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Field evaluation of *Bacillus thuringiensis* H-14 against *Aedes* mosquitoes

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Abstract. Studies were carried out on the residual efficacy of *Bacillus thuringiensis* H-14 (water dispersible granule, VectoBac® ABG 6511) as direct application in the control of *Aedes* larvae in the field. Field *Aedes* sp populations in the earthen and glass jars were predetermined before initiation of the trial. On confirmation of the presence of *Aedes* species in the designated area, Sungai Nibong Kecil, Penang Island, Malaysia, *Bti* was introduced in the 55L earthen and 3L glass jars). Two test designs were carried out. The first design had treated water replenished daily with 6L of seasoned water and the second design is without the replenishment of water but evaporated water was replenished. *Bti* was effective in the field for at least 35 days with more than 80% reduction in the *Aedes* larvae in the treated containers. For earthen jars with daily replenishment of water, 100% reduction was recorded for the first 3 days, while more than 80% reduction was recorded up to day 40. At day 60, *Bti* still provided an efficacy of 54.32±4.61 (%) of reduction. Whilst for earthen jars without daily replenishment of water, 100% reduction was recorded for the first 5 days, while more than 80% of reduction was recorded up to day 40. For the glass jars studied, similar efficacy was observed. In jars with daily replenishment of water a better larval control was observed. Percentage of reduction from day 50 to 60 for replenishment of water was between 50 to 70% compared to without-replenishment of water with less than 40%.

INTRODUCTION

Dengue hemorrhagic fever/dengue fever (DHF/DF) cases are on the increase. In year 2004, 33203 DHF/DF cases were reported with 67 mortalities in Malaysia. *Aedes aegypti* and *Aedes albopictus* are two major vector mosquitoes of DHF/DF. Although chemical control provided quick knockdown/mortality activity, resistance of mosquito to insecticides have been reported (WHO, 1992). Resistance of *Aedes* species against all major insecticide groups inclusive of organophosphate, pyrethroids, organochlorine and carbamate have been reported (Malcom & Wood, 1982; Georghiou *et al*., 1987; WHO, 1992, 1997; Wu Neng *et al*., 1992; Rodriguez Coto *et al*., 2000). As such, the usage of insecticide as one of the mosquito control tool should be reduced. The use of microbial agent as an additional mosquito control tool, because of effectiveness and non-resistance characteristics has been recommended (Rodcharoen & Mulla, 1996; Glare & O'Callaghan, 1998).

Control of vector mosquitoes using microbial agents such as *Bacillus thuringiensis* H-14 (*Bti*) is a relatively recent development in Malaysia (Foo & Yap, 1982, 1983; Lee, 2000). The effectiveness of *Bti* in the control of mosquitoes has been demonstrated (Van Essen & Hembree, 1980; Foo & Yap, 1982, 1983; Balaraman *et al*., 1983; Klowden & Bullar, 1984; Pantuwatana & Youngvanitsed, 1984; Fry-O'brien & Mulla, 1996). To top it all, *Bti* has been proven to be safe in drinking water (WHO, 1999). *Bti* showed quicker killing activity but short residual activity.

Therefore, further development of such microbial agents is needed as an additional tool for the overall vector mosquito control program. Recently, a new formulation of *Bti*, water dispersible granules (WDG; Vectobac ABG 6511), was evaluated as a larvicide against *Culex* species (Su & Mulla, 1999).

Although the effectiveness of *Bti* against vector mosquitoes has been proven, the actual usage of it is still inadequate. Lack of proper usage strategy and effective application methods are among the reasons why the usage of *Bti* is not encouraging. In Malaysia, *Bti* is still not widely used as part of dengue control program. This may be due to the lack of information on the residual efficacy of *Bti* formulations in the field. Most of the *Bti* formulations provide only short residual efficacy (Lee *et al.*, 2005), however, *Bti* water dispensable granules used in this study have shown longer residual activity in the laboratory. As such, the study on the actual residual efficacy of *Bti* in the field is needed especially in the tropical environment. The objective of this study was to investigate the residual bioefficacy of VectoBac ABG 6511 in the field against larvae of *Aedes* species. The applicability of *Bacillus thuringiensis* H-14 in the dengue endemic areas in order to control outbreaks of dengue fever/dengue haemorrhagic fever was evaluated.

