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Field effectiveness of *Bacillus thuringiensis israelensis* (Bti) against *Aedes (Stegomyia) aegypti* (Linnaeus) in ornamental ceramic containers with common aquatic plants

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Abstract. This study was undertaken to determine the impact of larvaciding using a Bti (*Bacillus thuringiensis israelensis*) formulation (VectoBac WG) against *Aedes aegypti* larvae in earthen jars containing aquatic plants. Aquatic plants commonly used for landscaping, *Pistia stratiotes* (L.) (Liliopsida: Araceae) and *Sagittaria* sp. (Liliopsida: Alismataceae) were placed inside earthen jars filled with 50 L tap water. All earthen jars were treated with Bti formulation at 8g/1000L. Untreated jars with and without aquatic plants were also set up as controls. Fifty laboratory-bred 2nd instar larvae were introduced into each earthen jar. All earthen jars were observed daily. Number of adults emerged was recorded and the larval mortality was calculated. The indicators of effectiveness of Bti for these studies were (i) residual activities of Bti, and (ii) larval mortality in earthen jars with or without aquatic plants. The treated earthen jars containing *P. stratiotes* and *Sagittaria* sp. showed significant residual larvicidal effect up to 7 weeks, in comparison to untreated control ($p < 0.05$). The larval mortality ranged from 77.34% – 100% for jars with aquatic plants vs 80.66% – 100% for jars without aquatic plant. Earthen jars treated with Bti without aquatic plants also exhibited significantly longer residual larvicidal activity of up to 10 weeks ($p < 0.05$). The larval mortality ranged from 12.66% – 100% for jars with aquatic plants vs 59.34% — 100% for jars without aquatic plant. Thus, earthen jars without aquatic plants exhibited longer residual larvicidal effect compared to those with aquatic plants. This study suggested that containers with aquatic plants for landscaping should be treated more frequently with Bti in view of the shortened residual activity.

INTRODUCTION

Dengue is a vector-borne disease, with increasing number of cases and mortality in Malaysia over the years. High human population growth in many areas has led to extensive deforestation, irrigation and urbanization. These high population densities and associated environmental modifications have created conditions that favor the proliferation of the dengue vectors. For the year 2008, 49,335 Malaysian cases of dengue were notified, amounting to an increase of 489 cases comparing to 48,846 cases reported in 2007. This disease claimed 112 lives in 2008. In the first 7 weeks of 2009, a total of 6,766 dengue cases were reported, showing an increase of 2,755 cases or 68.69% comparing to 4,011 cases reported in the same period in 2008 (Ministry of Health, 2009).

In Malaysia, temephos is recommended as a larvicide to control the dengue vectors, *Aedes aegypti* and *Aeedes albopictus* by Ministry of Health and has been used for the last 3 decades. However, *Aedes* larval resistance against temephos was reported by many workers in this country (Lee & Lime, 1989; Chen *et al*., 2005). Thus, other larvicide such as *Bacillus thuringiensis israelensis* (Bti) should be used as an alternative control measure to ensure the efficiency of program aimed at vector control and protection of human health.

Bti, known as a microbial control agent, has been used since the early 1980's. Bti toxins are highly and specifically effective against *Aedes* larvae. No *Aedes* resistance against Bti has been reported thus far.

There are no such reports on the effect of aquatic plants on the efficacy of Bti treatment to the larvae in the treated waters. This study was conducted to determine whether the presence of aquatic plants would be a limiting or a deterring factor for larvae to ingest the Bti toxins in the treated water.

MATERIALS AND METHODS

Test site

The study was conducted in the surrounding area of the Medical Entomology Unit, Institute for Medical Research (IMR), Jalan Pahang, Kuala Lumpur (N03°10.167', E101°41.919').

Test Specimen

Laboratory-bred late 3rd or early 4th instar larvae of *Ae. aegypti* were used to assess the larvacidal effect of Bti.

Insecticide

A Bti formulation, VactoBac WG (Lot No. 114-114-3L) provided by Valent BioSciences Corporation was used in this study. The manufacturer's recommended dosage of 8 g / 1000 L was used.

Aquatic plant

Aquatic plants commonly used for landscaping, *Pistia stratiotes* (L.) (Liliopsida: Araceae) and *Sagittaria* sp. (Liliopsida: Alismataceae) were used in this study.

