

EFFICACY OF *BACILLUS THURINGIENSIS ISRAELENSIS*, VECTOBAC[®] WG AND DT, FORMULATIONS AGAINST DENGUE MOSQUITO VECTORS IN CEMENT POTABLE WATER JARS IN CAMBODIA

To Setha, Ngan Chantha and Doung Socheat

National Malaria Center (CNM), Center for Entomology, Parasitology and Malaria Control, Phnom Penh, Cambodia

Abstract. This study reports the evaluation of *Bacillus thuringiensis israelensis* (*Bti*), a biological larvicide, in cement jars holding river, well and rain water. Two *Bti* formulations, VectoBac WG[®] and VectoBac DT[®], were evaluated in a village in Phnom Penh. Thirty-one households with cement jars supporting the colonization of *Ae. aegypti* immatures were chosen. In each house 3 jars were aligned next to each another and filled with the same type of water. One of the 3 jars was treated with VectoBac WG[®] at 0.4 g per 50 liters, a second jar was treated with VectoBac DT[®] at 1 tablet per 50 liters, and a third jar was an untreated control (UTC). The jars were not covered, kept outdoors and not subjected to water exchange activity. The efficacy of VectoBac[®] to control natural *Ae. aegypti* infestation was measured by *Ae. aegypti* pupae surveillance, conducted 3 days per week for 3 months post-treatment (June - September 2004). All pupae were removed, allowed to emerge in the Cambodia National Malaria Center insectarium and the emerged adults were identified and counted. The VectoBac treatments were more effective in river water, followed by well and rain water. The VectoBac treatments significantly reduced the pupae numbers for a minimum of 3 months in the river water and 2.5 months in the well water ($p < 0.05$). In the rain water, the pupae densities in the VectoBac WG[®] and DT[®] treated jars were not significantly different from the untreated jars, although the treated jars yielded 2.0 to 5.2 fold less pupae, respectively, than in the untreated jars during the 3 months post-treatment. The efficacy of VectoBac WG[®] to control *Ae. aegypti* was similar to the efficacy of VectoBac DT[®] in the 3 water types ($p > 0.05$). It was also observed that VectoBac WG[®] and DT[®] were target specific, without any adverse effects on aquatic predatory insects common in well and rain water. VectoBac WG[®] and DT[®] were found to be easy-to-use formulations, with no need to repackage them prior to use in the containers. The amounts of VectoBac WG[®] and DT[®] used were 12.5 fold less by weight than temephos (Abate 1.0% SG[®]).

INTRODUCTION

Large scale, timely application of larviciding using temephos has been the strategy for *Aedes aegypti* control in water storage jars since 2001. This larvicidal program targets the key containers and is an interim measure to

lower the potential risk of a dengue outbreak in the epidemiological stratified areas. With the prevalence of widespread temephos resistance in *Aedes* larval populations in the Americas (Rawlins, 2002), Malaysia (Lee *et al*, 1998) and Thailand (Saelim *et al*, 2005), an alternative control agent must be developed beforehand to control temephos-resistant *Ae. aegypti* in Cambodia. For effective vector control, the National DHF Control Program, Cambodia is field-testing several other potential larvicides, including biological larvicides, such

Correspondence: Dr To Setha, National Malaria Center (CNM), Center for Entomology, Parasitology and Malaria Control, # 372 Monivong Blvd (Corner St 322), Phnom Penh, Cambodia.
E-mail: to_setha@yahoo.com

as *Bacillus thuringiensis israelensis* (*Bti*). VectoBac® WG, a water-dispersible granular formulation of *Bacillus thuringiensis* H-14 containing 3,000 ITU/mg against *Aedes aegypti*, manufactured by Valent BioSciences Corporation (VBC) and approved by the WHOPEP for mosquito larval control. The product is successfully used annually in many mosquito control programs worldwide in temperate and tropical zones. This formulation has been effectively applied at low dosages via aerial, ground and direct application into water habitats for the control of nuisance and disease transmitting mosquitoes (First Asean Congress of Parasitology and Tropical Medicine, 2004). The National Dengue Control Program in Brazil uses VectoBac WG for routine treatment of reservoirs of drinking water to control temephos resistant *Ae. aegypti* larvae. The VectoBac WG formulation was the most suitable *Bti* formulation (Vilarinhos and Monnerat, 2004; Brazilian Ministry of Health, 2005).

