

Non-larvicidal effects of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* on oviposition and adult mortality of *Culex quinquefasciatus* Say (Diptera: Culicidae)

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ABSTRACT: Two microbial mosquito larvicides, *Bacillus thuringiensis* ssp. *israelensis* (Bti) and *Bacillus sphaericus* (Bsph), have been shown to be highly effective in controlling mosquito larvae and have been used in larvicidal programs for many years. In exploring other modes of action of these agents, we studied the ovipositional response of Bsph susceptible and resistant *Culex quinquefasciatus* to aqueous suspensions of Bti and Bsph water dispersible granules (WDG). We quantified the level of mortality of adult mosquitoes caused by exposure to Bti and Bsph suspensions during oviposition. Significantly lower numbers of egg rafts were laid and collected from the treatments than the control regimen. There was an inverse relationship between *Bacillus* product concentrations and oviposition. As the concentration of Bti or Bsph increased from 0.0 to 2.0 mg/L, treated waters received progressively fewer egg rafts. In addition to the negative effects of *Bacillus* on oviposition, both male and female adult mosquitoes suffered high mortality on landing and imbibing on Bti and Bsph suspensions, the extent of mortality directly proportional to concentration. These two microbial agents used solely as mosquito larvicides thus have the additional benefits of reducing mosquito oviposition and killing adult mosquitoes, especially gravid females that come in contact with the treated water either for oviposition or drinking. Reducing the number of gravid females may also result in reduced transmission rates of pathogens. The combined effects of reduced oviposition and adult mortality could result in higher control potential of these microbial agents. *Journal of Vector Ecology* 30 (1): 155-162. 2005.

Keyword Index: Oviposition, Bti, Bsph, adulticidal activity, *Culex quinquefasciatus*.

INTRODUCTION

Since the discovery of the mosquitocidal properties of *Bacillus thuringiensis* ssp. *israelensis* de Barjac (Bti) and *Bacillus sphaericus* Neide (Bsph), various commercial formulations of these agents have been evaluated and developed against mosquito larvae. Bti is an aerobic, gram positive, spore-forming bacterium found commonly in the environment. It produces crystalline protein toxins during sporulation, constituting the principal bioactive ingredients in Bti formulations (Gill et al. 1992). Upon ingestion by a susceptible species, the proteins in the crystals are solubilized in the midgut by a combination of alkaline pH and proteolysis (Aronson et al. 1986). The crystalline endotoxin particles of Bti contain lethal stomach toxins that are toxic to larvae of *Aedes*, *Anopheles*, *Culex*, *Psorophora*, and other mosquitoes (Mulla 1985, Lacey and Undeen 1986). This bacterium has been used operationally for the control of mosquitoes and blackflies since 1980 in the United States.

Bsph is also an aerobic, rod-shaped, spore-forming bacterium. This bacterium is found commonly in soil and other substrates. Over 300 strains, most of them non-entomopathogenic, have been isolated from different parts of the world. Several strains, however, show mosquitocidal properties (Davidson 1984, Singer 1985). During sporulation, Bsph produces a parasporal inclusion body that contains two mosquitocidal proteins that act as a binary toxin. Both are required for toxic activity upon ingestion by susceptible mosquito larvae (Porter et al. 1993). Bsph has found

widespread use in the control of mosquito larvae breeding in polluted and clear water sources. Bsph was selected for development, formulated, and introduced into mosquito control programs in the early 1990's in Europe, Asia, Africa, and South America (Sinègre et al. 1994, Mulla et al. 2003, Hougard et al. 1993, Silva-Filha et al. 1995), and in 1997 in the United States.

These two microbial agents have been used exclusively as larvicides. There are few studies on the effect of *Bacillus thuringiensis* ssp. *israelensis* on adult mortality in a variety of insect species, such as *Aedes aegypti* (Klowden et al. 1983), *Simulium vittatum* (Klowden et al. 1985), and house flies, stable flies, green lacewings (Wilton and Klowden 1985). No research data on the effects of this microbial agent on oviposition and adult mortality in *Culex* mosquitoes have been reported. In preliminary studies on the effects of these two microbial agents on oviposition activity of *Culex quinquefasciatus* Say, we noted that gravid mosquitoes laid fewer egg rafts in Bti and Bsph aqueous suspensions than on distilled water. We also noted that during Bti and Bsph oviposition tests against Bsph- susceptible and resistant *Cx. quinquefasciatus*, both male and female mosquitoes suffered mortality in the waters treated with Bti or Bsph suspensions. These results will likely have practical implications for mosquito control. It therefore became desirable to investigate the impact of Bti and Bsph on mosquito oviposition and mortality of adults in Bsph susceptible and resistant mosquitoes. In earlier studies it was noted that there was no cross-resistance in Bsph-resistant mosquitoes to Bti and it

