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Laboratory evaluation of *Bacillus thuringiensis* H-14 against *Aedes aegypti*

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Abstract. Laboratory efficacy and residual activity of a water dispersible granule formulation of *Bacillus thuringiensis israelensis* (*Bti*) at the dosages of 3000, 6000 and 15000 ITU/L were conducted in this study. The study was conducted in two different size containers, earthen jar (45 L) and glass jar (3 L) with or without daily replenishment of 6 L and 0.3 L of water in the earthen and glass jars, respectively. Results indicate that for both earthen jar and glass jar evaluations, *Bti* at the tested dosages, performed effectively against *Aedes aegypti*, giving a minimum of 42 days effective killing activity. When the dosage was increased from 3000 ITU/L to 6000 ITU/L or 15000 ITU/L, the effective periods of the *Bti* increased by an additional one to three weeks. The *Bti* water dispersible granule provided better larvicidal activity with replenishment of water compared with non-replenishment of water especially for the higher dosage (15000 ITU/L).

INTRODUCTION

Dengue hemorrhagic fever/dengue fever (DHF/DF) cases are on the increase. In the year 2001, 16368 DHF/DF cases were reported with 50 deaths in Malaysia (MOH, 2002). *Aedes aegypti* and *Ae. albopictus* are two major vector mosquitoes of DHF/DF. Vector control is one of the most effective approach in combating DHF/DF. As DHF/DF could be transmitted transovarially (Lee *et al.*, 1997; Lee, 2000), vector control of *Aedes* species at larval stage is very important.

Due to the resistance of mosquitoes to various insecticides (Malcom & Wood, 1982; Georghiou *et al.*, 1987; WHO, 1992, 1997), the use of adulticides as the sole tool in mosquito control may be inappropriate in many situations. With that, the use of microbial toxin as additional mosquito control tool because of the effectiveness and lack of resistance problem has become commonplace (Rodcharoen & Mulla, 1996; Glare & O'Callaghan, 1998). The effectiveness of *Bacillus thuringiensis* H-14 (*Bti*) against vector mosquitoes has been well documented (Van Essen & Hembree, 1980; Foo & Yap, 1982; Balaraman *et al.*, 1983; Klowden & Bullar, 1984; Pantuwatana & Yougvanitsed, 1984; Fry-O'brien & Mulla, 1996). However, *B. thuringiensis* H-14 was known to provide short control periods without adequate residual efficacy. The main objective in this study was to identify a suitable dosage for VectoBac WDG, *B. thuringiensis* H-14 formulation that is able to provide adequate residual activity.

In this study, the laboratory effectiveness and residual activity of VectoBac ABG 6511, a water dispersible granule formulation of *Bti* against laboratory bred susceptible strains of *Ae. aegypti* was studied using earthen and glass jars.

METHODS AND MATERIAL

Mosquitoes

Laboratory-bred, late third or early fourth instar larvae of *Ae. aegypti* from the Vector Control Research Unit, Universiti Sains Malaysia, (VCRU, USM) were used in this study. The above mentioned susceptible mosquito strain have been colonised in the laboratory of VCRU, USM since the 1980s and have not been exposed to insecticides for more than 500 generations.

Test containers

The study was conducted in two different size containers, i.e. earthen jar (45 L) and glass jar (3 L). The earthen jars were filled with 40 L of water and the glass jars with 2 L of water.

Bacillus thuringiensis H-14 formulation and dosages

The *Bti* formulation used in this study was water dispersible granule formulation VectoBac WDG (ABG6511), a formulation by Abbott Laboratories, Australia (now merged with Sumitomo (M) Sdn Bhd). VectoBac WDG has 3000 International Toxic Units (ITU) per mg against *Ae. aegypti*.

In this simulated study, three dosages [3000, 6000 and 15000 ITU/L] were tested, with untreated water as control. The formulation was weighed and sprinkled on the water surface of the containers.

For the earthen jar (40 L of water), 40, 80 and 200 mg and for the glass jar (2 L of water), 2, 4 and 10 mg were weighed and sprinkled on the water surface of the container for the dosages of 3000, 6000 and 15000 ITU/L, respectively.

Test Design

The water was allowed to season for at least 48 hours prior to the experiment. Then, VectoBac WDG was introduced into the test containers, while first evaluations of larvae were carried out after 24 hours of treatment. Twenty laboratory bred mosquito larvae (L3/L4) from well established laboratory colony were introduced into test jars at 24 hours post treatment, followed on, day 7, day 14, day 21, day 28 until the efficacy was less than 50%. Larval mortality was recorded daily up to six days at each introduction period.

Dead larvae were removed and all pupae formed were collected and kept in a cup with 200 ml of the respective water from the jars. Adult emergence were observed and recorded.

Three sets of experiments were conducted and the trials at each dose was terminated when the mortality rate fell below 50%.