MATERIALS AND METHODS

Field larval collection (Ovitrap)
The trials were conducted indoors in a squatter residential area in Sungai Nibong Kecil, a township in the southwestern coastal area on Penang Island. This field trial was conducted in collaboration with the Ministry of Health and related community councils. A total of 100-150 houses were chosen for mosquito breeding assessment. The ovitrap method (Yap, 1975a,b) was used to determine the presence of dengue vectors (*Ae. aegypti* and *Ae. albopictus*) at the experimental sites.

Larval breeding jars
After confirming the presence of *Aedes* breeding in the study site, residual efficacy study of *Bti* was initiated. A total of more than 120 earthen jars/glass jars were set at the locations which showed highest breeding population during the ovitrap study conducted earlier. The glass jars (3 L) and earthen jars (50 L) were placed under roofs around each of the selected houses. Water was introduced into the earthen and glass jars at 2000 ml and 40 liters, respectively. For the pre-treatment survey of the experimental jars, the number of larvae in the jars was counted five days after introducing water into the jars. The larvae/pupae were collected using a small fish net measuring 15 cm x 15 cm with the mesh size of the net of more than 25 (for every 1cm^2).

Test containers
The earthen jars were filled with 40 L of water and the glass jars with 2 L of water.

Replenishment and non-replenishment of water
Two treatment regimes were adopted: A set of jars, 30 per treatment concentration with 30 controls were not subjected to any replenishment of water but evaporated water was replaced; and in the 2nd set, 30 jars per concentration with 30 controls were given a daily replenishment of 6 L and 0.3 L of water for the earthen and glass jars, respectively, these providing a weekly turnover of the whole volume. The daily replenishment is to simulate daily usage of water.

***Bacillus thuringiensis* H-14 formulation and concentrations**
The *Bti* formulation used in this study was water dispersible granule formulation VectoBac WDG (ABG6511) [Lot No: 72-57B-PG]. VectoBac WDG has 3000 International Toxic Units (ITU) per mg against *Ae. aegypti*.

***Bti* treatment and treatment duration**
After confirmation of the larvae breeding in the test jars, *Bti* water dispersible

granules was introduced into the study jars at 6000 ITU/L. The treatment was carried out by introducing 4 mg and 80 mg of *Bti* into the glass jars and earthen jars, respectively. In the post-treatment assessment, the number of larvae in the jars (treatments and control) was assessed every 5 days for a period of 2-3 months. In the initial post-treatment, larvae collection was carried out at 24 and 72 hours in addition to the 5 day collection.

Laboratory culture of field collected larvae

All of the larvae (L2-L4) and pupae in the jars were collected and brought back to the laboratory for further observation. The larvae/pupae were cultured under laboratory condition (relative humidity at 65±10% and temperature at 26±2ºC) with food provided until emergence into adult. Mosquito species was identified after emergence into adult. The percentage of adult emergence was also recorded.

Data analysis

The percentage reduction of larvae and adult emergence was corrected using Mulla's formula (Mulla *et al.*, 1971):

RESULTS AND DISCUSSION

The larvae and pupae were collected at five day intervals until the end of the trial from all of the jars placed in the selected houses. The collected larvae/pupae were colonised in the laboratory. The emerged adults were identified. Based on the 30 houses chosen for the study (untreated and treated houses included), a total of 3 species of mosquitoes *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* were collected (Table 1). Out of the total mosquito species collected, the ranking of mosquito species collected based on numbers ranged from *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* (Table 1). However, almost 98% of the collected mosquito species were *Ae. albopictus* and *Ae. aegypti*, while *Cx. quinquefasciatus* only showed a small percentage.

For the earthen and glass jars treated with *Bti* at 6000 ITU/L as well as the untreated jars, the summary percentage of reduction based on larvae appearance up to 65 days are as presented in Table 2, whereas the percentage of reduction based on adult emergence up to 65 days are as presented in Table 3.

For the bioefficacy of *Bti* at 6000 ITU/L in earthen jar and glass jar with daily replenishment of water, the effective protection period to prevent the *Aedes* mosquito larvae from breeding was up to 40 days with the percentage of reduction between 80 to 100%. Whilst for earthen jar without daily replenishment of water, the *Bti* efficacy was up to day 40 with the percentage of reduction between 80 to 100%. However, for glass jar without daily replenishment of water, the *Bti* efficacy was up to day 35. Replenishment of water showed significantly better efficacy after day 45 up to day 60 compared to non-replenishment of water (Table 2).

