TOXICITY OF *BACILLUS SPHAERICUS* STRAIN 2362 ON *MANSONIA* SPP. LARVAE

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Abstract. The efficiency of *Bacillus sphaericus* strain 2362 (Vectolex®) as larvicide against *Mansonia* spp. was studied. Bioassay studies showed that the toxicity of *B. sphaericus* on both age groups (I-II instar and III-IV instar) of *Mansonia* spp. larvae occurred within 24 hours. Probit analysis revealed that LC_{100} (one hundred per cent lethal concentration) for both age groups of *M. boneae* were higher than those of *M. dives*. Small scale field trials were done at Kreng Village, Cha-uat District, Nakhon Si Thammarat Province, one of the most serious filarial infected areas. It was indicated that 100% kill of *Mansonia* spp. larvae in the field occurred within 9 days after the larvicide application. When a dose of 5 times of LC_{100} value was used, 100% control was acheived up to about one month.

INTRODUCTION

Human lymphatic filariasis occurs in humid tropical area of Africa, the Americas, Asia and numerous islands in the Pacific Ocean (WHO, 1989). Filariasis in Thailand is caused by *Wuchereria bancrofti* and *Brugia malayi* (Harinasuta, 1984). The principal mosquito vectors of both types of filariasis in Thailand are *Anopheles* spp. and *Mansonia* spp. Brugian filariasis was reported from six provinces along the eastern coast of the peninsula region of Southern Thailand (Chumphon, Surat Thani, Nakorn Si Thammarat, Phatthalung, Pattani and Narathiwat) (Guptavanij *et al,* 1977). In this area of the country there are many small and large swampy areas with various kinds of aquatic plants, grasses and weeds which serve as good breeding sites for *Mansonia* mosquitos. The Filariasis Control Program in Thailand was initiated in 1961 (Harinasuta, 1984). The aim of the program is to control Brugian filariasis in the entire endemic areas in these six provinces. The chemical control measure against mosquito vectors was made as a by-product of the DDT spraying of the National Malaria Eradication Program.

As a result of the concern for environmental safety and for resistance to chemical insecticides among important vector species, some microbial agents for vector control have been developed. Of the various potential microbial agents, bacteria appear to have the most potential as larvicides for mosquitos.

During the past decade, *Bacillus thuringiensis* H-14 (Bt. H-14) has been proven to be an important larvicide in mosquito vector control programs. However Bt. H-14 is relatively ineffective in polluted water and its residual activity in most habitats is limited to a few days after treatment (WHO, 1985). Recently, approximately 40 strains of *B. sphaericus* have been found to possess larvicidal activities against some mosquito species, especially culicine mosquitos (Lacey and Singer, 1982). Despite a narrower spectrum of activity than Bt. H-14, *B. sphaericus* possesses some characteristics which make it a better candidate for production and field application. Its spores and toxins can persist for a considerable length of time in the environment (Hornby *et al,* 1981; Muligan *et al,* 1980). *B. sphaericus* is able to recycle in certain aquatic environments and grows in cadavers of *Culex* spp. (Hertlein *et al,* 1979; Davidson *et al,* 1984; Des Rochers and Garcia, 1984). It also possesses the advantage of nonpathogenicity to many species of predaceous mosquitos (Lacey, 1983).

The molecular mode of action of *B. sphaericus* is not clear. The histopathology study showed that midgut epithelium should be the primary site of action (WHO, 1985). After ingestion of the spores and/or cells of the insecticidal strains, the epithelium distends and the gut is paralysed. This is followed by epithelial cell lysis and the mosquito larva is killed within a matter of hours. Cost analysis study of *B. sphaericus* preparations has been done by the Vector Control Research Center, Pondichery, India. It was concluded that briquette formulation of *B. sphaericus* is best, as economical as fenthion for larval control, and is widely used at present. These formulations are also cheaper than malaria oil, Paris green or abate (WHO, 1989).

Reported here is the efficiency of *B. sphaericus* strain 2362 (Vectolox®, Abbott Laboratories) in controlling *Mansonia* spp. larvae.

MATERIALS AND METHODS

Culture of immature *Mansonia* spp.

The method used in the maintenance of the colonies of immature *Mansonia* spp. was modified from Limsuwan, (1987). Water lettuce (*Pistia stratiotes* L.) was used as an aquatic plant for immature *Mansonia* to attach and acquire oxygen.

