Efficacy of New Formulations of the Microbial Larvicide
*Bacillus sphaericus* against Polluted Water Mosquitoes in Thailand

Mir S. Mulla\(^1\), Tianyun Su\(^1\), Usavadee Thavara\(^2\), Apiwat Tawatsin\(^2\), Wichai Kong-ngamsuk\(^2\) and Prakong Phan-Urai\(^2\)

1 Department of Entomology, University of California, Riverside, USA.
2 Department of Medical Sciences, National Institutes of Health, Ministry of Public Health.


**Abstract**

Two new water dispersible granular (WDG) formulations of the microbial control agent *Bacillus sphaericus* (strain 2362) were extensively evaluated in polluted waters against *Culex quinquefasciatus* in Thailand. The studies were carried out in stagnant as well as flowing waters during August 1997 to July 1998. The trail period covered both rainy and dry seasons. The two WDG formulations of *B. sphaericus* tested were low potency (350 ITU/mg) and high potency (630 ITU/mg) products. Both formulations were used at various rates to determine initial efficacy and longevity. The high potency formulations provided excellent control (80 to 90%) of immature mosquitoes at the rates of 50 to 100 mg/m\(^2\), while the less potent formulation yielded similar control at the rates of 89 to 250 mg/m\(^2\). Longevity of control was anywhere from one week to four weeks or longer depending on the dosage, habitat, and environmental conditions. Two treatments with low dosages of *Bacillus thuringiensis* ssp. *israelensis* WDG provided short-term control lasting for one week. Frequent episodes of heavy rains facilitated longterm suppression of immature mosquitoes in klongs by flushing out the larvae. In the absence of rain, the longevity of treatments in flowing waters was shorter than in the presence of rain. Rain, however, had some but not pronounced effect on longevity in the stagnant water habitats. Operation of floodgates controlling water flow from stagnant water habitats into the Chao Phraya River had greater influence on the abundance of mosquito immatures in the ponded polluted water under dwellings. Precipitation, adding large amounts of water, was probably responsible for diluting the control agents and also resulted in lower counts of immatures per unit volume of water. These variables could influence the efficacy (especially longevity) of treatments employing formulations of
microbial agents in operational control programs. The WDG formulations are preferred over other formulations, such as granules, because the former contain larger quantities of toxins per unit mass than the latter and are easily applied as aqueous sprays. Potent formulations with greater content of active ingredients are less costly to ship and transport to distant areas.

**Keywords**

Mosquitoes, *Bacillus thuringiensis israelensis, Bacillus sphaericus*, control polluted water, formulations

**Introduction**

In recent years, two mosquitocidal microbial control agents, *Bacillus sphaericus* Neide (*B. sphaericus*) and *Bacillus thuringiensis* ssp. *israelensis* (B.t.i.) de Barjac have been developed for the control of mosquitoes and other insects of public health importance (Mulla 1990, 1991). Both agents are spore-forming bacteria, which produce parasporal proteinaceous crystal toxins showing high activity against larvae of certain dipterans. The latter agent (B.t.i.) was developed and labeled for the control of mosquitoes and blackflies around 1980 and has been used since then, while the former (*B. sphaericus*) was registered in the United States in 1991 and found its use in mosquito control programs in 1996. *Bacillus sphaericus* is highly specific and shows maximum activity against *Culex* and a few other groups of mosquitoes (Barbazan et al. 1998, de Barjac and Sutherland 1990, Mulla 1991, Mulla et al. 1988, 1997, Skovmand and Bauduin 1997). Some of the desirable attributes of *B. sphaericus* are its persistence in the habitat after treatment and its recycling potential in the habitat or in larval cadavers (Becker et al. 1995, Corea and Yousten 1995, Lacey 1990, Mulla et al. 1988, Nicolas et al. 1987, Skovmand and Bauduin 1997).