The percentage of reduction for the larvae breeding was still higher than 50% at day 60 compared to without replenishment where the efficacy dropped to lower than 40% after day 50. Better efficacy in water replenished jars is due to re-suspension of *Bti* particles in the water. Thus, the *Bti* particles were readily made available to the larvae in the jars (Mulla *et al.*, 2004).

For the glass jar study, the same trend has been indicated. *Bti* at 6000 ITU/L with replenishment of water provided good larvicidal effect up to day 40 with more than 80% reduction, while for non-replenishment of water, *Bti* showed more than 80% reduction at day 35 post-treatment (Table 2).

From all of the jars placed in the selected houses, all of the larvae and pupae were collected at five day intervals until the end of the trial. The collected larvae/pupae were colonised in the laboratory. The emerged adults were positively identified. When the percentage of reduction was calculated based on the adult emergence from the larvae collected and colonised in the laboratory, earthen jar

Table 1. Total number and percentage of adult mosquito emergence under laboratory condition and mean percentage of adult mosquito species composition

	Earthen jar		Glass jar		Total
	Control	Treatment	Control	Treatment	
Number of larvae collected	41035	5789	34077	7408	88309
Number of adult emergence	32578	3295	26220	4745	66838
Percentage of adult emergence	79.39	56.92	76.94	64.05	75.69
Ae. aegypti					
Number	15618	1196	8857	1554	27225
Percentage	47.94	36.30	33.78	32.75	40.73
Ae. albopictus					
Number	16745	2088	17292	3191	39316
Percentage	51.40	63.37	65.95	67.25	58.82
Culex quinquefasciatus					
Number	215	11	71	0	297
Percentage	0.66	0.33	0.27	0.00	0.44

Table 2. Mean percentage of reduction based on larvae collected from earthen jars and glass jars after treated with *Bacillus thuringiensis* H-14 at 6000 ITU/L in Sungai Nibong Kecil, Penang Island. The calculation of mean percentage of reduction was adjusted with control jars for the pre-treatment and post-treatment

Day	Earthen Jar		Glass jar	
	Replenishment	Non replenishment	Replenishment	Non-replenishment
1	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
3	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
5	99.58±0.17[a]	100.00±0.00[a]	95.37±1.50[b]	95.89±0.96[b]
10	84.35±2.94[b]	96.49±1.00[a]	96.42±0.92[a]	98.70±0.42[a]
15	100.00±0.00[a]	96.70±1.13[b]	88.18±3.89[c]	96.50±1.33[b]
20	81.04±3.78[b]	93.77±1.12[a]	93.90±1.22[a]	90.55±1.36[a]
25	92.14±2.00[a]	91.40±1.83[a]	93.82±1.11[a]	90.24±1.59[a]
30	85.46±2.76[b]	93.25±1.45[a]	87.46±2.07[b]	94.96±0.85[a]
35	92.45±1.53[a]	80.20±3.41[c]	88.80±2.49[b]	89.18±1.65[b]
40	80.30±1.90[a]	84.28±2.39[a]	83.95±2.73[a]	69.89±2.06[b]
45	70.66±3.44[a]	58.17±4.10[b]	72.26±4.40[a]	52.83±2.16[b]
50	73.70±3.40[a]	39.98±4.52[b]	67.00±4.13[a]	40.34±2.61[b]
55	64.75±3.64[a]	35.39±3.38[b]	62.30±3.89[a]	27.92±2.87[c]
60	54.32±4.61[a]	33.78±3.02[b]	51.96±3.46[a]	33.43±2.57[b]
65	39.56±2.96[a]	37.31±3.46[a]	28.31±3.54[b]	28.66±2.11[b]

* Mean percentages of reduction followed by the same letters within the same rows are not significantly different (P>0.05, LSD test using computer program SPSS).

Table 3. Mean percentage of reduction based on adult emergence in the laboratory from field larvae collected from study jars treated with *Bacillus thuringiensis* H-14 at 6000 ITU/L in Sungai Nibong Kecil Kecil, Penang Island

