced at 48, 96 and 168 h post-treatment into the sentinel cages, there was no statistical difference between the untreated and treated number of live larvae for the formulations of BSP 1, BSP 2, ABG 6184 and ABG 6232 (F test, $\alpha = 0.05$). However, for the corncob granular formulation (ABG 6185), there appeared to be significant residual effects at 48 h post-treatment at higher dosages (Table 4).

For the paddy field ditches trials, the percentage reduction of larvae recovered from shaking of *Eichhornia* plants in treated ditches versus the untreated (control) ditch for all 3 formulations tested ranged from 86.4 to 98.8% at 72 h post-treatment (Table 5). In addition, residual activities for all 3 formulations tested at days 7 and 14 provided mean percentage reduction of larval population of 91.9% and 80.4%, respectively (Table 5). The calculation of the above

Table 3. Dosage-response values (kg or liter/hectare) of *Bacillus sphaericus* (strain 2362) formulations against *Mansonia uniformis* larvae in small plots.

Formulation	Lethal dosage (kg or liter/ha)				
	LD50	95% C.L.	LD90	95% C.L.	Slope ± SE
Aqueous suspension (BSP 1)	0.96	0.81–1.12	6.93	5.67–8.84	1.49 ± 0.10
Aqueous suspension (BSP 2)	11.31	9.62–13.27	95.32	68.68–148.23	1.38 ± 0.11
Technical powder (ABG 6184)	0.20	0.11–0.30	1.45	0.98–2.49	1.48 ± 0.15
Corncob granules (ABG 6185)	3.01	2.65–3.38	11.92	10.42–13.96	2.15 ± 0.13
Wettable powder (ABG 6232)	0.60	0.53–0.68	2.86	2.41–3.49	1.89 ± 0.11

percentage reduction was based on a formula (Mulla et al. 1971) that took into consideration the mosquito population fluctuation of untreated ditches during the experimental period.

DISCUSSION

Results on the environmental parameters of the 2 test locations (Table 1) basically indicated the norm of tropical aquatic conditions of swampy and paddy field ditches in northern Malaysia (Yap and Ho 1977). As a whole, the water was characteristically weakly acidic with moderately high organic content (as indicated by the relatively low dissolved oxygen levels) and high ion contents (high conductivity values). The relatively high organic and ionic content of the water is necessary to sustain the natural growth of water hyacinth plants and their associated *Mansonia* immatures in such locations. The interaction of environmental factors with the microbial insecticides was reviewed earlier (Yap 1987).

In small-plot trials, dose-response studies on efficacy of *B. sphaericus* formulations tested indicated high degrees of differences due primarily to the varied potency of the bacterial formulations used (Tables 2 and 3). However, direct comparison of efficacy of various formulations on a weight-to-weight basis is not warranted due to variations of potency of the formulations tested. Overall, a dose of 10 kg or 10 liters per ha should achieve effective control (as measured by 90% mortality) for all the formulations tested except that of aqueous suspension BSP 2. Computer probit analysis of the efficacy data provided very flat regression slopes for all formulations tested (Table 3). The flat slopes indicate that the formulations are effective over a wide dosage range. This is in accordance with laboratory and field efficacy tests of insecticides and *B. thuringiensis* H-14 against *Mansonia* species (Yap et al. 1968; Yap and Sulaiman 1976; Foo and Yap 1982, 1983).

In small-plot trials, the residual effects of *B. sphaericus* (strain 2362) formulations are generally not obvious except for that of the corncob granules (ABG 6185) at exceedingly high dosages of 20 and 40 kg per ha (Table 4). The discrepancy may be due to the assessment technique of using floating screened-cages and older larvae. Except for the granular formulation (ABG 6185), the active ingredients of other formulations used would pass and sink through the screened cages and hence would not be available to the feeding larvae inside the cages. In contrast, the granules would be trapped inside the cages to provide slightly prolonged residual effect at higher dosages.