The first formulation of *B. sphaericus* that received EPA registration for mosquito control in the United States was VectoLex CG (Abbott Laboratories N. Chicago, IL), a corn grit formulation with 7.5% active ingredients and a potency of 50 ITU/mg. This formulation is now a commercial product and is the only product currently used in the USA. Since mosquitoes are a diverse group of insects breeding in a variety of developmental sites, the manufacturer (Abbott Laboratories, N. Chicago, IL) embarked upon the development and production of new formulations with high potency for the purpose of developing
and facilitating additional use patterns. There is a great need for formulations that can be applied with water to a variety of habitats where VectoLex CG is not the material of choice. Among these new products, two new water dispersible granular (WDG) formulations of *B. sphaericus* (strain 2362) were produced and made available for laboratory and field evaluation in 1997. Field evaluation of the less potent early WDG formulation was initiated in Thailand in late 1996 and the results reported elsewhere (Mulla et al. 1997), and efficacy data on WDG formulations of both *B. sphaericus* and B.t.i. were generated in mesocosms (Su and Mulla 1999) under controlled conditions.

We here report on extensive field evaluation of the two new WDG formulations of *B. sphaericus* carried out in 1997-1998 against polluted water mosquitoes (*Culex quinquefasciatus* Say) in Thailand. The WDG formulations evaluated had potencies much higher than the currently used VectoLex CG (corn grit) formulation. They were extensively evaluated in stagnant water habitats as well as flowing water canals that received polluted water and supported heavy population of *Cx. quinquefasciatus* larvae.

**Materials and methods**

These studies were carried out in ponded domestic wastewater accumulations and two polluted water klongs (canals) in the Nonthaburi Province (adjacent to Bangkok) in Thailand. The studies were initiated in August 1997 and terminated in July 1998. This period encompassed both rainy (May-October) and dry (November-April) seasons. During the dry season there were episodes of rain, but the frequency and intensity were less than that in the rainy season. Several treatments (with the exception of Klong Keha-Chumchon) were made to each site to determine longevity and optimum dosage of the formulations to control mosquito larvae and pupae. The general methods and procedures employed in the treatments and assessments are those used in our earlier studies in Thailand (Mulla et al. 1997). In brief, these methods are presented below.
Experimental Sites

Soi Jumpa (ponded water).

The community located in the Pak Kret District of the Nonthaburi Province in Thailand has a cluster of low income housing without and adequate wastewater disposal system. The dwellings are all raised on posts, and domestic wastewater accumulates over the entire area under the houses as well as between houses. The water depth ranged from 10-25 cm and water level fluctuated with rains and opening or closing of flood control gates in the klongs which drain into the Chao Phraya River. At the outset, two plots of 300 m² and 600 m² at Soi Jumpa were selected. Each plot was treated several times with different dosages; the dosages are shown in figures for each treatment. In subsequent experiments, most of Soi Jumpa (3,600 m²) water accumulation was used as one plot and treated several times with various dosages of *B. sphaericus* WDG formulation and two treatments of B.t.i. WDG formulation. The latter formulation was used for comparison with the *B. sphaericus* formulation. Concomitantly with this large test, and area (Raevadee) similar to Soi Jumpa was designated as a control, which supported heavy production of mosquitoes as did the Soi Jumpa habitat.

Klongs (canals).

Two klongs were subjected to treatments of the WDG formulations. A portion (200 m long, 4 m wide, 800 m²) of Klong Keha Chumchon located in the Pak Kret District of Nonthaburi, was treated only once on August 22, 1997 with the older WDG formulation (350 ITU). Due to frequent flushing caused by rains, clean out, and dredging three weeks after treatment, mosquito density did not increase enough to require another treatment until June 1998. Water in this portion of the klong was 10-50 cm deep, and water flow was sluggish (2m/min.) in the dry season. Water velocity increased during the rainfalls. Most of the larval aggregations were noted along the margins in vegetation or in accumulations of solid wastes in the water; and, therefore, only the margins were sampled for larvae and samples (frequency of samples shown in figures) were taken only in spots where mosquito larvae were abundant.

The second klong (Bangkraso) is located in the Muang District of Nonthaburi Province. A small, accessible, L-shaped section of this klong was selected where heavy populations of mosquitoes occurred. The treated section
measured 170 m long by 4.7 m wide. Water depth was 10-50 cm and water flow was 6 m/min. in the dry season. Mosquito larvae prevailed along the margins in vegetation or in accumulations of solid wastes and the samples (for frequency and number see sampling below) were taken in these areas.