was further noted that Bsph resistant mosquitoes were more susceptible to Bti than were Bsph susceptible mosquitoes (Rodcharoen and Mulla 1996, Zahiri et al. 2002). Since there is no cross-resistance between Bti and Bsph in larvae, it is important to determine oviposition and adult mortality profiles in Bsph susceptible and resistant *Cx. quinquefasciatus* on exposure to these agents.

The current study was initiated to investigate the oviposition responses of Bsph-susceptible and Bsph-resistant *Cx. quinquefasciatus* strains to Bti and Bsph. In addition, the adult mortality caused by exposure to these two larvicides during oviposition was quantified in the two strains.

MATERIALS AND METHODS

Mosquito colonies

A colony of *Cx. quinquefasciatus* susceptible to Bsph was established in 2001 at the National Institute Health (NIH) Thailand. Egg rafts of this colony were collected from Sirachai District, Northaburi province, Thailand. A sub-colony of this strain has been maintained in our laboratory for studies on Bsph resistance (Su and Mulla 2004). A highly Bsph-resistant colony of this species from Wat Pikul (Northaburi province) was also brought to our laboratory from NIH, Thailand in 2001 without further selection with Bsph. This sub-colony has also been maintained in our laboratory since then and used in resistance management studies (Su and Mulla 2004).

The susceptibility of the Sirachai strain to Bsph WDG (water dispersible granules) using 4th instar larvae was quite high where the LC₅₀ and LC₉₀ values were 0.004 and 0.013 mg/L respectively. The highly Bsph resistant sub-colony showed LC₅₀ (>2000 mg/L) and LC₉₀ (>8000 mg/L) values respectively. From these two parental colonies, two sub-colonies were established for oviposition tests and study of effects on adult mortality. For culturing these subcolonies, egg rafts (8-10) from each sub-colony were placed in an enamel pan (50 x 25 x 7cm) containing 2 L of tap water and 2 g of rabbit pellets added as larval food. Larvae were reared under a photoperiod of 14:10 (L:D) at 27±2 °C. The larvae were reared to the 4th instar and allowed to pupate. The pupae were transferred into cups with tap water and placed in screen cages (23 x 30 x 23 cm) where the adults emerged. The adults were provided with 10% sucrose solution, and on day 5 after emergence, females were allowed to feed overnight on restrained 2 to 5-d-old chicks (Animal Use Protocol Number A-50205015-2, University of California, Riverside, CA). Adults from these two sub-colonies were used in oviposition and adult mortality studies.

Test materials

Water dispersible granules of Bti (VectoBac WDG-6511 Lot# 84-536-BJ) and Bsph (VectoLex WDG Lot# 82-625-PG) received on August 20, 2002 from Valent BioSciences Corporation, North Chicago, IL were used in oviposition tests and assessment of adulticidal activity.

Oviposition and adult mortality tests

Single and multiple concentrations of Bti and Bsph WDG

suspensions were assessed for their effects on oviposition behavior in screen cages (23 x 23 x 32cm) at 27 ± 2 °C and a photoperiod of 14:10 (L:D) h. Fifty males and 50 females from both susceptible and resistant strains to Bsph were separated in pupal stage (by size of the pupae) and introduced into each cage separately, and the pupae were allowed to emerge into adults. In oviposition tests, females were blood fed on 2 to 5-d-old chicks 5 d after emergence. Oviposition tests were carried out in the cages four d after blood feeding, and oviposition tests were set up in the late afternoon and terminated the next morning (duration of oviposition was 16 h) when the egg rafts were counted. Before the start of the oviposition tests, sugar solutions were removed and the adults were sugar starved during the exposure period for 16 h, and this group was designated as 0 h starvation. In some tests, sugar was removed 8 h before the start of the tests and in this case adults that were starved for a total of 24 h were designated as 8 h starvation.