Replenishment and non-replenishment of water

Two treatment regimes were adopted: A set of jars; 3 per treatment dosage with 3 controls were not subjected to any replenishment of water but evaporated water was replaced; and in the 2nd set, 3 jars per dosage with 3 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.

RESULTS AND DISCUSSIONS

In the simulated study, earthen jar assessment indicated that this *B. thuringiensis* H-14 WDG formulation at 15000 ITU/L with daily replenishment of 6 L of water, performed effectively against *Ae. aegypti* up to day 56 with more than 89.17±3.52% larval mortality, whereas at day 63, 72.50±2.14% mortality was recorded (Table 1). However, for the non-replenishment of water, *B. thuringiensis* H-14 WDG formulation at 15000 ITU/L indicated more than 84.17±3.00% larval mortality up to day 49, on day 56, a 68.33±9.89% larval mortality was recorded (Table 2). Low larval mortality (less than 20%) was recorded after day 63 (Table 2). *B. thuringiensis* H-14 WDG formulation at 6000 ITU/L performed effectively against *Ae. aegypti* with replenishment of water up to day 42 with more than 82.50±7.61%

Table 1. Earthen and glass jars treated with *Bacillus thuringiensis* H-14 water dispersible granule formulation and treated water was subjected to daily replenishment of water in laboratory

	Post-treatment (days)	Mean mortality[1] (%) of *Ae. aegypti* ± SE			
		Control	3000 ITU/L (1 mg/L)	6000 ITU/L (2 mg/L)	15000 ITU/L (5 mg/L)
Earthen jars	1	3.33 ± 1.67[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	7	4.17 ± 1.54[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	14	5.00 ± 2.58[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	21	3.33 ± 1.67[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	28	5.00 ± 2.58[c]	100.00 ± 0.00[a]	94.17 ± 3.27[b]	100.00 ± 0.00[a]
	35	5.83 ± 2.01[b]	90.83 ± 5.69[a]	90.00 ± 3.87[a]	96.67 ± 1.67[a]
	42	5.83 ± 3.27[b]	87.50 ± 6.02[a]	82.50 ± 7.61[a]	90.83 ± 2.39[a]
	49	3.33 ± 2.11[d]	44.17 ± 5.83[c]	55.83 ± 3.96[b]	90.83 ± 3.00[a]
	56	6.67 ± 4.22[e]	26.67 ± 5.43[d]	48.33 ± 6.01[c]	89.17 ± 3.52[a]
	63	4.17 ± 2.71[d]	21.67 ± 8.82[b]	10.00 ± 2.89[b]	72.50 ± 2.14[a]
	70	5.83 ± 2.39[b]	2.50 ± 1.75[b]	2.50 ± 1.71[b]	15.00 ± 4.28[a]
Glass jars	1	4.17 ± 2.71[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	7	8.33 ± 4.01[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	14	5.83 ± 2.01[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	21	4.17 ± 2.71[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	28	11.00 ± 4.18[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	35	4.17 ± 2.71[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	42	5.00 ± 2.58[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	49	2.50 ± 1.71[c]	75.00 ± 9.83[b]	90.00 ± 2.89[a]	95.00 ± 2.58[a]
	56	7.50 ± 3.59[c]	73.33 ± 7.60[b]	83.33 ± 3.57[b]	93.33 ± 3.57[a]
	63	2.50 ± 1.71[c]	6.67 ± 2.47[b,c]	7.50 ± 2.14[b]	83.33 ± 2.47[a]
	70	1.67 ± 1.05[c]	9.17 ± 3.52[b]	10.00 ± 2.89[b]	54.17 ± 11.50[a]

[1] Mean percentage mortality followed by the same letters within the same rows are not significantly different (P=0.05, LSD test using computer program SPSS)
Results of pupae mortality and adult emergence are not presented because more than 95% of the emerged pupae in this study emerged as adults.

larval mortality, whereas at day 49 onwards, less than 55.83±3.96% mortality was recorded (Table 1). However, for the non-replenishment of water, *B. thuringiensis* H-14 at 6000 ITU/L showed more than 85.00±7.75% larval mortality up to day 42, but after day 49, less than 52.50±5.59 % larval mortality was recorded (Table 2). Low larval mortality (less than 20%) was recorded after day 63 (Table 2). *B. thuringiensis* H-14 at 3000 ITU/L performed effectively against *Ae. aegypti* up to day 42, giving more than 87.50±6.02% larval mortality for both replenishment and non-replenishment of water, whereas from day 49 onward, less than 50% mortality was recorded (Tables 1 and 2).

For the glass jar assessment, results indicated that *B. thuringiensis* H-14 at 15000 ITU/L with daily replenishment of 0.3 L of water, performed effectively against *Ae. aegypti* up to day 63 with more than 83.33±2.47% larval mortality, whereas from day 70 onward, 54.17±11.50% mortality was recorded (Table 1). However, for the non-replenishment of

Table 2: Earthen and glass jars treated with *Bacillus thuringiensis* H-14 water dispersible granule formulation and treated water without daily replenishment of water in laboratory.

