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THE PROSPECT OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND *BACILLUS SPHAERICUS* IN MOSQUITO CONTROL IN THAILAND

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ABSTRACT

Malaria, Dengue hemorrhagic fever, encephalitis and filariasis are mosquito borne diseases that cause serious problems in Thailand. Because we are still lacking practical immunization or therapeutic measures for preventing or treating these diseases, control of these diseases relyentirely on the control of mosquito vectors, on the early identification of cases and, if possible, on the isolation of cases from contact with vector mosquitoes. In some cases (i.e. treatment of malaria), drug-resistant organisms are now becoming a severe problem for the treatment and control of malaria.

Chemical insecticides are conventionally used to control mosquitoes in Thailand. However, concerns over the environmental consequences, the resulting legal constraints regarding the use of non-selective and persistent pesticides, and the development of insecticide resistance by target species have brought about intensified efforts to develop biological control agents for the suppression of vectors.

Interest in the potential of pathogens for the control of medically important arthropods has been encouraged by the results recently achieved in the development and experimental use of certain spore forming bacteria such as the serotype H-14 of *Bacillus thuringiensis (B.t.i.)* and strains of *B. sphaericus.* With mosquitoes, it has been demonstrated that susceptibility *to B.t.i.* and *B. sphaericus* varies considerably according to the species tested. *B.t.i.* is more effective against *Aedes* and *Culex* than *Anopheles* larvae, but it does not persist in the environment. In contrast, most *Culex* species, including *Cx. quinquefasciatus,* are highly susceptible to *Bacillus sphaericus,* but *Aedes* larvae are little affected. In addition, *B. sphaericus* demonstrates the ability to recycle in certain conditions and appears to persist in the environment better than *B.t.i.,* Small scale field trials using these 2 bacterial agents demonstrated good prospects in using these microbial agents against mosquitoes in the near future.

INTRODUCTION

In Thailand, the four major mosquito-borne diseases are dengue haemorrhagic fever which is transmitted by *A edes aegypti* and Ae. *albopictus,* Japanese encephalitis which is transmitted by *Culex tritaeniorhynchus and Cx. gelidus,* malaria which is transmitted by anopheline mosquitoes especially *Anopheles minimus and An. dirus,* and filariasis whichis transmitted by *Culex* and *Mansonia species.* All of these mosquito vectors occur in different and wide varieties of water habitats that make efforts to control mosquitoes more difficult.

Chemical agents were used as effective weapons to control mosquitoes since 1939. However, soon evidence of resistance to chemical insecticides in mosquito species (including anopheline species) was increasingly encountered. This gradual and relentless spread of resistance to insecticides promoted an increase in research to find new insecticides and to find alternative ways to control mosquitoes to slow the rate of development of resistance.

Two spore-forming bacterial agents are considered promising agents in mosquito control programmes. These two bacilli are *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and *Bacillus sphaericus*. They produce endotoxins and can be cultured relatively easily. The endotoxins act as stomach poisons when ingested by insect larvae. These products can be formulated, stored and shipped like other chemical agents which is a great advantage over living organisms which must be handled more carefully. Several large companies are now manufacturing and formulating *B.t.i.* and *B. sphaericus* on a large scale for public health use and field tests and operational trials of these agents are under investigation. Therefore, there is a prospect for the operational use of these two bacterial agents to control mosquitoes in the near future in Thailand.

In order to put a prospect for the operational use of *B.t.i.* and *B. sphaericus* for mosquito control into practice in Thailand, there is a need to develop new methodologies and an appropriate technology for mosquito control programmes as soon as possible. Thus, field trials are carried out against target species of mosquitoes in different environmental conditions in order to control these two bacterial agents.

Field evaluation and efficacy of *B. sphaericus*

Bacillus sphaericus was isolated and its larvicidal activity was elucidated. Recently, two potent strains (1593 and 2362) of *B. sphaericus* have been evaluated against mosquitoes under laboratory conditions with satisfactory results (Davidson et al., 1975; Singer, 1973; Pantuwatana and Youngvanitsed, 1984). In most of these studies, it was determined that *Aedes* species were less suscpetible or unaffected by *B. sphaericus*. It was also documented that younger instars were more susceptible to a given preparation or formulation than the older instars and susceptibility decreased with increasing larval age.