This klong was treated four times, the first treatment on September 4, 1997 and the last treatment on March 4, 1998. Although this klong was flushed out several times during the rainy season, it was not dredged or cleaned out during the course of this study, and mosquito larval resurgence was noted after the effectiveness of the treatment had ceased.

Formulations

Two water dispersible granular formulations (WDG) of *B. sphaericus* and one WDG formulation of B.t.i. were field tested. These formulations were produced by Abbott Laboratories, N. Chicago, Illinois. The first WDG formulation of *B. sphaericus* (ABG 6491, lot no. 30-073-BR, 350 ITU/mg) became available in mid-1997, while the second WDG formulation (ABG-6491, lot no. 32-094BR, 630 ITU/mg) was received in October 1997. A third formulation of B.t.i. WDG (lot no. 31-078-BR, 4000 ITU/mg) was also tested at very low dosages to see if it will provide satisfactory (short-term) control of polluted water mosquitoes. All three formulations readily dispersed in water on shaking in the spray tank, no further mechanical agitation was needed during spraying. The suspension was easily applied through a cone nozzle that shot the spray stream to a distance of 4-5 m onto the target area. Areas farther away could not be reached by the spary streams.

Applications

The required amount of the WDG formulations was placed in an 8-liter compression spray tank and the required amount of tap water added. During addition of water, the mixture was stirred with a stick and then shaken in the tank. The tank was then sealed and pumped to pressurize the spray mixture. The WDG suspension was sprayed through a cone nozzle that facilitated the spray stream to travel and hit target areas up to 4 to 5 m away.

The plots in Klong Keha Chumchon (200 m x 4 m) and in Klong Bangkraso (170 m x 4.7 m) were also treated with 7 liters of suspension, spraying both sides and the middle of the klongs. The small plot (300 m²) in
Soi Jumpa was sprayed with 4 liters of the suspension, while the larger plot (600 m²) was sprayed with 7 liters of the spray. The whole stagnant water area (3600 m²) in Soi Jumpa was treated with three 7-liter tanks (21 liters) of the spray. In treating Soi Jumpa from walkways and catwalks, the spray stream could only reach 4-5 m away, and in some cases all the water underneath the houses could not be reached by the spray. The dosages, however, were based on the total area of the habitat and are noted in the figures.

**Sampling**

All sampling for larvae and pupae was done by dipping with a standard 400 ml dipper. Samples were taken in a biased manner, taking samples only in those spots where heavy aggregations of mosquito larvae occurred. The contents of each dipper were transferred into white plastic trays (30 x 15 x 4 cm deep), and the immatures were counted and categorized into 1st - 2nd instars, 3rd - 4th instars, and pupae. At Soi Jumpa, 10 dip samples were taken in each of the small plots, but 20 dips were taken when the total breeding habitat was treated. Similarly, 20 dip samples were taken each time in the Raevadee control plot. The two klongs were sampled by taking 20 samples at each interval as shown in the figures.

**Efficacy Assessment**

In order to determine the magnitude of reduction in immatures, the densities of posttreatment intervals were compared with those of the pretreatment. It was not possible to establish untreated plots in the klongs, as most of the klongs upstream or downstream from the treated area were not readily accessible. Similarly, it was not possible to establish untreated plots in the Soi Jumpa area in the small tests, as comparable mosquito breeding areas were not available in the vicinity. However, when all the Soi Jumpa mosquito developmental sites were treated, we selected a comparable area (Raevadee) as a control. The two areas were noted to be similar in their potential of producing mosquitoes. Retreatments were administered when immature mosquitoes (larvae and pupae) resurged, reaching approximately the 40-50% mark or higher of the pretreatment populations.