Bioassay for lethal concentration of *Mansonia* spp. larvae

Bioassay for lethal concentration (LC) was done according to WHO protocol (WHO, 1985). In this study mosquito larvae of each species were divided into I-II and III-IV instars. *B. sphaericus* strain 2362 (Vectolex®, Abbott laboratories) at 5 different concentrations (0.005, 0.050, 0.500, 5.000 and 7.000 mg/l) were used as larvicidal agent. Each experiment had replications. Three plastic cups (or 3 plots) were used for each concentration. Each plastic cup (7 cm diameter, 5 cm height) contained 100 ml of rearing media (guinea pig dung water solution), 1 or 2 small water lettuce plants, and 25 mosquito larvae of the same species. Bacterial preparation of known volume was added into each cup for the desired concentration. One or two drops of yeast solution were added every two days as bacterial food source. Numbers of dead larvae were checked every 24, 48 and 72 hours. Net percent kill of mosquito larvae was obtained using Abbott's formula. The LC_{50}, LC_{90}, and LC_{100} values of I-II and III-IV instars of each species were determined by probit analysis (Finney, 1952).

Small scale field trials

Two old fish-breeding ponds about 8m × 30m and 7m × 45m with a depth of 0.6 m at Kreng village, Cha-uat District, Nakhon Si Thammarat were selected as field trial sites. These two ponds were located about 10 m from each other and were covered with water hyacinth [*Eichhornia crassipes* (Mart) Solms.]. BOD (Biochemical Oxygen Demand) and pH (potential of hydrogen) of water in each pond were 10 mg/l and 6.3, respectively. The experimental design used in this study was randomize complete block (Fig 1).

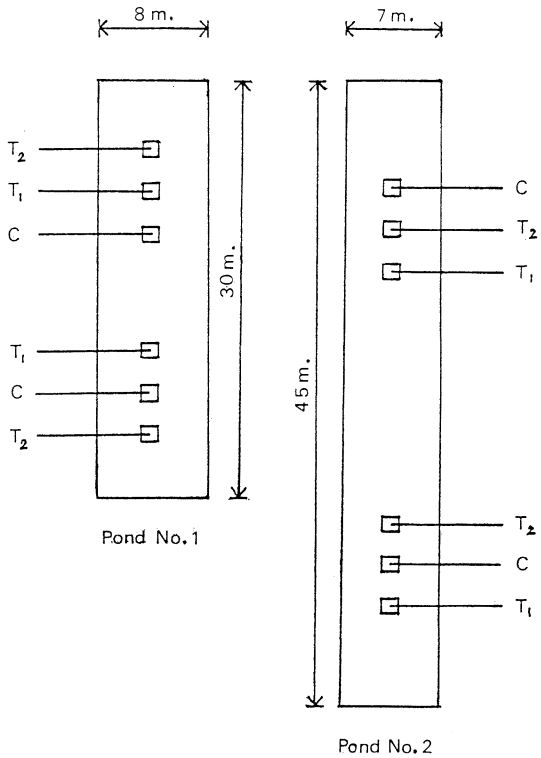

Fig 1—Experimental sites and design for small scale field trials of *Bacillus sphaericus* at Kreng Village, Cha-uat District, Nakhon Si Thammarat.
C = Control
T_1 = 5 ml/m^2
T_2 = 25 ml/m^2

There were six plots (2 replications) in each pond. Each plot (1 m^2) was fenced off by bamboo frame (1m × 1m), and was about 2 m away from one another. Numbers of mosquito larvae, other aquatic insects and natural enemies of mosquito larvae were recorded before the bacterial application. Three water hyacinth plants were randomly picked up from each plot and the lower part of the plants shaken vigorously in water in a plastic bowl (50 cm diameter, 30 cm depth). Immature *Mansonia* spp. attaching themselves to the roots of the weed would then be detached. Three levels of concentration of bacteria (0, 1 and 5 times of laboratory LC_{100}) were applied as shown in Fig 1. A manually operated knapsack sprayer was used in this field application.

Two days after, the number of mosquito larvae was observed in a similar manner. The data were collected continuously every 7 days until the bacteria lost their residual effect (which could be indicated by the appearance of a new set of mosquito larva).

RESULTS

Bioassay for lethal concentrations of *Mansonia* spp. larvae

Only two *Mansonia* species were used in the bioassay study *(M. boneae* and *M. dives)*. Numbers of dead larvae of each species at different periods of time and different concentrations of bacteria are shown in Tables 1, 2, 3 and 4.

B. sphaericus seemed to act rather slowly such that the lethal concentration at 48 hours had to be considered. Probit analysis for lethal concentration of *B. sphaericus* showed that *M. dives* was more tolerant than *M. boneae* (Tables 1, 2, 3 and 4).