Test containers

Earthen jars were used as mosquito breeding containers in this study. Earthen jars each with an opening of 52 cm in diameter, base diameter of 35 cm and 47 cm in height were prepared and placed outdoor. Three replicates of each were used in each research arm of the study (Table 1). Each earthen jar held 50 L tap water. Before initiating the study, all containers were washed with tap water and tested for the presence of contaminant by introduction of 50 *Ae. aegypti* 2nd instar larvae. The larvae were observed until complete emergence as adults.

Trial procedures

Aquatic plants commonly used for landscaping, *P. stratiotes* and *Sagittaria* sp. were placed inside earthen jars filled with 50 L tap water according to Table 1. A total of 5 units of aquatic plants were introduced into each earthen jar. Bti formulation at 8g/1000L was introduced to the earthen jars. Untreated jars with and without aquatic plants were also set up as controls. Fifty laboratory-bred 2nd instar larvae were introduced into each earthen jar. All earthen jars were observed daily. Pupae were

Table 1. Setup of earthen jars for testing

Earthen jar	Treated with Bti	Aquatic plant	Number of replicates
Untreated	No	Without aquatic plant	3
	No	*Pistia stratiotes*	3
	No	*Sagittaria* sp.	3
Treated	Yes	Without aquatic plant	3
	Yes	*Pistia stratiotes*	3
	Yes	*Sagittaria* sp.	3

collected, recorded and transferred into paper cups covered with net. The total number of adults emerged was recorded and the larvae mortality was calculated. The same procedure was repeated by adding fresh batch of larvae (50 larvae) into each earthen jar weekly.

Data analysis

The indicators of effectiveness of Bti for these studies were (i) residual activities of each dosage, and (ii) mortality of larvae. A cut-off point of larval mortality $\geq$ 50% was considered to be effective.

RESULTS AND DISCUSSION

Table 1 shows the mean number (± SE) of adult *Ae. aegypti* that emerged from the earthen jars with and without plants. Mortality of *Ae. aegypti* larvae in earthen jars treated with Bti with or without aquatic plants were showed in Figure 1 to Figure 3. The treated earthen jars containing *P. stratiotes* and *Sagittaria* sp. showed significant residual larvicidal effect up to 7 weeks, in comparison to untreated control ($p < 0.05$) (Table 2). The larval mortality ranged from 77.34% – 100% for jars with aquatic plants (Figure 2 and Figure 3) vs 80.66% – 100% for jars without aquatic plant (Figure 1). Earthen jars treated with Bti without aquatic plants also exhibited significantly longer residual larvicidal activity of up to 10 weeks ($p < 0.05$) (Table 2). The larval mortality ranged from 12.66% – 100% for jars with aquatic plants (Figure 2 and Figure 3) vs 59.34% – 100% for jars without aquatic plant (Figure 1).

The human and environmental safety of Bt, including Bti, has been assessed by WHO (1999). The insecticide crystal proteins (ICP) spores and vegetative cells of the Bti subspecies, when administered by different routes, were found to be mostly non-pathogenic and non-toxic to various animal species. Owing to their specificity, Bti products are unlikely to pose any hazard to human, other vertebrates and non-target invertebrates, provided that they are free from non-Bt microorganisms and biologically active products other than the ICPs. They are safe for use in aquatic environments, including drinking-water and reservoirs, for the control of mosquitoes, blackflies, and larvae of nuisance insects (WHO, 2004).

The laboratory (Lee & Zairi, 2005; Fansiri *et al.*, 2006) and field (Benjamin *et al.*, 2005; Armengol *et al.*, 2006; Setha *et al.*, 2007) effectiveness of Bti in the control of mosquitoes has been well documented. Lee & Zairi (2006) reported that more than 80% reduction of mosquitoes was recorded in earthen jars treated with Bti up to 40 days. This is also seen in this study, where more than 80% larvae mortality was observed in earthen jars without plants up to 49 days. Lima *et al.* (2005) also reported earthen jars treated with Bti showed mortality levels of 70% or more were attained for 2 – 5 weeks; while field study of the efficacy of Bti studied by Lee & Cheong (1987) showed that Bti formulaions provided up to 6 weeks efficacy. The result agreed with this study where Bti provide residual activity in the field, in which earthen jars without plants treated with Bti showed larval mortality of more than 80% up to 7 weeks, and more than 50% up to 10 weeks.