VBC has also developed a commercial tablet formulation based on *Bti*, the VectoBac DT. The tablet weighing 0.34 g (2,300 ITU/mg against *Ae. aegypti*) is irradiated and formulated to be used by direct application in artificial containers holding potable water at a dosage of 1 tablet per 50 liters water. Efficacy of the tablet has been evaluated for the potential control of dengue vectors, *Ae. aegypti* and *Ae. albopictus* larvae, in various types of potable water containers (Vilarinhos and Monnerat, 2004; Benjamin *et al*, 2005). The tablet significantly reduced the temephos resistant *Aedes* population, in tablet treated earthen, HDPE and plastic containers holding tap water, for a minimum period of 2 months (Benjamin *et al*, 2005).

This paper reports the research findings of a pilot study that was conducted from April to September 2004 by the National Malaria Center, Ministry of Health, Cambodia (CNM) to determine the efficacy of VectoBac WG and VectoBac DT to control *Ae. aegypti* in cement jars holding well, rain and river water. The ef-

fect of VectoBac products on non-target insects was also determined by monitoring the presence/absence of aquatic predatory insects in the *Bti* treated waters.

MATERIALS AND METHODS

Study site

Phum Thmei, a village located 16 km from Phnom Penh, has 366 households and a population of 1,875 people. Each household in this village stores potable water in cement jars placed outside their homes, and each home has an average of 4 jars with capacities of 500, 400, 300 or 150 liters. The jars store well, rain or river water. All the jars in this village were last treated with temephos in April 2003.

In April 2004, an entomological survey was conducted to determine the presence of dengue mosquito vectors in this village. Forty-six houses, with a total of 166 cement jars, were surveyed for the presence of dengue mosquito vector(s). All pupae were collected and allowed to emerge in the CNM insectarium and the adults were identified.

Study jars

In April 2004, 93 jars with L3-L4 larvae and pupae were chosen from 31 houses for the study. The presence of larvae and pupae indicates the jars were free from chemicals or other larvicidal contamination. The jars supported the colonization of natural dengue mosquito vectors. The 93 jars were acquired from the villagers and replaced with new jars.

Most of the study jars were removed from their original position to another site in the compound, usually about 1 to 5 m from the original position. The move was done to prevent the villagers from using the water in the study jars and to keep the jars from being in the way of the villagers.

In each house, 3 chosen study jars (1 set) were aligned next to one another and filled with the same type of water. The jars were filled with water at least 3 weeks before *Bti* treat-

ment. There were 11 sets of jars with well water, 10 sets with rain water and 10 sets with river water. The jars were observed routinely for dengue mosquito vectors before the initiation of *Bti* treatment.

***Bacillus thuringiensis israelensis* and entomological surveillance**

B. thuringiensis israelensis (*Bti*) formulations, VectoBac WG (Lot No: 104-986-3L) a water dispersible granule formulation and VectoBac DT (Lot No: 04-004-VB) a tablet formulation, were used to treat the study jars.

On 11 June 2004, the jars were treated as scheduled. In each set, jar 1 was treated with VectoBac WG at a dosage of 0.4 g per 50 liters, jar 2 was treated with VectoBac DT at a dosage of 1 tablet per 50 liters, and jar 3 was untreated (UTC). The jars were not covered and were exposed to sunlight and rain. There was no water exchange activity throughout the study.

The efficacy and persistence of VectoBac WG and VectoBac DT were each measured by entomological surveillance for 3 months post-treatment from 12 June to 6 September 2004. Entomological surveillance, consisting of larval and pupae surveillance, was conducted 1 day after treatment (12 June 2004), followed by routine surveillance 3 days per week. Larval observation was recorded for dengue mosquito vectors naturally colonizing the jars in L1 to L4 stages. The actual larval numbers were not recorded. Pupae surveillance included removing of all the pupae from the jars. These pupae were then allowed to emerge in the CNM insectarium. The adults were then identified and counted.

To overcome the problem of some jars not having any natural dengue mosquito vectors, all study jars were introduced with 30 (L3/L4) *Ae. aegypti* larvae, 3 days per week. The larvae were collected from villagers' in-use jars. The larvae were introduced on Wednesdays, irrespective of whether the jars had natu-

ral colonization or not. Larval mortality and pupation were observed on the following Fridays and Mondays.