Several tests were carried out on adult mortality; one test was carried out at the same time as the oviposition test, where females were blood fed and not deprived of sugar during oviposition. The number of dead adults was counted at the same time as the number of egg rafts in the oviposition cups. Another experiment was carried out where adults were blood-fed and starved for 8 h prior to the start of the experiment, and the number of dead adults was counted at the same time as the number of egg rafts. Also, tests for adult mortality were carried out to quantify adult mortality in Bti suspensions without assessing oviposition where females were not blood fed and adults were starved for a total of 16 (0 h starvation group) and 24 (8 h starvation group) h. In these tests only adult mortalities were determined 16 and 40 h post-exposure.

Effects of starvation on oviposition

Two experiments were carried out to study the oviposition activity and also the effect of starvation in Bti suspensions in both strains of mosquitoes. In the first experiment using Bsph susceptible and resistant mosquitoes, two waxed paper cups (120 ml) were placed in each cage (23 x 23 x 32 cm), one cup containing 80 ml of distilled water and treated with 1.0 mg/L Bti and the other cup without Bti serving as a control. The cups were placed in the cage one at each end of the cage, and the paired cups position in the replicates was reversed in each cage to eliminate positional cues. In this experiment, set in the late afternoon, sugar solution was not removed from the cages during the test period. The treatments were replicated three times. The number of egg rafts in each cup was recorded the following morning (16 h).

In the second experiment, to determine the effects of sugar deprivation, two waxed paper cups were placed in the cages, each holding 100 Bsph susceptible strain adults only. One treated cup contained 100 ml of distilled water with 2.0 mg/L Bti and the other cup without Bti served as a control. The treatments were replicated four times. In this experiment, set in late afternoon, sugar solution was removed at the start of the oviposition tests (0 h starvation). The number of egg rafts in each cup was recorded the following morning (16 h).

Effects of starvation/ adult mortality

Three experiments were carried out to study the adult mortality in Bti suspensions in both strains of mosquitoes. In the first experiment, adult mortality was recorded as in the previous tests. Two waxed paper cups, one containing 80 ml of distilled water with 1.0 mg/L Bti, and the other cup without Bti serving as a control, were placed in the cages with blooded gravid mosquitoes. In this experiment, set in the late afternoon, sugar solution was not removed from the cages during the test period. The treatments were replicated three times. The number of egg rafts in each cup and number of dead adults were recorded the following morning (16 h).

In the second experiment of this series, the adults were blood fed and also starved from sugar solution for 8 h prior to the start of the test (8 h starvation) and also deprived of sugar during the exposure period. The treatments were repeated four times. The number of egg rafts and adult mortality in each cage was recorded after 16 h. Adult mortalities and oviposition responses were recorded at the same time.

In the third experiment of this series, we wanted to know if lack of blood feeding (no oviposition) and sugar starvation had any effect on adult mortality. To study adult mortality of non blood-fed mosquitoes in Bti suspensions, two cups were placed in each cage (23 x 23 x 32cm) holding 5-d-old non-blooded females along with males (50 each) from the susceptible BspH strain mosquitoes. Sucrose solution was removed from each cage at 0 and 8 h prior to the start of adult mortality tests. One cup containing 100 ml distilled water with 2 mg/L Bti and the other cup without Bti served as a control. Adult mortality was recorded at 16 and 40 h post-exposure. The treatment was replicated four times.

Concentration effects

To study the oviposition activity and adult mortality in different concentrations of Bti and BspH in both strains of mosquitoes, four waxed paper cups, one containing 80 ml of distilled water as a control (no Bti), and the other three containing 80 ml of Bti suspensions of 0.1, 1.0, and 2.0 mg/L were placed in each cage. Distilled water cups (control) were placed at one corner of the cage, and the other three treated cups were placed in the other three corners of the cages at random. Sugar solution was removed before the start of the test and the adults were sugar starved for 16 h (0 h starvation) during the test. Each treatment was replicated five times. The number of egg rafts in the test cups and distilled water control were recorded the following morning. Adult mortality by counting the number of dead mosquitoes per cup was recorded at the same time as the number of egg rafts.