Although many field studies have been conducted on the efficacy of Bti in the drinking water containers (cement containers, earthen jars and plastic containers), however, no study has been conducted on the efficacy of Bti treated in the earthen jars containing aquatic plants for landscaping. This study has demonstrated the efficacy of Bti in the earthen jars containing aquatic plants commonly used in Malaysia, such as *P. stratiotes* and *Sagittaria* sp. The result obtained from this study shows larval mortality in earthen jars containing both aquatic plants achieved more than 50% mortality up to 7 weeks, indicating that earthen jars without aquatic plants exhibited longer residual larvicidal effect compared to those with aquatic plants. This study suggested that containers with aquatic plants for landscaping should be treated more frequently in view of the shortened residual activity.

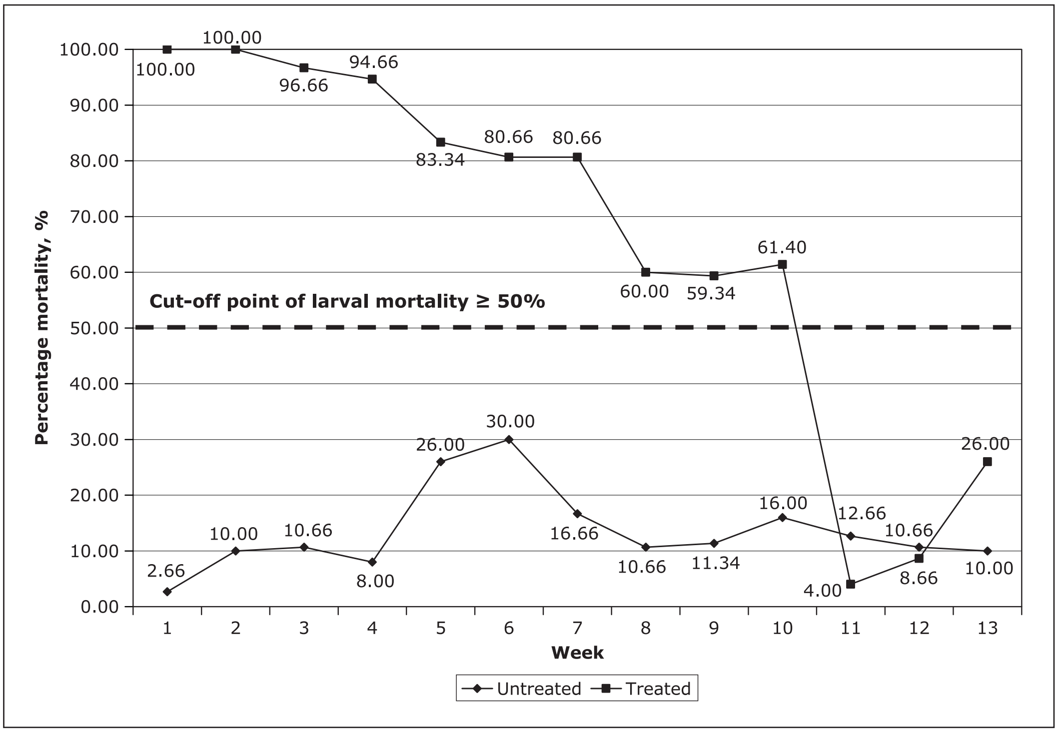

Figure 1. Mean percentage mortality of *Ae. aegypti* larvae in earthen jars treated with Bti without aquatic plants.