The efficacy and persistency of VectoBac treatments were determined by larval observation and comparing the significant differences in *Ae. aegypti* pupae density between the treated and untreated jars.

RESULTS

Upon initiation of the study, 46 houses, with a total of 166 cement jars, were surveyed for the presence of dengue mosquito vectors. A total of 1,394 pupae were collected and allowed to emerge in the CNM insectarium and the emerged adults were identified as *Ae. aegypti*; this was the only dengue mosquito vector found present in this study village.

Pre-treatment entomological surveillance

Ninety-three jars with *Ae. aegypti* larvae (L3/L4) and pupae were selected. Most of the 93 jars were moved from their original position to a different site within the compound, 1 to 5 m away from the original position. New jars were given to the villagers in exchange for the 93 jars and were placed in the original positions. Pre-treatment entomological surveillance revealed a decreasing number of jars with natural *Ae. aegypti* colonization. On the day of *Bti* treatment (11 June 2004) there were only 25 jars (out of 93) that were colonized by *Ae. aegypti*. All the new jars placed in the original position of the study jars had immature *Ae. aegypti*. Some of the study jars that were not moved from their original position also had good colonization with *Ae. aegypti*.

Absence of colonization in the study jars was not due to other mosquito control activities in the study village. In 2004 the Cambodia Local Health Council did not treat the study village with temephos or any other larvicide or adulticide. Reason(s) for the absence of colonization in the jars that were moved from their original position and reason(s) for good colo-

nization of the jars in their original position still remains unknown.

Since some study jars did not have active colonization with *Ae. aegypti* mosquitoes, all study jars had 30 (L3/L4) *Ae. aegypti* larvae introduced, which were collected from the villagers' in-use jars.

Infestation of aquatic predatory insects

One day after *Bti* treatment, aquatic predatory insects were observed in the study jars. These predators: corixid bugs (Family: Corixidae) and water scavenger beetles (Family: Hydrophilidae) were observed to grasp *Ae. aegypti* larvae and suck the hemolymph from their prey. The presence of aquatic predatory insects in the study jars and their population density increasing with time, contributed to fewer *Ae. aegypti* larvae in the jars, thus interfering with this study to determine the efficacy of VectoBac WG and VectoBac DT treatment on the control of natural *Ae. aegypti* infestation.

Efficacy of VectoBac WG and VectoBac DT treatments for 3 months post-treatment

The post-treatment *Ae. aegypti* larvae and pupae were observed for a total of 34 days from 12 June to 6 September 2004; 1 day post-treatment, and 3 days per week for 11 consecutive weeks, thus covering a period of 3 months post-treatment. During post-treatment larval surveillance, colonization of aquatic predatory insects was also observed.

Aedes aegypti larval surveillance (Table 1)

Table 1 describes the mean number of days positive for *Ae. aegypti* larvae and aquatic predatory insects in the cement jars during the 3-month post-treatment entomological surveillance.

VectoBac WG and VectoBac DT significantly reduced the number of days positive for *Ae. aegypti* in comparison to the untreated jars with all 3 water types: well, rain and river water, for the first 4 weeks post-treatment ($p < 0.05$). After that, the number of VectoBac

treated jars positive for *Ae. aegypti* were not significantly different from the untreated jars with the well and rain water. The number day positive for larvae in the VectoBac treated jars with river water at 12 weeks post-treatment was significantly fewer than the untreated jars ($p < 0.05$).

The potential for VectoBac treated waters to control *Ae. aegypti* larvae in well and rain water was not evident due to the presence of aquatic predatory insects. Aquatic insects were observed to prey on *Ae. aegypti* larvae causing inconsistent *Ae. aegypti* larval densities in the jars. The density of aquatic predatory insects increased with time, ie the number of positive colonization days for predatory insects, in both untreated and VectoBac treated jars holding well and rain waters, significantly increased from an average of 3.20-3.55 days in the first one month post-treatment to an average of 9.20-10.00 days by the 3rd month post-treatment ($p < 0.05$). Thus, the positive *Ae. aegypti* larval colonization days decreased with time in the untreated jars holding well and rain waters. The river water containers had significantly fewer colonization days with aquatic predators, an average of 2.2-4.5 days for 12 weeks post-treatment, in comparison to well and rains water ($p < 0.05$). Thus, there was consistent colonization of *Ae. aegypti* larvae in the jars holding river water and the efficacy of VectoBac treatment was significantly evident in treated jars with river water for 12 weeks post-treatment.