Dilution plate count technique indicated that bacterial formulation contained 1.05 × 10^{10} cell/gm.

Small scale field trials

LC_{100} of *M. dives* (Table 3, 4) was selected for use in field application because it showed the highest rate of bacteria (501.19 mg/l or 5 ml/m^2). Field applications were done at 0, 1 and 5 times of LC_{100} of *M. dives* or 0.00 ml/m^2, 5.00 ml/m^2, and 25.00 ml/m^2.

Three species of *Mansonia* mosquitos were found in Kreng village. They are *M. uniformis, M. dives* and *M. boneae* (61.4%, 29.7%, and 3.7% respectively). However *Mansonia* spp. pupae, dragonfly nymphs, and adults of predaceous beetle were found in very low numbers. The efficiency of *B. sphaericus* against field population of *Mansonia* spp. is indicated by means of reduction percent of mosquito larvae in each four plots of the same bacterial concentration (Rodcharoen, 1987). Population reduction of both age-group I-II and III-IV instar of *Mansonia* spp. larvae in both treatments (5 ml/m^2 and 25 ml/m^2) occurred within two days after application (Table 5, 6 and Fig 2, 3). One hundred per cent reduction was obtained within nine days after application in both treatments. Fading out of bacterial efficiency in lower concentration (5 ml/m^2) was found within 23 days after application instead of 30 days for the higher concentration (25 ml/m^2).

Table 1

Bioassay study on the effectiveness of *Bacillus sphaericus** on the 1st/2nd instar of *Mansonia boneae.*

Concentration (mg/l)	Mean percent kill			LC_{50}	LC_{90} (mg/l, at 48 hours)	LC_{100}	Regression equation
	24 hours	48 hours	72 hours				
0.000	0.17	0.67	0.83	0.0032	1.4125	147.9108	b = 0.4958 ± 0.4759
0.005	3.28	13.06	18.50				Y = 4.7781 ± 0.4958X
0.050	10.67	19.84	24.39				
0.500	14.78	21.89	24.45				
5.000	19.50	22.72	24.83				
7.000	19.89	24.56	25.00				

* Vectolex®, 1.05 × 10^{10} cell/gm.

Table 2

Bioassay study on the effectiveness of *Bacillus sphaericus** on the 3rd/4th instar of *Mansonia boneae*.

Concentration (mg/l)	Mean percent kill			LC_{50}	LC_{90} (mg/l, at 48 hours)	LC_{100}	Regression equation
	24 hours	48 hours	72 hours				
0.000	0.00	1.42	1.00	0.0002	0.0331	2.4547	b = 0.5584 ± 0.8614
0.005	1.45	19.33	24.22				Y = 4.8773 ± 0.5584X
0.050	10.11	24.22	25.00				
0.500	10.78	24.22	25.00				
5.000	9.56	24.89	25.00				
7.000	10.34	24.89	25.00				

* Vectolex®, 1.05 × 10^{10} cell/gm.

Table 3

Bioassay study on the effectiveness of *Bacillus sphaericus** on the 1st/2nd instar of *Mansonia dives*.

Concentration (mg/l)	Mean percent kill			LC_{50}	LC_{90} (mg/l, at 48 hours)	LC_{100}	Regression equation
	24 hours	48 hours	72 hours				
0.000	0.00	0.50	1.00	0.0050	3.3884	501.1872	b = 0.4570 ± 0.5014
0.005	1.00	13.00	16.67				Y = 4.68 ± 0.4570X
0.050	3.00	18.67	21.00				
0.500	5.33	24.00	24.67				
5.000	8.00	24.67	25.00				
7.000	8.33	25.00	25.00				

* Vectolex®, 1.05 × 10^{10} cell/gm.

Table 4

Bioassay study on the effectiveness of *Bacillus sphaericus** on the 3rd/4th instar of *Mansonia dives*.

Concentration (mg/l)	Mean percent kill			LC_{50}	LC_{90} (mg/l, at 48 hours)	LC_{100}	Regression equation
	24 hours	48 hours	72 hours				
0.000	0.00	0.75	1.25	0.0006	0.8913	501.1872	b = 0.4018 ± 0.7740
0.005	6.67	17.22	20.56				Y = 4.6758 ± 0.4018X
0.050	6.56	19.33	22.78				
0.500	6.44	22.78	24.89				
5.000	9.67	22.78	24.89				
7.000	8.33	23.22	25.00				

* Vectolex®, 1.05 × 10^{10} cell/gm.