Standard error (SE) values of the mean for each sampling date were determined by using Stat View SE+Graphics (Abacus Concepts, Inc., Berkeley, CA), and these values are inserted in the figures.
Results and Discussion

Klong Keha Chumchon

Klong Keha Chumchon received a single treatment of the WDG formulation of *B. sphaericus*. (350 ITU/mg) at the rate of 250/m² on August 21, 1997. This single treatment yielded excellent control of all immatures especially the pupae (*B. sphaecicus* does not kill pupae, pupal control is a result of the larval control) for four days with moderate resurgence of 3rd and 4th instars, 7 and 12 days posttreatment (Figure 1) and declining 14 days posttreatment. This slight to moderate resurgence is characteristic of *B. sphaericus* treatments, where the populations rise slightly and then decline subsequently. This secondary decline is probably due to the recycling of *B. sphaericus* in larval cadavers (Becker et al. 1995, Corea and Houston 1995, Skovmand and Bauduin 1997). A drastic reduction was noted on days 14 and 21 posttreatment before the dredging and clean-out operation. On day 21, the klong was cleaned out and dredged and experienced frequent flooding due to rains up to 53 days posttreatment. Thereafter the immature populations, especially the pupae, remained zero to very low with the exception of a slight resurgence on day 97 posttreatment. It seems that a treatment with *B. sphaericus* WDG formulation combined with clean-out and/or flooding action can provide long-lasting control of *Cx. quinquefasciatus* in polluted klongs. The immatures (3rd and 4th instars and pupae) did not reach the pretreatment level over the entire period of 10 months or so (not shown in Figure 1). From this experiment it seems that the WDG formulation of *B. sphaericus* at 250 mg/m² provided almost complete control of pupae for 21 days or so, and thereafter immature poputations remained very low or nil due to heavy rains or combination of rains and the treatment. After cessation of rain (53 days posttreatment), the poputation of larvae increased moderately on days 84 and 97 and then declined naturally thereafter.

Klong Bangkraso

This klong, with higher water velocity than the previous one, supported high populations of *Cx. quinquefasciatus* along the margins. It was treated twice with the less potent WDG formulation (350 ITU/mg). The first treatment was made on September 4, 1997 at the rate of 100 mg/m², while the second treatment at the same rate was made on November 19, 1997. The first
treatment appears to have yielded excellent suppression of all immature stages
up to 43 days or longer posttreatment (Figure 2), a period which also experienced
frequent rains. On day 86 (dry season) after the first treatment, there was a
moderate resurgence of immatures, when the klong was retreated with 100
mg/m² of the *B. sphaericus* WDG formulation (350 ITU/mg). This treatment
provided good control of larvae for about 28 to 35 days, larval populations
increasing on day 40 and beyond this period, reaching extremely high numbers
on February 19, 1998 (82 days posttreatment), when it was retreated with the
more potent WDG formulation (see Figure 3).

![Figure 1](image)

**Figure 1. Evaluation of Bacillus sphaericus WDG formulation (350 ITU/mg) against Culex quinquefasciatus in Klong Keha Chumchon, Pak Kret District, Nonthaburi Province, Thailand (Starting August 21, 1997).**

It seems that precipitation influenced the efficacy and longevity of the
first treatment with the low potency WDG formulation. Eight days after the
first treatment (September 13, 1997), heavy rains came practically every two
to three days up to 43 days posttreatment. As can be seen from the chart (Fig.
2), immature populations remained very low during the heavy rainy period,
with moderate resurgence occurring 86 days posttreatment, during a period
when no heavy rains were encountered. This dry period continued up to
March 10, 1998 (82 days post second treatment) when the immatures recovered markedly. The results of the second treatment are in contrast to the first treatment suggesting that heavy rains and flooding flushed out immatures of mosquitoes, resulting in apparent long-term suppression of immatures during the first treatment period. This trend was similar to that observed in Klong Keha Chumchon.