Day	Earthen Jar		Glass jar	
	Replenishment	Non-replenishment	Replenishment	Non-replenishment
1	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
3	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
5	99.95±0.05[a]	100.00±0.00[a]	97.76±0.71[b]	97.76±0.60[b]
10	93.39±1.79[b]	97.39±1.01[a]	98.12±0.49[a]	99.48±0.16[a]
15	100.00±0.00[a]	98.66±0.40[b]	97.20±1.29[b]	99.04±0.33[b]
20	88.20±1.69[c]	95.72±0.83[a,b]	97.32±0.64[a]	93.85±1.15[b]
25	94.12±1.22[b]	95.25±1.16[b]	97.07±0.62[a]	94.54±1.01[b]
30	90.90±1.53[b]	97.21±0.73[a]	91.67±1.80[b]	96.62±0.58[a]
35	93.48±1.26[b]	88.14±2.05[c]	96.20±1.12[a]	94.33±0.91[a,b]
40	87.72±1.45[b,c]	90.17±2.10[a,b]	90.56±2.55[a]	78.26±2.43[c]
45	83.34±2.08[a]	75.79±2.98[b]	87.34±2.55[a]	66.44±2.70[c]
50	83.26±3.02[a]	65.01±3.55[b]	84.54±3.04[a]	48.69±3.50[c]
55	85.23±2.57[a]	47.10±3.18[c]	79.07±3.20[b]	48.10±3.04[c]
60	58.75±4.05[b]	45.05±4.07[c]	70.32±3.70[a]	30.60±4.03[d]
65	44.00±3.20[a]	31.31±5.63[b]	44.17±5.31[a]	37.29±2.95[b]

* Mean percentages of reduction followed by the same letters within the same rows are not significantly different (P>0.05, LSD test using computer program SPSS)

with replenishment of water showed longer protection period up to 55 days with more than 80% of reduction (Table 3). For glass jar assessment, *Bti* at 6000 ITU/L with replenishment of water successfully prevented adult emergence at more than 80% of reduction up to 50 days (Table 3). However, for the assessment without replenishment of water, the efficacy with more than 80% of reduction were only up to day 40 and 35 for earthen and glass jar, respectively.

Simulated field evaluations of *Bti* against vector mosquitoes are relatively important to ensure the applicability of the *Bti* formulation in the actual use. Research has shown that *Bti* is also effectively used in the field. Small (Rampal *et al.*, 1983) or large (Lacey & Inman, 1985) scale field trials showed that *Bti* at proper dose provided effective control against mosquitoes

Although *Bti* is proven to be effective against many mosquito species, the operational application showed that the microbial agent is more suitable for *Aedes* species (Yap *et al.*, 2003). The use of *Bti* more toward *Aedes* control rather than other mosquito species may be due to the bottom feeding behavior of the *Aedes* species (Christophers, 1960) as most of the *Bti* toxins sediment to the base of the container during treatment (Su & Mulla, 1999).

According to WHO recommendation, a formulation providing more than 90% mortality against treated larvae is considered as effective (WHO, 1996). In this simulated field study, both earthen jars and glass jars evaluations indicate that *Bti* at the tested concentrations, performed effectively against both *Ae. aegypti* and *Ae albopictus* for a minimum of 25 days. The residual activity of *Bti* in this field evaluation agrees with the laboratory assessment before (Lee & Zairi, 2005).

However, the longer residual efficacy of *Bti* found in this study vary from others where *Bti* is supposed to provide short residual efficacy (Lee *et al.*, 2005). Basically, *Bti* showed very short residual activity under natural condition (Ignoffo *et al*. 1981; Van Essen & Hembree 1982). Although immunofluorescent staining research indicated that *Bti* is able to grow and produce toxins in the larvae of *Ae. aegypti* (Pantuwatana & Sattabongkot, 1990), the residual activity of *Bti* is reported to give a short period in field conditions (Margalit *et al.*, 1983; Gharib & Hilsenhoff, 1988; Pantuwatana & Sattabongkot, 1990; Mulla *et al.*, 1993; Ali *et al.*, 1994).

In contrast, field study of the efficacy of *Bti* (sand granules and plaster of Paris pellets) against *Ae. albopictus* carried out in Kuala Lumpur, Malaysia showed that both *Bti* formulations provided up to six weeks efficacy (Lee & Cheong, 1987). The result agrees with this study where *Bti* provides residual activity in the field.

In this study, the *Bti* formulation used is water dispersible granules which contain *Bti* with their toxin and spores. The *Bti* water dispersible granules provided better larvicidal activity with replenishment of water compared with non-replenishment of water. Replenishment of water probably improved the distribution and availability of *Bti* in the earthen jar.

As a conclusion, the *Bti* formulation provided larvicidal activity with good residual effect. The use of *Bti* had been proven to be safe in drinking water (WHO, 1999). Furthermore, the concentration used in this study did not present any visible sign that the water have been treated, possibly encouraging the usage of *Bti* in households.

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