	Post-treatment (days)	Mean mortality[1] (%) of *Ae. aegypti* ± SE			
		Control	3000 ITU/L (1 mg/L)	6000 ITU/L (2 mg/L)	15000 ITU/L (5 mg/L)
Earthen jars	1	8.33 ± 3.80[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	7	4.17 ± 2.71[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	14	9.17 ± 3.00[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	21	5.83 ± 1.54[c]	92.50 ± 4.79[b]	97.50 ± 1.71[b]	100.00 ± 0.00[a]
	28	5.83 ± 2.01[c]	96.67 ± 2.11[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	35	6.67 ± 3.57[c]	90.83 ± 3.00[b]	90.83 ± 3.75[b]	100.00 ± 0.00[a]
	42	7.50 ± 2.50[c]	90.00 ± 3.42[b]	85.00 ± 7.75[b]	94.17 ± 4.17[a]
	49	5.00 ± 2.58[d]	44.17 ± 2.39[b]	52.50 ± 5.59[b]	84.17 ± 3.00[a]
	56	6.67 ± 3.57[d]	30.83 ± 3.00[c]	48.33 ± 6.67[b]	68.33 ± 9.89[a]
	63	4.17 ± 2.71[c]	3.33 ± 1.67[b]	4.17 ± 2.01[b]	14.17 ± 5.23[a]
	70	4.17 ± 2.71[b]	2.50 ± 1.12[b]	3.33 ± 1.67[b]	17.50 ± 5.88[a]
Glass jars	1	10.00 ± 4.47[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	7	3.33 ± 2.47[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	14	3.33 ± 2.47[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	21	2.50 ± 1.71[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	28	5.83 ± 3.75[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	35	2.50 ± 1.71[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	42	6.67 ± 2.47[c]	100.00 ± 0.00[a]	85.00 ± 8.16[b]	100.00 ± 0.00[a]
	49	3.33 ± 1.67[c]	96.67 ± 1.67[a]	89.17 ± 4.36[a]	94.17 ± 3.27[a]
	56	9.17 ± 4.17[c]	71.67 ± 4.22[b]	89.17 ± 4.36[a]	92.50 ± 2.14[a]
	63	4.17 ± 2.01[c]	41.67 ± 8.23[b]	57.50 ± 10.55[a,b]	65.00 ± 11.33[a]
	70	2.50 ± 1.71[c]	17.50 ± 6.55[b]	25.83 ± 5.97[b]	56.67 ± 11.88[a]

[1] Mean percentage mortality followed by the same letters within the same rows are not significantly different (P=0.05, LSD test using computer program SPSS).
Results of pupae mortality and adult emergence are not presented because more than 95% of the emerged pupae in this study emerged as adults.

water, *B. thuringiensis* H-14 at 15000 ITU/L showed more than 92.50±2.14% larval mortality up to day 56, and from day 63 onwards, less than 65.00±11.33% larval mortality was recorded (Table 2). *B. thuringiensis* H-14 at 6000 ITU/L performed effectively against *Ae. aegypti* with replenishment of water up to day 56 with more than 83.33±3.57% larval mortality, whereas from day 63 onwards, less than 10.00±3.89% mortality was recorded (Table 1). However, for the non-replenishment of water, *B. thuringiensis* H-14 at 6000 ITU/L showed more than 89.17±4.36% larval mortality up to day 56, but after day 63, less than 57.50±10.55 % larval mortality was recorded (Table 2). *B. thuringiensis* H-14 at 3000 ITU/L performed effectively against *Ae. aegypti* up to day 49 with more than 96.67±1.67% larval mortality for both replenishment and non-replenishment of water, whereas from day 63 onwards, less than 50% mortality was recorded (Tables 1 and 2).

As a conclusion, both earthen jar (40L) and glass jar (2L) evaluations indicated that *Bti* at the tested dosages, performed effectively against *Ae. aegypti* for a minimum of 42 days. The effective periods of the *Bti* increased by an additional one to three weeks when the dosage increased from 3000 ITU/L to 6000 ITU/L or 15000 ITU/L. However, by considering the cost of the products and its residual efficacy, the best dose to be recommended for used in this study, would be 3000 or 6000 ITU/L.

The *Bti* water dispersible granules provided better larvicidal activity with daily replenishment of water compared to without replenishment of water especially for the higher dosage (15000 ITU/L). Replenishment of water probably improved the distribution and availability of *Bti* in the earthen jar.

Furthermore, the *Bti* formulation provided larvicidal activity with good residual effect. The use of *Bti* has been proven safe in drinking water (WHO, 1999). In addition, the dosage 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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