Detailed studies on field efficacy of *B. sphaericus* formulations reviewed here are obtained from field trials conducted in Thailand consisting of simulated field tests and small- and large-scale field evaluations.

Trials with *B. sphaericus* on *Cx. quinquefasciatus* larvae breeding in polluted and sewage water produced good reduction in larval populations. The treated sites varied from small pools to 1,600 m^2. In one trial, the bacterial preparation (cell count, 3.53×10^{10} viable spores/ml) was evaluated in 1 m^2 ponds containing polluted sewage water against *Cx. quinquefasciatus* in Bangkok. One hundred second, third and fourth-instar *Cx. quinquefasciatus* larvae from a colony or field-collected were placed in sentinel cages made from plastic boxes 7 cm × 7.5 cm × 10 cm in size, and covered with nylon screen on all sides. The mortality of the sentinal larvae was recorded 48 hrs after being placed in the test pond. The dosage applied ranged from 3.0×10^5 to 1.8×10^8 cells/ml. Results are summarized in Figure 1. *B. sphaericus* gave excellent larvicidal activity against sentinel larvae for 60 days and mortality higher than 50% lasted up to 190 days in the first pool. In the second pool, *B. sphaericus* also gave the excellent activity against sentinel larvae for 53 days. The larvicidal activity fluctuated above and below 50% mortality from 60 days onward. The populations of bacteria fluctuated in both pools. The COD values for the water taken from the test pools also fluctuated but were in the range of non-polluted water. By the sentinel technique, the concentrations used produced 80–100% mortality at 48 hr post-treatment intervals for at least 190 days.

A concentrated liquid formulation of *B. sphaericus* 1593 consisting of 1.0×10^{10} to 3.0×10^{10} viable spores/ml was applied at 16 liters/1,600 m^2 to polluted sewage ponds supporting population of *Cx. quinquefasciatus* in Bangkok during September and November 1985. The bacterial agent yielded 80–100% control in all 4 trials, with control persisting up to 10 days post-treatment. Results are summarized in Figure 2. It was interesting to note that *B.t.i.* did not reduce *Cx. quinquefasciatus* larvae in the test pond as opposed to *B. sphaericus*.

In another study, a concentrated liquid formulation of *B. sphaericus* 1593 consisting of 2.4×10^7 to 5.7×10^8 viable cells/ml was applied at 15 liters/2 hrs and 9 liters/30 min to clear-water slow-running stream in Nakorn Ratchasima Province, Thailand. This stream was supporting populations of *An. minimus* and *An. maculatus* (99% was *An. minimus*). This small stream was about

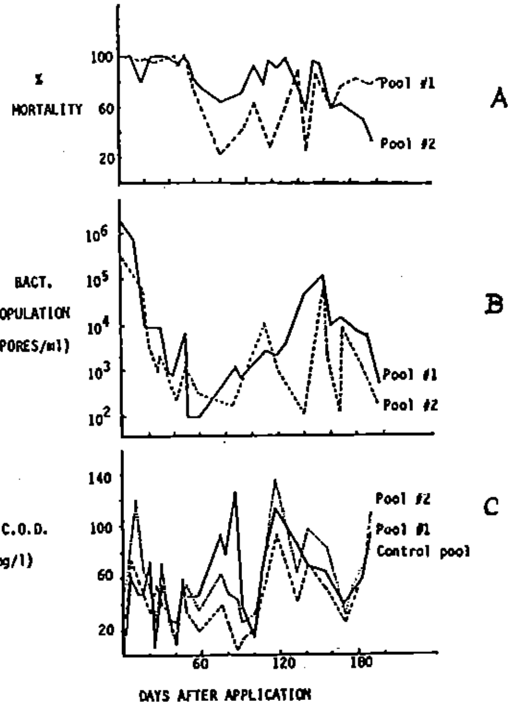

Fig. 1. The relationship among the larvicidal activity of inoculated water against sentinel *Cx. quinquefasciatus* larvae in the test pools (A), the bacterial population of *B. sphaericus* 1593 in the test water taken from the pools (B) and the COD values of the test water taken from the test pools after the application of *B. sphaericus* 1593 (C) in the pools during April–October, 1985 in Bangkok.