When *B. sphaericus* (strain 2362) formulations were tested against natural populations of *Mansonia* mosquitoes in impounded paddy field ditches, they seem to provide better efficacy and longer residual effects (up to 14 days) at dosages of 1 kg or 1 liter per ha when compared with the small-plot trials. This may be due to the very long immature stages of *Mansonia* mosquitoes, which last about 20–25 days (Wharton 1962). Laboratory culture of both *Ma. uniformis* and *Ma. indiana* indicates that the mosquitoes require 12–16 days to reach the third instar larval stage. Hence, the longer residual effects may reflect the period needed by the late instar larval population to recover from the initial treatments. The field population of *Mansonia* larvae collected indicated a greater preponderance of *Ma. indiana* (80%) as compared with *Ma. uniformis*. However, laboratory tests of *B. sphaericus* against the 2 species did not indicate any differences in susceptibility between them (H. H. Yap, unpublished data).

It is thus seen that *B. sphaericus* can reduce the field population of *Mansonia* species in different habitats in nature in small-scale trials.

Table 4. Residual effects of formulations of *Bacillus sphaericus* (Strain 2362) against *Mansonia uniformis* larvae in floating cages in small plots, introduced at 48, 96 and 168 h post-treatment and exposed in the plot for 48 h.

Formulation	Treatment received (kg or liter/hectare)	Post-treatment recovery of larvae of 50 introduced		
		48–96 h	96–144 h	168–216 h
Aqueous suspension (BSP 1)	Untreated	32	33	30
	0.1	40	37	26
	0.5	31	36	29
	1.0	28	36	32
	2.5	32	25	31
	5.0	26	22	38
	10.0	29	28	42
Aqueous suspension (BSP 2)	Untreated	36	—[1]	—[1]
	1.0	39	—	—
	2.5	36	—	—
	5.0	40	—	—
	10.0	35	—	—
	20.0	32	—	—
	40.0	30	—	—
Technical powder (ABG 6184)	Untreated	38	39	44
	0.1	40	44	32
	0.5	47	32	36
	1.0	39	40	21
	2.5	36	37	28
	5.0	38	32	42
	10.0	24	38	36
Corncob granules (ABG 6185)	Untreated	38	32	37
	1.0	30	35	34
	2.5	41	29	37
	5.0	22	24	41
	10.0	24	22	33
	20.0	7	29	29
	40.0	4	22	30
Wettable powder (ABG 6232)	Untreated	39	—[1]	—[1]
	0.1	41	—	—
	0.25	34	—	—
	0.5	39	—	—
	1.0	32	—	—
	2.5	26	—	—
	5.0	29	—	—

[1] Residual effect not assessed.

Table 5. Larvicidal and residual effects of *Bacillus sphaericus* (strain 2362) formulations against natural populations of *Mansonia* species in impounded paddy field ditches at Bagan Serai, Peninsular Malaysia.

Treatment received	No. and % reduction of larval population in days (%)[1,2]						
	0[3]	1	3	5	7	14	21
Untreated	16.7[a]	27.3[a]	21.0[a]	18.7[a]	16.0[a]	24.0[a]	9.7[a]
Aqueous suspension BSP1 (1 liter/ha)	25.3[a]	8.3[b] (55.9)	4.3[b] (86.4)	4.3[b] (84.7)	3.7[b] (84.9)	11.7[b] (68.0)	12.3[a] (16.1)
Technical powder ABG 6184 (1 kg/ha)	13.3[a]	8.7[b] (60.4)	1.0[b] (94.1)	1.3[b] (91.1)	1.0[b] (92.2)	2.4[b] (85.7)	8.0[a] (±3.5)
Corncob granules ABG 6185 (1 kg/ha)	25.0[a]	4.3[b] (89.4)	0.3[b] (98.8)	0.0[b] (100)	0.3[b] (98.6)	3.0[b] (87.5)	13.3[a] (8.0)

[1] No. of larvae were means of dislodged late 3rd and early 4th instar larvae recovered from 3 batches of 10 *Eichhornia* plants each. Mean within a column followed by the same alphabet are not significantly different ($P < 0.05$; Duncan's multiple range test).

[2] Plus sign (+) indicates an increase in the population.

[3] Means of pretreatment counts immediately before spraying.

Medium and large-scale trials of existing and newer formulations of both *Bacillus* species against *Mansonia* should be carried out to exploit the full potential of such biocontrol agents for *Mansonia* vector control.

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