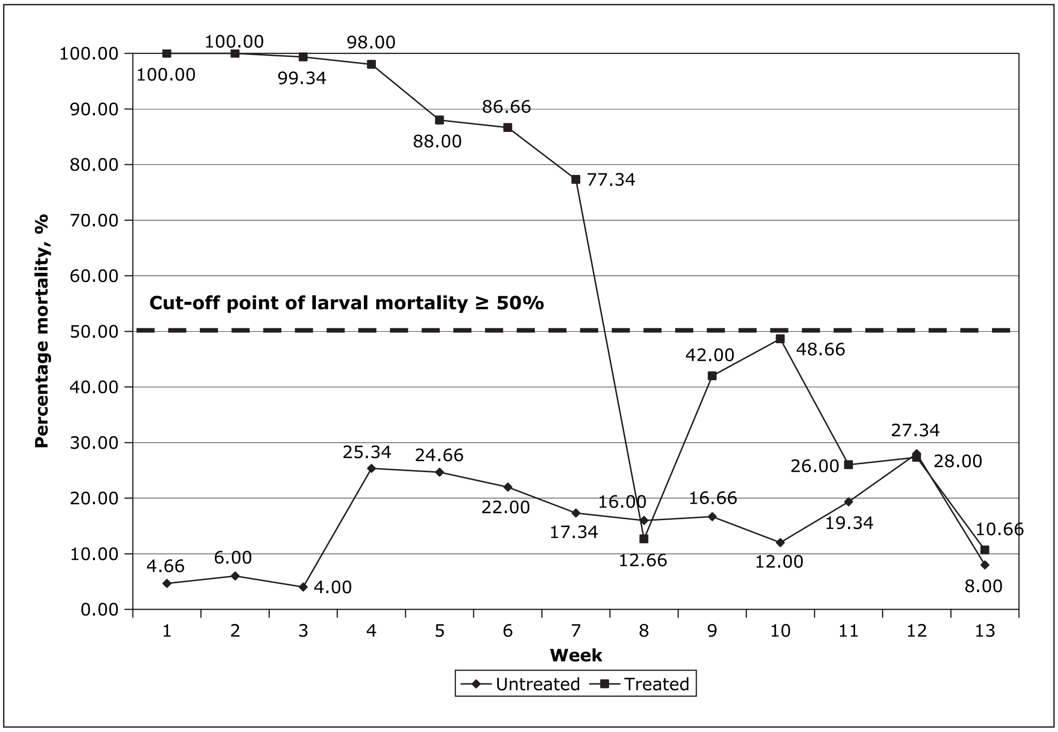

Figure 2. Mean percentage mortality of *Ae. aegypti* larvae in earthen jars treated with Bti with aquatic plant, *Pistia stratiotes.*