After recovery of the immatures on day 82 post second treatment, Klong Bangkraso was administered a third treatment on March 10, 1998 (dry season), using the more potent WDG formulation (630 ITU/mg) at the rate of 106 mg/m². This treatment yielded excellent control of immatures for up to 26 days posttreatment (Figure 3) despite the fact that there was no significant precipitation during this period. It is likely that the immature suppression was solely due to B. sphaericus treatment. On day 33 (posttreatment), the immature population increased somewhat (although not to the pretreatment level) and the klong was administered a fourth treatment at the rate of 125 mg/m² of the more potent WDG formulation (630 ITU/mg). This fourth treatment, as the third treatment, yielded excellent control of immatures for up to 28 days or

**Figure 2. Evaluation of Bacillus sphaericus WDG formulation (350 ITU/mg) against Culex quinquefasciatus in Klong Bangkraso, Muang District, Nonthaburi Province, Thailand (starting September 4, 1997).**
longer posttreatment. During these 28 days of the dry season, no heavy rains were encountered, but the rainy season began soon thereafter. During this ensuing rainy period, no resurgence of immatures was noted up to 56 days post this treatment, at which time the fifth treatment at the low dosage of 50 mg/m² of the *B. sphaericus* WDG (630 ITU/mg) was made. This low dosage treatment yielded good control for seven days posttreatment (Figure 3). The decline in immatures continued up to 14 days posttreatment when the experiment was terminated.

From the previous five treatments (Figure 2, 3) in Klong Bangkraso, it is apparent that under dry season conditions the more potent WDG formulation provided longer-lasting control of immatures than the less potent WDG formulation at approximately similar dosages. A dosage of 100 mg/m² of this potent formulation seems to be adequate to provide larval and pupal reduction for three to four weeks or longer.

**Soi Jumpa (Plots 1 and 2)**

An area of shallow, stagnant wastewater totaling 3,600 m² was selected in this community. In the first series of tests, a subarea was divided into plot 1 (300 m²) and 2 (600 m²). They were treated on August 21, 1997 at the rate of 250 mg/m² and 100 mg/m² of the less potent WDG formulation (350 ITU/mg), respectively. In plot 1, a high level of control was achieved up to 14 days posttreatment (Figure 4). A slight resurgence was noted 21 days posttreatment; but the immature populations declined on the 25th day posttreatment, increasing again 34 days posttreatment, when the plot was retreated at the very low rate of 10 mg/m² of the WDG (350 ITU/mg). This low rate of application as expected yielded excellent suppression for two days only, the population rebounding eight days posttreatment (Figure 4). The population reached a high level of density 50 and 54 days post second treatment when this plot and plot 2 were made a part of a large plot, which was treated with the same WDG formulation on November 18, 1997 (see Figure 6). The purpose of this test was to treat the whole mosquito infested water and to include another similar area as a control for comparison.

The second plot in Soi Jumpa (supporting extremely high densities of immatures than plot 1) treated on the same date as plot 1 with the *B. sphaericus* WDG formulation (350 ITU/mg) at the rate of 100 mg/m², experienced almost
complete control for 12 days posttreatment and better than 84% control (based on 3rd and 4th instars and pupae) for 14 days posttreatment (Figure 5). The level of control achieved was about 45% or better up to 34 days posttreatment. Immature populations prevailed at low levels, but the plot was retreated with a low dosages (25 mg/m²) of the WDG formulation (350 ITU/mg) on September 24, 1997 (34 days post first treatment). This second treatment administered at the low rate of 25 mg/m² yielded excellent control of immatures on day 2 of sampling, populations resurging moderately 8 to 14 days posttreatment and reached maximum numbers 50 and 54 days post second treatment (see Figure 5). This plot was then combined with plot 1 (see above) and additional breeding sites were also added to make a larger plot that was teated on November 18, 1997 (see Figure 6).

During this test period in Soi Jumpa (34 days) there were seven episodes of heavy rains. Immature populations fluctuated and remained low in both plots. The second treatment at the very low rates of 10 and 25 mg/m² to plot 1 and 2, respectively, yielded short-term control, indicating that these rates of this WDG (low petency) formulation are not sufficient to provide long term control, but could suppress populations markedly for a very short period.

**Figure 3. Evaluation of Bacillus sphaericus new WDG formulation (630 ITU/mg) against the polluted water mosquito Culex quinquefasciatus in dry season in Klong Bangkraso, Muang District, Nonthaburi Province, Thailand (starting March 10, 1998).**
Figure 4. Evaluation of *Bacillus sphaericus* WDG formulation (350 ITU/mg) against *Culex quinquefasciatus* in domestic waste water accumulation, Soi Jumpa (Plot 1), Pak Kret District, Nonthaburi Province, Thailand (starting August 21, 1997).