Various concentrations of BspH were also studied in oviposition tests and assessment of adult mortality in BspH susceptible and resistant *Cx. quinquefasciatus*. This experiment was carried out in the same way as the Bti experiment above using the same concentrations of 0.0, 0.1, 1.0, and 2.0 mg/L of BspH. Both oviposition and adult mortalities were recorded in each cup at the same time.

Effects of larval food enrichment on oviposition

To study the relationship of microbial agents with or

without larval food provision on oviposition, four oviposition cups were placed inside each cage. One cup held only distilled water (100 ml) as a control, while the second cup held distilled water with one drop of 10% larval food (ground rabbit pellets). The third cup contained aqueous Bti suspension at 1.0 mg/L, while the fourth cup had Bti suspension at 1.0 mg/L plus one drop of 10% rabbit pellet food. Each treatment was replicated five times. Sugar solution was removed before the test started (0 h starvation). The number of egg rafts in each cup was recorded the following morning.

Oviposition Activity Index (OVI)

The results of oviposition are expressed as mean number of egg rafts and oviposition activity index calculated according to Kramer and Mulla (1979), where the activity index = $(NT - NS)/(NT + NS)$; NT denoted the number of egg rafts laid in the test cups and NS the number egg rafts laid in distilled water control cups. Index values lie within the range from +1 to -1. Positive values indicate that more egg rafts were deposited in the test cups than in the distilled water controls, and that the test waters were attractive. Conversely, more egg rafts in the controls than in the test cups yielded negative index values, indicating that the test waters were repellent.

Data analysis

The mean number of egg rafts/cup and number of dead adults in the Bti and BspH tests were statistically analyzed using the paired *t*-test in the two-choice test and a one-way analysis of variance (ANOVA) (Scheffe-F-test) in the multiple concentration tests.

RESULTS AND DISCUSSION

Effects of starvation on oviposition (pair tests)

Data from the first experiment in the pair tests assessing the magnitude of oviposition in Bti by BspH-susceptible and resistant *Cx. quinquefasciatus* in suspensions of Bti at 1.0 mg/L as compared to distilled water are summarized in Table 1. Gravid females of BspH-susceptible mosquitoes laid significantly fewer egg rafts in Bti cups than in the control. Gravid females of BspH-resistant mosquitoes also laid significantly fewer numbers of eggs in Bti suspensions than in the distilled water controls. It should be noted that the gravid mosquitoes of both strains preferred to lay more egg rafts in distilled water than in Bti suspension. This suggests that ovipositing *Cx. quinquefasciatus* were repelled by Bti suspension at 1.0 mg/L or that the reduced oviposition was due to the greater die off of adults in Bti suspension before ovipositing. The total number of egg rafts laid by 50 gravid females in both treatment and control cups in this test were relatively low. Pre-oviposition drinking by *Culex* mosquitoes has been reported by Weber and Tipping (1993), a phenomenon that we have also noted in our studies (unpublished report).

Results of the second pair-test assessing the effects of starvation on oviposition are presented in Table 2. BspH-susceptible females laid fewer egg rafts in the Bti suspension cups than in the distilled water controls. The number of egg

Table 1. Ovipositional response and adult mortality of *Cx. quinquefasciatus* BspH-susceptible (S-Thailand) and resistant (R-Thailand) in Bti (VectoBac WDG) suspension at 1.0 mg/L.^a

Mosquito strain	Treatment	Mean no. egg rafts/cup ± SE	Mean no. of dead adults/cup ± SE
S-Th	Control	3.56 ± 0.27	1.89 ± 0.14
	Bti	2.22* ± 0.38	4.56* ± 0.19
R-Th	Control	3.33 ± 0.15	3.01 ± 0.14
	Bti	2.33* ± 0.12	3.67* ± 0.19

*Significantly different from control at 0.05 level. The two strains were analyzed separately.

^aAdults were not deprived of sugar solution during exposure.

rafts laid by BspH-susceptible strain in Bti suspension and distilled water (DW) at 8 h of starvation was not significantly different from each other at the 0.05 level. However, the numbers of egg rafts laid by BspH-susceptible strain on Bti-treated water and control at 0 h of starvation were significantly different from each other (Table 2).