9 km long starting from the foot hill. During the peak season of *An. minimus* the water level was very low and the average discharge of water was 1,228.5 cm^3/min in the area of slow current and was 0.61 m^3/min measured at the fastest running section of the stream. The width of the stream varied from 30 cm to 2 meters. The average temperature of the water during the course of the study was 22 ± 2°C and pH of water was 7.5. The average temperature during the course of the study was 24.8 °C. The upper section of stream was used as untreated control area, about 3 km in length from the foot hill. The *B. sphaericus* 1593 was applied twice, at one week intervals, at a site in the middle of a village. The first application was made from the container into the stream at 15 liters/2 hrs; the second application was made by spraying the margin of the stream for 450 meters. Twenty-four hours prior to the application of *B. sphaericus*, larval population of *An. minimus* was estimated by taking 25 dips with a 350 ml plastic dipper from each site along the stream. Individual sites for population estimates were marked with color paint 50 meters apart. In the untreated control area above the releasing point (opposite to the direction of water current), 21 sites were marked and in the treated area, 21 sites were marked in the first 1,000 meters and 20 sites were marked in the second 1,000 meters starting from the releasing point. At each site, 25 dips were randomly made from the edge of the stream on both sides 24 and 48 hrs after application, then at 1-week intervals for the first month and then 2-week intervals thereafter for 2 months. In addition to dipping method, two sentinel cages containing twenty 3rd and 4th instar *An. minimus* larvae were placed in each of 32 marked

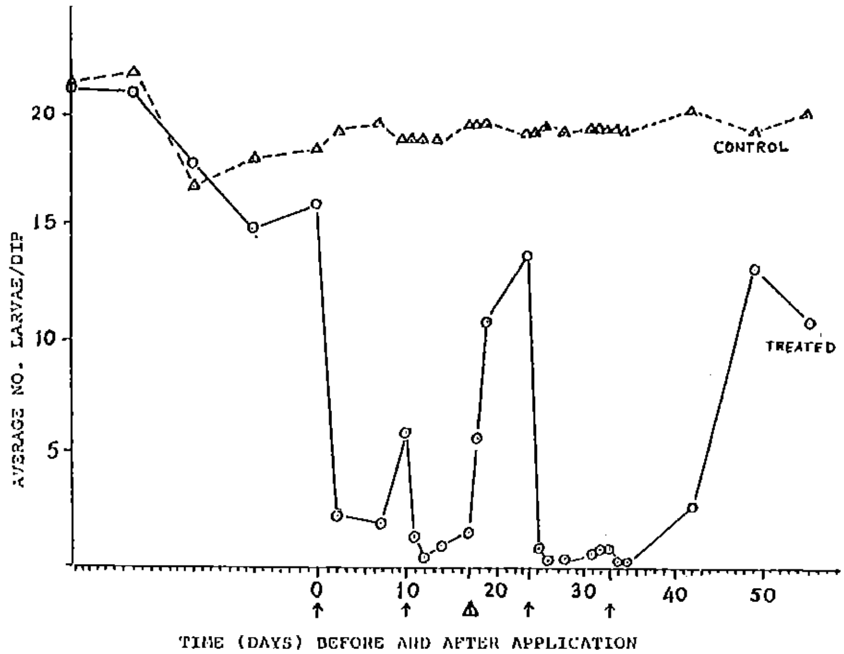

Fig. 2. Average number of *Cx. quinquefasciatus* larvae in the treated water in comparison with average number of *Cx. quinquefasciatus* larvae in the untreated control pond before and after the application of *B. sphaericus* and *B. thuringiensis* H-14 during August–November 1985, in Bangkok. ↑ = the day in which *B. sphaericus* was applied. ▲ = the day in which *B.t.i.* was applied. Average number of mosquito larvae was expressed as number of larvae per dip.

sites in the treated area and 2 sites in the untreated control area. The mortality of sentinel larvae was recorded 48 hrs after exposure to the test stream. The application yielded 60–100% control of treated larvae with the level of control persisting for up to 9 weeks post-treatment. *B. sphaericus* gave excellent larvicidal activity against sentinel *An. minimus* larvae for at least 10 weeks. Results are summarized in Figures 3 and 4.