Table 5

Number of immature *Mansonia* spp. (I-II instar) observed before and after the application of *Bacillus sphaericus* strain 2362 at different rates (0.00, 5.00 and 25.00 ml/m^2).

Bacterial Concentration (ml/m^2)	Days after application						
	-2	2	9	16	23	30	37
	L1/2	L1/2	L1/2	L1/2	L1/2	L1/2	L1/2
0	47.50	48.50	79.00	104.00	103.25	94.25	92.25
5	57.88	9.25 (71.76)	0.00 (100.00)	0.00 (100.00)	2.75 (94.94)	8.00 (85.11)	14.75 (72.91)
25	42.38	6.50 (85.38)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.50 (98.59)	6.00 (86.24)

* Vectolex®, 1.05 × 10^{10} cells/gm.
% reduction in parenthesis

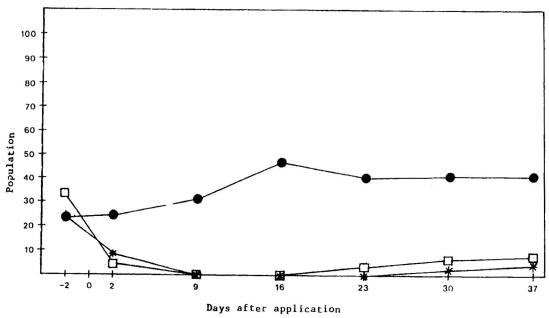

Fig 2—Change in field population of the first and second instar *Mansonia* spp. larvae after the application of *Bacillus sphaericus* strain 2362. Vectolex®, 1.05 × 10^{10} cell/gm.
● Control
□ 5 ml/m^2
* 25 ml/m^2

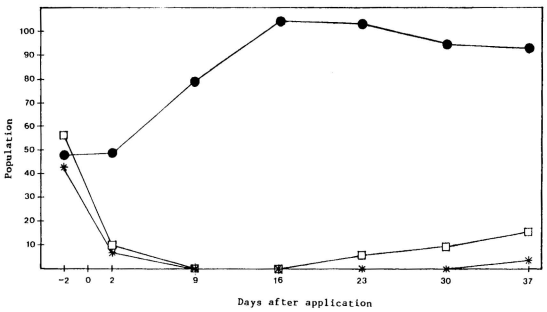

Fig 3—Change in field population of the third and fourth instar *Mansonia* spp. larvae after the application of *Bacillus sphaericus* strain 2362. Vectolex®, 1.05 × 10^{10} cell/gm.
● Control
□ 5 ml/m^2
* 25 ml/m^2

DISCUSSION

At Kreng village swamps with aquatic weeds are found all around. At this village to get a hundred of mosquito bites within a few hours is very common. Personal communication with the village public health officer revealed that only about 20% of the infected patients will come back to get medication to continue the chemotherapy. Living in such an environment makes it difficult to avoid disease transmission.

Vector control, especially in the summer season when water in swamps is drying up, seems to be a promising way to decrease the vector population. During that period of the year a tremendous number of *Mansonia* spp. larvae were found attached to the roots of aquatic weeds such as waterhyacinth and waterlettuce in artificial fish breeding ponds. In this study it was shown that *Bacillus sphaericus* strain 2362 (Vectolex®) gave good control against *Mansonia* spp. larvae. *B. sphaericus* also has no hazardous effect to the environment which makes it safe to use in mosquito breeding places such as canals, ponds and swamps. Bacterial spraying in mosquito breeding sites in summer time should be a useful method to help decrease the transmission of filariasis in such conditions.

Table 6

Number of immature *Mansonia* spp. (III-IV instar) observed before and after the application of *Bacillus sphaericus* strain 2362 at different rates (0.00, 5.00 and 25.00 ml/m^2).

Bacterial Concentration (ml/m^2)	Days after application						
	-2	2	9	16	23	30	37
	L3/4	L3/4	L3/4	L3/4	L3/4	L3/4	L3/4
0	23.25	23.75	31.00	47.50	40.25	42.25	42.25
5	33.13	8.75	0.00	0.00	2.75	6.50	7.00
		(71.76)	(100.00)	(100.00)	(91.79)	(85.23)	(78.59)
25	23.88	8.25	0.00	0.00	0.00	2.00	4.00
		(65.76)	(100.00)	(100.00)	(100.00)	(92.06)	(83.44)

* Vectolex®, 1.05 × 10^{10} cells/gm.
% reduction in parenthesis

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