Effects of starvation on adult mortality (pair test)

During the oviposition test, we repeatedly noted that more adults (both susceptible and resistant) died on the surface of the Bti water suspension than in distilled water (Table 1). Both strains of mosquitoes experienced some mortality in the untreated water, which was lower than in the Bti suspension.

To determine the effects of starvation and duration of exposure on adult mortality in the oviposition test for the 0 and 8 h of starvation, we assessed adult mortality 16 h post-exposure. Much higher mortality of non-starved adults occurred in Bti than in the control cups (Table 2). Adult mortality in the 8 h of sugar starvation showed the same trend between the treated cups (4.50) and control (0.25) as in the 0 h starvation. Overall, the total number of dead adults at 8 h of pre-oviposition sugar starvation was lower than 0 hrs of sugar starvation (Table 2). A possible explanation for this difference in oviposition and adult mortality at 16 and 24 h of total starvation could be due to the gravid female needs for sugar during the pre-oviposition period. Although Canyon et al. (1999) indicated that the presence of sugar delayed or inhibited oviposition in *Aedes aegypti* they did not, however, indicate the needs of sugar for the pre-oviposition period in *Culex* mosquitoes. It should be noted that in the total 24 h of starvation, the yield of egg rafts in both control water and treated water were lower than the 16 h starvation during the

exposure period which may confirm our hypothesis that gravid females need sugar during the pre-oviposition period for the induction of oviposition activity.

In the third pair test with starvation, only adult mortality was measured in 5-d-old adults where the females were not blooded and where they were starved 16 and 24 h in total. Adult mortality was assessed 16 and 40 h (spanning two nights) post-exposure. Both males and females suffered higher mortality in Bti suspension than in control. At 16 h of exposure, we noted a greater number of dead adults in the Bti suspension than in distilled water at both 16 and 24 h total sugar starvation (Table 3). It should be noted that, in general, at 40 h post-exposure the number of dead adults increased over the 16 h in Bti suspension with no increase in the control mortality (Table 3). The numbers of dead males counted were greater than the females at 40 h exposure at both durations of starvation but the differences were not significant (Table 3).

Concentration effects

Two multiple concentration tests were run on susceptible and resistant strains, using Bti and BspH suspensions. In the first test of this series, BspH-susceptible strain laid significantly fewer egg rafts in Bti suspensions at three concentrations than in the controls (Table 4). There was an inverse relationship between oviposition activity and concentration. Oviposition activity index (OAI) values for all concentrations were significantly below neutrality (Figure 1). Although water containing Bti suspension of 0.1 and 1.0 mg/L were not significantly different from each other, they were, however, significantly different from the controls. A similar trend was shown in BspH-resistant mosquitoes, where cups containing Bti suspensions of 0.0, 0.1, 1.0, and 2.0 mg/L received a mean

Table 2. Effect of adult starvation on the ovipositional response and adult mortality of BspH-susceptible (S-Thailand) *Cx. quinquefasciatus* in Bti (VectoBac WDG) suspension at 2.0 mg/L.

Starvation ^a	Treatment	Mean no. egg rafts/cup ± SE	Mean no. of dead adults/cup ± SE		
			Male	Female	Total
0 h	DW	12.25 ± 1.19	1.00 ± 0.29	0.25 ± 0.12	1.25 ± 0.38
	Bti	4.00* ± 0.45	13.00* ± 1.13	7.50* ± 0.92	20.50* ± 1.48
8 h	DW	3.75 ± 0.24	0.25 ± 0.12	0.0	0.25 ± 0.12
	Bti	2.75 ± 0.65	2.75* ± 0.43	1.75* ± 0.24	4.50* ± 0.59

^aStarvation period prior to exposure.

*Significant differences between treatment and control at the 0.05 level, analyzed separately for no starvation (0 h) and starvation (8 h).

Table 3. Effects of adult starvation and duration of exposure on adult mortality of non-blooded Bsph-susceptible (S-Thailand) *Cx. quinquefasciatus* in Bti (VectoBac WDG) suspension at 2.0 mg/L.