Longevity of *B. sphaericus* was studied in different biotypes to which the pathogen was applied, and the bacterial agent was found to survive in the polluted water of the treated habitats for at least 6 months after treatment. In tap water and clear water, the organisms also survived for at least 9 and 3 months after treatment, respectively (Silapanuntakul et al., 1983).

Field trials with *Bacillus thuringiensis* subsp. *israelensis*

This microbial control agent was first isolated by Goldberg and Margalit (1977). After the isolation, this strain was used in numerous laboratory studies demonstrating its activity against mosquitoes and black fly larvae. In general, *Aedes* species were most susceptible, followed by *Culex* and *Anopheles*, in that order. It was also documented that younger instars were most susceptible to a given preparationf than the older instars, similar to results found with *B. sphaericus*. In every case, susceptibility decreased with increasing instars.

In Thailand, it was similarly demonstrated that *Ae. aegypti* larvae were the most susceptible to *B.t.i.*. Detailed studies on field efficacy of *B.t.i.* formulations were limited, since *Ae. aegypti* larvae were found mainly in jars or artificial water containers indoors. Field trials consisted of simulated

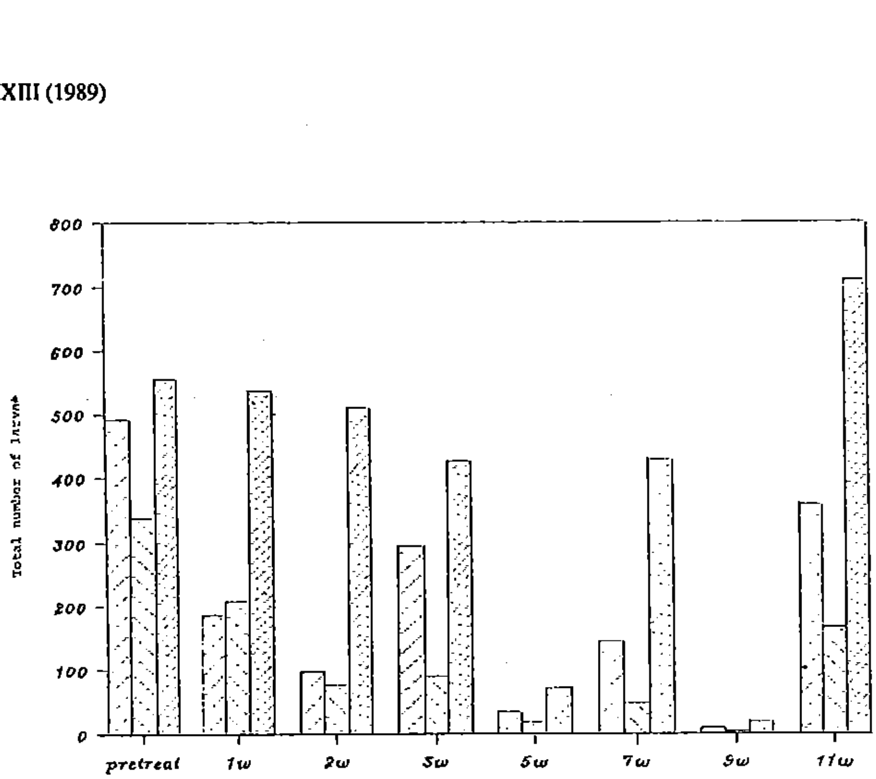

Fig. 3. Total number of *An. minimus* larvae detected by dipping method in treated area and untreated control area after application of *B. sphaericus* 1593.

= total number in control area/525 dips. = total number in 1–1,000 meters below the releasing point/525 dips. = total number in 1,001–2,000 meters below the releasing point/500 dips.

field tests and indoor conditions in jars using field water and *Ae. aegypti* larvae from colonies or field-collected specimens.