Figure 5. Evaluation of *Bacillus sphaericus* WDG formulation (350 ITU/mg) against *Culex quinquefasciatus* in domestic waste water accumulation, Soi Jumpa (Plot 2), Pak Kret District, Nonthaburi Province, Thailand (starting August 21, 1997).
Soi Jumpa (large plot)

After termination of the experiments in plots 1 and 2 in Soi Jumpa on November 18, 1997, the two plots as well as additional areas of breeding sources were combined into one large plot amounting to 3,600 m². At the same time an equivalent and similar area in the Revadee community close to Soi Jumpa was established as a control and sampled for larvae along with the treated area after treatment (intervals shown in figures). The large plot (Soi Jumpa) was treated with the less potent WDG formulation (350 ITU/mg) of *B. sphaericus* at the rate of 88.9 mg/m². Very high levels of control were achieved up to nine days posttreatment (Figure 6), and the extent of control was still appreciable (based on 3rd and 4th instars and pupae) up to 15 days posttreatment. The immature populations in the control plot in contrast increased tremendously at this time. The immatures in the treatment further increased on day 24, when the plot was treated the second time. The second treatment with the same formulation was administered at 100 mg/m² on December 12, 1997. This treatment yielded excellent control (based on 3rd and 4th instars and pupae) for 14 days (Figure 6). Thereafter, the immature populations fluctuated remaining low to moderate for up to 54 days posttreatment. At this time, the plot was retreated for the third time with the same formulation. This third treatment with the less potent WDG formulation was made at 100 mg/m² on February 5, 1998. This treatment yielded good control (80% plus) of 3rd and 4th instars and pupae for up to 30 days. After 30 days the level of control dropped to about 75% and the plot was treated with B.t.i. WDG on March 10, 1998 (30 days post third treatment) (Figure 7). It should be noted that during these treatments (Figure 6) of Soi Jumpa, the population of immatures in the control plot, Raevadee, remained very high. The treated and control plots had similar populations at the time of the first treatment. The controls remained very high (except for one dip) most of the time.

Due to the potential for resistance development to *B. sphaericus* (Rodcharoen and Mulla 1994) it was deemed desirable to determine the initial short-term efficacy of B.t.i. WDG (4000 ITU/mg) by making two treatments. The first treatment of B.t.i. WDG at the very low rate of 28 mg/m² was made on March 10, and the second treatment at the rate of 42 mg/m² was made on April 1, 1998. The first B.t.i. treatment yielded about 89% reduction as measured on day 7 posttreatment, but the immatures resurged reaching high densities 14
and 21 days posttreatment (Figure 7). The second treatment with B.t.i. yielded 76% control up to seven days posttreatment, but immature populations increased reaching close to the pretreatment level and the plot was retreated with the more potent WDG formulation (630 ITU/mg) of *B. sphaericus* at 50 mg/m² on April 17, 1998. This third treatment of the large plot produced excellent control of the immatures up to 20 days or longer posttreatment (Figure 7). It gave 92% control 20 days posttreatment. The rainy season started and there were frequent episodes of heavy rains during the period 14 to 27 days posttreatment.

![Figure 6. Evaluation of *Bacillus sphaericus* WDG formulation (350 ITU/mg) against the polluted water mosquito *Culex quinquefasciatus* in dry season in Raevadee (as control area) and Soi Jumpa (treated total area), Pak Kret District, Nonthaburi Province, Thailand (starting November, 1997).](image)

During the course of these three treatments of the large plot at Soi Jumpa (two treatments with B.t.i. and one treatment with *B. sphaericus*), the population of immatures, with the exception of one dip, remained quite high in the control. Larval cohorts prevailed at more than 80/dip of each cohort during this test.
period. It is thus apparent that B.t.i. and *B. sphaericus* treatments effectively suppressed immatures.