Starvation ^a	Treatment	Mean no. of dead adults/cup ± SE			
		24 h		48 h	
		Total	Female	Male	Total
0 h	DW	0.75 ± 0.21	0.50 ± 0.14	0.75 ± 0.12	1.25 ± 0.24
	Bti	7.50* ± 0.84	7.00* ± 0.74	8.25* ± 0.59	15.25* ± 1.26
8 h	DW	1.50 ± 0.22	0.50 ± 0.25	1.50 ± 0.25	2.00 ± 0.28
	Bti	4.50* ± 0.37	5.50* ± 0.62	6.75* ± 0.47	12.25* ± 1.15

^aStarvation period prior to exposure.

*Significant differences between treatment and control at the 0.05 level, analyzed separately for 0 h and 8 h starvation. Male and female numbers in 48 h exposure in Bti and distilled water (DW) at both starvation durations were not significantly different from each other.

Table 4. Ovipositional response and adult mortality of *Cx. quinquefasciatus* susceptible (S-Thailand) and resistant (R-Thailand) to Bsph in Bti (VectoBac WDG) suspensions at various concentrations.

Bti WDG concentration mg/L	S-Th*		R-Th*	
	Mean no. egg rafts/cup ± SE	Mean no. dead adults/cup ± SE	Mean no. egg rafts/cup ± SE	Mean no. dead adults/cup ± SE
0.0	21.00 ^a ± 1.69	3.33 ^a ± 0.35	22.25 ^a ± 2.07	4.00 ^a ± 0.96
0.1	7.25 ^b ± 0.74	5.00 ^b ± 0.91	15.00 ^b ± 0.98	6.25 ^b ± 0.83
1.0	7.00 ^b ± 0.93	11.11 ^c ± 0.54	4.50 ^c ± 0.43	8.75 ^b ± 1.07
2.0	3.50 ^c ± 0.92	15.50 ^d ± 0.92	5.75 ^c ± 0.88	16.26 ^c ± 1.10

*Means followed by the same letter in a column are not significantly different at the 0.05 level.

Table 5. Ovipositional response and adult mortality of *Cx. quinquefasciatus* Bsph-susceptible (S-Thailand) and resistant (R-Thailand) in Bsph (VectoLex WDG) suspensions at various concentrations.

Bsph WDG concentration mg/L	S-Th*		R-Th*	
	Mean no. egg rafts/cup ± SE	Mean no. dead adults/cup ± SE	Mean no. egg rafts/cup ± SE	Mean no. dead adults/cup ± SE
0	21.26 ^a ± 2.20	3.55 ^a ± 0.66	17.50 ^a ± 1.52	2.77 ^a ± 0.43
0.1	10.27 ^b ± 1.56	6.27 ^b ± 0.94	15.75 ^a ± 0.51	4.00 ^a ± 1.01
1.0	9.00 ^b ± 1.06	14.75 ^c ± 1.23	11.00 ^b ± 0.34	3.55 ^a ± 0.59
2.0	4.29 ^c ± 0.66	15.85 ^c ± 2.16	2.75 ^c ± 0.24	7.25 ^b ± 0.66

*Means followed by the same letter in a column are not significantly different at the 0.05 level.

Table 6. Ovipositional response of *Cx. quinquefasciatus* Bsph-susceptible (S-Thailand) and resistant (R-Thailand) in Bti (VectoBac WDG) suspension (1.0 mg/L) and distilled water (DW) with or without enrichment.

Enrichment	Treatment	Mean no. of egg rafts/cup ± SE*	
		S-Th	R-Th
With	DW	15.81 ^a ± 0.63	18.21 ^a ± 0.95
	Bti	5.00 ^c ± 0.58	4.20 ^c ± 0.62
Without	DW	8.22 ^b ± 0.79	6.20 ^b ± 0.79
	Bti	4.63 ^c ± 0.46	4.66 ^c ± 0.58

* Means followed by the same letter in a column are not significantly different at the 0.05 level.