Preliminary small-scale field trials were conducted in Ban Plu village, Song Khla Province, using water jars containing 50 liters of underground water. Three breeding sites were selected and 7 concentrations were used in each set with one additional jar serving as a control. The tests were run in duplicate. *B.t.i.* powder in the amount required to make a given final concentration (400, 100, 10, 4, 1, 0.4, and 0.1 mg/liter) was added to water and mixed vigorously. Fifty 4th instar laboratory reared *Ae. aegypti* larvae were added to each jar every other week. The mortality of larvae was observed at 48 hours. It was shown that *B.t.i.* gave good activity against *Ae. aegypti* larvae for about 41 days at the concentration of 0.4 mg/liter (initial concentration) as shown in Table 1 (Pantuwatana et al., 1985). In another simulated indoor condition in Bangkok, 100% reduction was obtained in *Ae. aegypti* and *Cx. quinquefasciatus* at the rate of 1.33 × 10^4 spores/ml made from Abbott wettable powder formulation. The level of control persisting up to 100 and 70 days for *Aedes aegypti* and *Culex quinquefasciatus*, respectively (Silapanuntakul et al., 1983). Poor control of *Cx. quinquefasciatus* was demonstrated in polluted waste water in Bangkok at the rate of 16 liters/1,600 m^2 of the concentrated liquid formulation made locally (2 × 10^{10} viable spores/ml). Field studies reported by others clearly demonstrated that currently available formulations of *B.t.i.* produce an immediate high level of control of larvae, with the extent of control declining rapidly after 7 days. Thus, repeated applications (weekly treatment) are needed in situations where continuous breeding and recruitment are taking place.

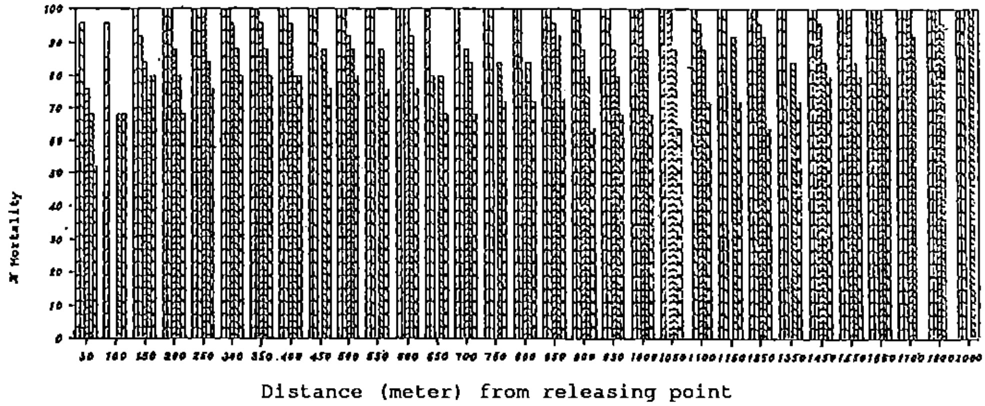

Fig. 4. Percent mortality of sentinel larvae, *A. minimus*, in the floating cage at individual site that tested from 3rd week to 10th week after application of *B. sphaericus* 1593.

= 3rd week
= 4th week
= 6th week
= 8th week
= 10th week

TABLE 1

The persistence of toxicity of *Bacillus thuringiensis* H-14 against 4th instar larvae of *Aedes aegypti* in a small scale field trial at Ban Plu, Hat Yai District, Song Khla Province during June–October 1982

	Per cent mortality					
	Concentration (mg/liter)					
Days after application	0.1	0.4	1.0	4.0	10.0	Control
0	10	96	100	100	100	0
18	0 T	70	80	80	90	0
23	0	60	76	80	72	0
33	0	64	78	76 C	80	0
41	0 T	50	62	74	84	0
48	0 T	40	36	68 T	84	0
55	0	20 CTA	32	34	40 T	0

T = *Toxorhynchites* larvae were found in the container.
C = *Culex* larvae were found in the containter.
A = *Aedes* larvae were found in the container.

Based on field studies reported elsewhere and results obtained in Thailand, it is apparent that *B. sphaericus* and *B.t.i.* have good potential as effective weapons to control mosquitoes in the future. However, further studies under field conditions are needed.

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