![Graph showing the evaluation of B.t.i. WDG (4,000 ITU /mg) and Bacillus sphaericus (630 ITU/mg) WDG against the polluted water mosquito *Culex quinquefasciatus* in dry season in Raevadee (as control area) and Soi Jumpa (treated total area), Pak Kret District, Nonthaburi Province, Thailand (starting March 10, 1998).]

**Figure 7. Evaluation of B.t.i. WDG (4,000 ITU /mg) and Bacillus sphaericus (630 ITU/mg) WDG against the polluted water mosquito *Culex quinquefasciatus* in dry season in Raevadee (as control area) and Soi Jumpa (treated total area), Pak Kret District, Nonthaburi Province, Thailand (starting March 10, 1998).**

On day 27 (May 14, 1998) post third treatment to July 23, 1998, this large plot was treated three times (May 14, June 4, and July 2, 1998) with the potent *B. sphaericus* WDG formulation (630 ITU/mg), each time at the low rate of 50 mg/m². The data for these low rate treatments are presented in Figure 8. Briefly, during the nine-week period of the three treatments (May 14 to July 23), populations of immature mosquitoes fluctuated at low levels, each treatment suppressing the immature populations for a week or ten days and then resurging moderately. The immature populations in general exceeded the densities observed on the day of the first treatment of this series of tests (May 14) indicating the 50 mg/m² rate was not too effective. During the course of
observation during these and previous tests, we have noted that once a habitat is treated with *B. sphaericus*, mosquito populations in general even after resurgence prevail at a low equilibrium level. The population trends in the control remained stable with the exception of one dip. They prevailed in much higher numbers in the control plot than in the treated plot even though the two areas had similar potential in supporting larvae.

![Graph showing population trends](image)

**Figure 8.** Evaluation of *Bacillus sphaericus* WDG formulation (630 ITU/mg) against the polluted water mosquito *Culex quinquefasciatus* in Raevadee (as control area) and Soi Jumpa (treated total area), Pak Kret District, Nonthaburi Province, Thailand (starting May 14, 1998).

**Summary**

In summary, it can be concluded that *B. sphaericus* (strain 2362) WDG formulations yielded excellent control of immature mosquitoes in stagnant as well as flowing water situations. The range of effective dosages was 50-100 mg/m². The more potent WDG formulation was effective at lower dosages
than the less potent formulation. This relationship was also noted in microcosm studies (Su and Mulla 1999) on these formulations. Due to uncontrollable field conditions (similar to those under operational paradigms) the results as expected were quite variable and typical of what might be expected in operational control programs. Thiery et al. (1997) in outdoor small scale experiments on *B. sphaericus* reported even greater variability among tests in different locations and habitats and different times. Such variability in population trends of mosquitoes is to be expected especially when one subjects large areas to treatments.

In our trials, rains, especially episodes of heavy precipitation, caused flooding and flushing of the treated habitats (to a greater extent in the klongs), washing away immature stages of mosquitoes. Experimental evidence showed that *B. sphaericus* treatments administered during the rainy season could provide long-lasting suppression of immature mosquitoes. In addition to rains, water level changes due to closure or opening of floodgates to let water flow into the Chao Phraya River could also influence abundance of immature mosquitoes. Light rains did not seem to have marked effects on the efficacy of treatments especially in the relatively stagnant water habitats. One other impact of rain could be due to the rise in water level, leading to reduced larval density per unit volume of water resulting in lower density counts. Increase in the volume of water could also cause a dilution of the toxins. These uncontrollable elements have to be dealt with in the administration of treatments in operational control programs. Appropriate surveillance programs have to be employed to determine the need for treatment of a given habitat for mosquito control in operational control programs.

**Acknowledgments**

These studies were conducted in cooperation with the Department of Medical Sciences, National Institutes of Health, Ministry of Public Health, Nonthaburi, Thailand. We recognize and appreciate the encouragement and assistance of Dr. Pijit Warachit, Director of NIH and other staff who assisted in carrying out treatments and timely samplings. We are indebted to Abbott Laboratories, N. Chicago, IL, USA, for providing experimental formulations for these trials.
References