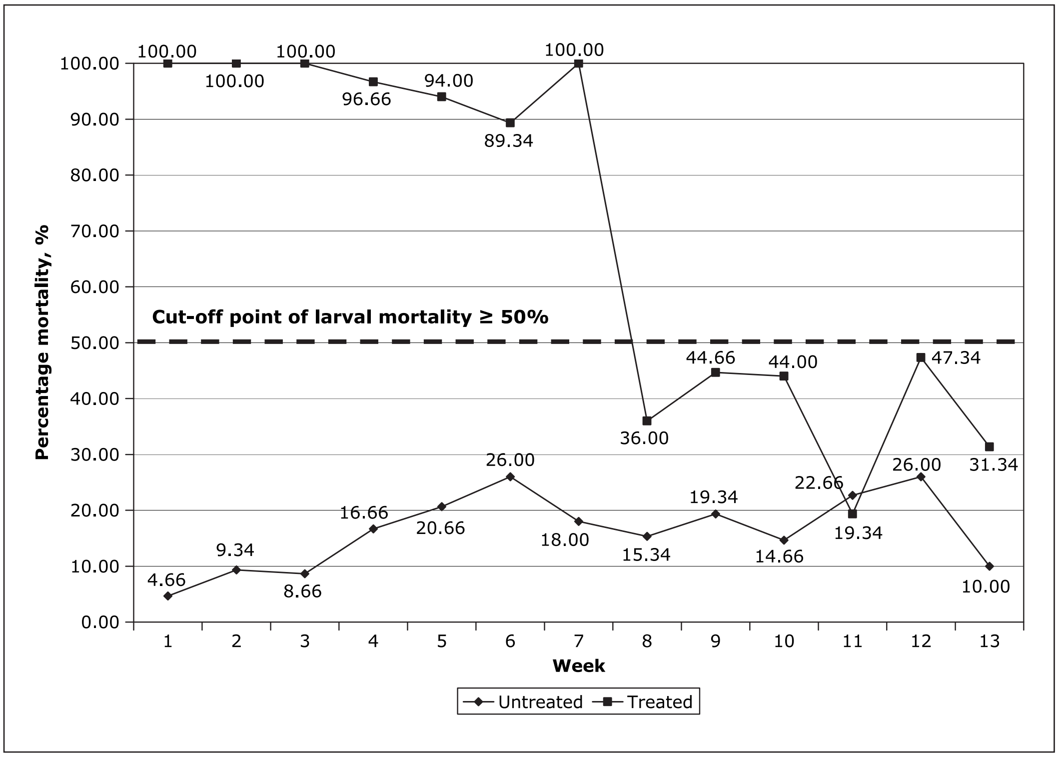

Figure 3. Mean percentage mortality of *Ae. aegypti* larvae in earthen jars treated with Bti with aquatic plant, *Sagittaria* sp.

Table 2. Mean number (± SE) of adult *Ae. aegypti* emerged from the earthen jars with and without plants

Week	Without aquatic plants			With aquatic plants					
				Pistia stratiotes			*Sagittaria* sp.		
	Untreated	Treated	t-test	Untreated	Treated	t-test	Untreated	Treated	t-test
1	48.67 ± 1.33	0.00 ± 0.00	P = 0.000	47.67 ± 2.33	0.00 ± 0.00	P = 0.000	47.67 ± 2.33	0.00 ± 0.00	P = 0.000
2	45.00 ± 1.53	0.00 ± 0.00	P = 0.000	47.00 ± 2.52	0.00 ± 0.00	P = 0.000	45.33 ± 2.40	0.00 ± 0.00	P = 0.000
3	44.67 ± 0.88	1.67 ± 0.67	P = 0.000	48.00 ± 1.53	0.33 ± 0.33	P = 0.000	45.67 ± 1.76	0.00 ± 0.00	P = 0.000
4	46.00 ± 4.00	2.67 ± 0.33	P = 0.000	37.33 ± 2.60	1.00 ± 1.00	P = 0.000	41.67 ± 4.91	1.67 ± 0.67	P = 0.001
5	37.00 ± 2.52	8.33 ± 3.53	P = 0.003	37.67 ± 0.88	6.00 ± 1.15	P = 0.000	39.67 ± 1.20	3.00 ± 0.58	P = 0.000
6	35.00 ± 1.00	9.67 ± 0.67	P = 0.000	39.00 ± 2.08	6.67 ± 0.67	P = 0.000	37.00 ± 1.53	5.33 ± 1.67	P = 0.000
7	41.67 ± 0.88	9.67 ± 2.67	P = 0.000	41.33 ± 1.45	11.33 ± 4.67	P = 0.004	41.00 ± 1.53	0.00 ± 0.00	P = 0.000
8	44.67 ± 1.33	20.00 ± 1.15	P = 0.000	42.00 ± 2.65	43.67 ± 2.85	P = 0.690	42.33 ± 2.60	32.00 ± 6.11	P = 0.195
9	44.33 ± 2.40	20.33 ± 3.18	P = 0.004	41.67 ± 2.40	29.00 ± 6.03	P = 0.123	40.33 ± 1.76	27.67 ± 4.81	P = 0.069
10	42.00 ± 2.00	19.33 ± 2.67	P = 0.002	44.00 ± 4.00	25.67 ± 6.84	P = 0.144	42.67 ± 2.67	28.00 ± 5.03	P = 0.062
11	43.67 ± 3.18	48.00 ± 0.58	P = 0.251	40.33 ± 2.85	46.00 ± 2.00	P = 0.179	38.67 ± 1.20	40.33 ± 4.81	P = 0.755
12	44.67 ± 2.19	45.67 ± 1.45	P = 0.723	36.00 ± 2.08	36.33 ± 3.38	P = 0.938	37.00 ± 2.89	26.33 ± 3.18	P = 0.068
13	45.00 ± 2.65	36.67 ± 6.36	P = 0.293	46.00 ± 1.53	44.67 ± 3.38	P = 0.738	45.00 ± 2.52	34.33 ± 8.11	P = 0.277

P > 0.05, not significantly different   P ≤ 0.05, significantly different

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