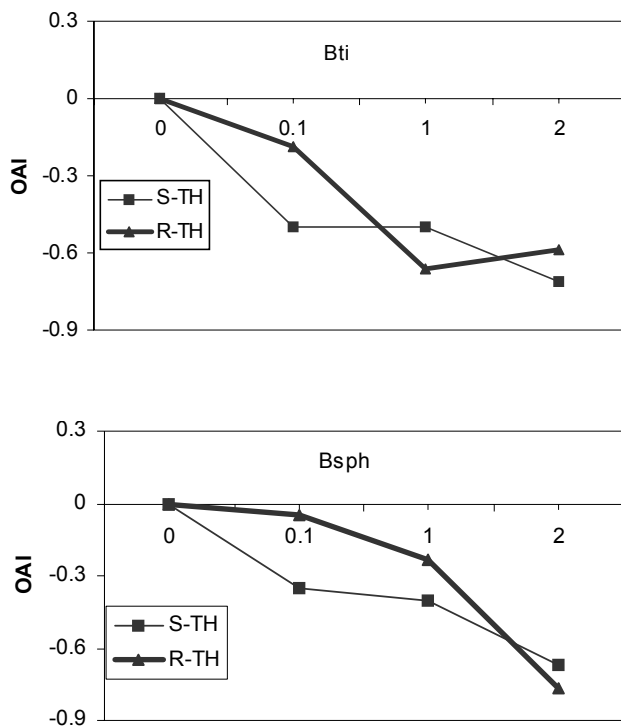


Figure 1. Oviposition activity indices (OAI) of Bsph S-Th and R-Th of *Cx. quinquefasciatus* females on waters with suspensions of Bti and Bsph WDG.

number of 22.25, 15.00, 4.50, and 5.75 egg rafts/cup respectively (Table 4). Among the Bti-treated waters, the oviposition activity index values declined to a level below zero (Figure 1). It is evident that as the concentration of Bti suspensions increased, the number of egg rafts deposited in the cups decreased, and there was a significant difference between treatments and controls.

In the second test of this series using multiple concentrations of Bsph, Bsph-susceptible *Cx. quinquefasciatus* laid significantly fewer egg rafts as was the case with Bti suspensions compared to the controls (Table 5). Oviposition activity index values for treated waters were below zero (Figure 1). The 0.1 and 1.0 mg/L Bsph suspensions were not significantly different from each other, but they were significantly lower than the controls. Gravid females of Bsph-resistant *Cx. quinquefasciatus* laid fewer egg rafts in suspension of 0.1 mg/L Bsph than in the controls (Table 5), as did the Bsph-susceptible. There was no significant difference between 0.1 mg/L Bsph suspension (15.75 per cup) and distilled water control (17.50 per cup). However, the mean number of egg rafts collected from suspensions of 1.0 and 2.0 mg/L Bsph (11.00 and 2.75 per cup, respectively) were significantly lower than in the distilled water control (Table 5). Bsph-resistant mosquitoes laid very few egg rafts in the suspension of 2.0 mg/L Bsph and were significantly lower than in the other suspensions of Bsph (Table 5, Figure 1).

In general, oviposition activity in both strains had an inverse relationship with Bti and Bsph concentrations. As the concentration of both agents increased, the number of egg

rafts deposited in the cups decreased. Su and Mulla (1999) have shown similar effects on oviposition reduction with a wettable powder formulation of Neem (Azad WP) against *Cx. quinquefasciatus*. Also, Beehler and Mulla (1993) observed oviposition repellency by the insect growth regulator, methoprene, against *Cx. quinquefasciatus* in the laboratory. The microbial agents used in these oviposition tests could act as oviposition repellents and/or deterrents to *Cx. quinquefasciatus*, as the designed tests using egg rafts as the end results could not differentiate between repellency and deterency. It is also possible that adult mortality prior to oviposition could result in a lower number of eggs in Bti and Bsph suspensions.

The multiple concentrations of Bti and Bsph induced varying levels of mortality in the adult mosquitoes. In Bsph-susceptible *Cx. quinquefasciatus*, the mean number of dead adults was 3.33, 5.00, 11.11, and 15.50 in Bti concentrations of 0.0, 0.1, 1.0 and 2.0 mg/L respectively (see Table 4). These mean values were significantly different from each other ($P < 0.05$). As the Bti concentration increased from 0.0 to 2.0 mg/L, dead adults retrieved from the suspensions increased progressively. The same held true for Bsph-resistant *Cx. quinquefasciatus*, where Bti suspension of 0.0, 0.1, 1.0, and 2.0 mg/L yielded 4.00, 6.25, 8.75, and 16.26 dead adults/cup respectively. Adult mortality in the control was significantly lower than the Bti suspensions in both strains of mosquitoes.

Results of adult mortality in Bsph-susceptible and resistant in suspensions of Bsph are summarized in Table 5. The mean numbers of Bsph-susceptible dead adults were 3.55, 6.27, 14.75, and 15.85 in Bsph suspensions of 0.0, 0.1, 1.0, and 2.00 mg/L respectively (Table 5). Adult mortalities in the treated water were significantly higher than the distilled water control cups ($P < 0.05$). It is interesting to note that the Bsph-resistant strain of *Cx. quinquefasciatus* suffered low mortality in the two lower concentrations of Bsph, not significantly different from the control. However, the number of dead adults at the highest Bsph concentration (2 mg/L) was still relatively low, but it was significantly higher than the lower two concentrations and the control (Table 5).

Effects of enrichment on oviposition

Previous investigations have shown that using fermented Bermuda grass infusion in water or other organic infusions caused increased oviposition by gravid *Cx. quinquefasciatus* (Millar et al 1992, Kramer and Mulla 1979). We studied the relationship between enrichment and the microbial agent (Bti) affecting oviposition. Results of ovipositing gravid Bsph-susceptible and Bsph-resistant in suspensions of Bti and controls with or without the enrichment are summarized in Table 6. Waters with enrichment but without Bti as expected received more egg rafts (15.81 per cup) than waters without enrichment (8.22 per cup). Suspensions of Bti with or without the enrichment (5.00 and 4.63 per cup, respectively) received essentially the same, but significantly lower, oviposition than the controls. A similar relationship was observed when gravid Bsph-resistant *Cx. quinquefasciatus* were allowed to oviposit in suspensions of Bti with or without the enrichment. Distilled water with enrichment received more egg rafts (18.21 per

cup) than distilled water without enrichment (6.20 per cup) or suspensions of Bti with or without enrichment (4.20 and 4.66 per cup, respectively). Bti suspensions with or without the enrichment reduced oviposition by both strains of mosquitoes.

In conclusion, both larvicidal agents tested for oviposition showed a significant effect on reducing oviposition in two paired tests or in tests with a range of concentrations from 0.1 to 2.0 mg/L. The concentrations used here were not much higher than the normal application rates used in mosquito control programs suggesting that these agents may also have a significant negative impact on oviposition under field conditions. There seems to be a relationship between reduced oviposition and magnitude of adult mortality. Adult mortality could be related to surface tension since both microbial formulations have surface active agents that aid in the mixing and suspension of the active ingredients in water. In such situations, these agents can reduce the surface tension of the water, making it difficult for gravid females or males to rest on the surface in order to oviposit. It was noted that not only gravid females but both males and non-gravid females were drowning in greater numbers in the treated cups than in the controls, indicating that adult mosquitoes land on water for drinking. However, it is important to note that Bsph-resistant adults did not suffer high mortality at the two lower dosages of Bsph suspensions while the susceptible strain experienced high mortality at all dosages. It seems that these microbial agents also have adulticidal activity in addition to their larvicidal effects. The evidence provided in our study documents these effects of the microbial agents.

In summary, Bti and Bsph WDG suspensions induced a lower level of oviposition in gravid females. There was an inverse relationship between the number of egg rafts and concentrations of Bti and Bsph. As the concentrations increased, the number of egg grafts oviposited in the treated water decreased. Adult mortality, on the other hand, indicated a direct relationship with concentrations of both microbial agents. As the concentration of Bti or Bsph increased, the number of dead adults on the surface of the treated water also increased. Weber and Tipping (1993) reported that about 93% of female *Culex* mosquitoes drank from the oviposition site in the field before beginning to oviposit. Most individuals drank more than once while completing the last 2/3 of their egg rafts (Weber et al. 1991). It is not clear whether the reduced oviposition in *Bacillus* suspensions is due to female die-off or to repellency and deterency by the bacteria. This aspect of oviposition needs further investigation.

The usefulness of Bti and Bsph as larvicides has been shown in mosquito control programs for a long time. However, our new discovery of the effects of Bti and Bsph formulations on mosquito oviposition, and their bioactivity against adult mosquitoes, suggest additional advantages when these products are used as larvicides in mosquito control programs. These significant findings open new areas of research as related to the ovipositional and toxic effects of Bti and Bsph against adult mosquitoes. Additional studies are warranted to elucidate the mode of entry and the mechanism of action of these microbial agents in adult mosquitoes.

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