VectoBac WG and VectoBac DT did not have any effect on the colonization of non-target insects, aquatic predatory insects. In the 3 water types, the number of colonization days with the aquatic predatory insects in the VectoBac treated jars was not significantly different from the untreated jars ($p > 0.05$).

Aedes aegypti pupae surveillance (Table 2)

All pupae collected from study jars were allowed to emerge in the CNM insectarium and identified. The successfully emerged *Ae.*

Table 1
 Mean number of days positive for breeding *Aedes aegypti* and aquatic predatory insects in cement jars during 12 weeks post-treatment entomological surveillance, at 3 days surveillance per week.

	Well water			Rain water			River water		
	1-4 weeks	5-8 weeks	9-12 weeks	1-4 weeks	5-8 weeks	9-12 weeks	1-4 weeks	5-8 weeks	9-12 weeks
	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a	post-treatment mean no. of days \pm SE (p < 0.05) ^a
<i>Ae. aegypti</i>									
1. UTC	3.82 \pm 0.86	2.00 \pm 1.00	1.27 \pm 0.73	3.70 \pm 0.75	1.70 \pm 0.79	1.70 \pm 1.10	3.10 \pm 1.04	3.50 \pm 1.09	3.70 \pm 1.08
2. VectoBac WG treated	1.27 \pm 0.30 (p=0.011)	0.27 \pm 0.14 (p=0.103)	1.36 \pm 0.41 (p=0.914)	1.10 \pm 0.31 (p=0.005)	0.40 \pm 0.40 (p=0.159)	0.90 \pm 0.53 (p=0.525)	0.40 \pm 0.22 (p=0.02)	0.60 \pm 0.31 (p=0.019)	1.10 \pm 0.41 (p=0.036)
3. VectoBac DT treated	0.64 \pm 0.24 (p=0.002)	0.82 \pm 0.55 (p=0.314)	1.09 \pm 0.49 (p=0.84)	0.80 \pm 0.29 (p=0.002)	0.70 \pm 0.40 (p=0.274)	0.90 \pm 0.71 (p=0.549)	0.40 \pm 0.16 (p=0.019)	0.80 \pm 0.39 (p=0.031)	0.80 \pm 0.49 (p=0.025)
Aquatic predatory insects									
1. UTC	3.55 \pm 1.02	8.45 \pm 1.13	10.00 \pm 1.10	3.20 \pm 0.98	8.40 \pm 0.98	9.20 \pm 1.19	2.20 \pm 0.94	4.00 \pm 1.18	4.50 \pm 1.20
2. VectoBac WG treated	2.64 \pm 0.86 (p=0.503)	7.45 \pm 1.36 (p=0.579)	9.45 \pm 1.16 (p=0.736)	2.40 \pm 0.96 (p=0.567)	6.40 \pm 1.27 (p=0.228)	7.80 \pm 1.59 (p=0.49)	2.10 \pm 0.72 (p=0.934)	3.60 \pm 1.03 (p=0.802)	5.60 \pm 1.28 (p=0.538)
3. VectoBac DT treated	2.55 \pm 1.02 (p=0.496)	7.36 \pm 1.11 (p=0.499)	9.55 \pm 1.05 (p=0.77)	2.20 \pm 0.96 (p=0.475)	6.90 \pm 1.19 (p=0.343)	10.0 \pm 0.92 (p=0.601)	2.10 \pm 0.98 (p=0.942)	4.80 \pm 1.28 (p=0.651)	7.10 \pm 1.06 (p=0.122)

^a : T-test was done to determine significant difference between the respective populations in treated and in untreated jars

aegypti adults were counted. The pupae surveillance analysis is summarized as the mean number of *Ae. aegypti* pupae collected per week for 3 months.

A day after treatment, larger numbers of pupae were collected from the treated jars compared to subsequent weeks. This is due to late L4 larvae not ingesting or having minimal ingestion of the *Bti* toxins, thus successful pupation. After this the pupae numbers remained low in the treated jars until the 6th week post-treatment.

The efficacy and persistence of the VectoBac treated containers were compared to the untreated containers (UTC). The efficacy and persistence was most evident in river water, followed by the well and rain water. A single application of VectoBac significantly reduced the pupae numbers for a minimum

of 3 months in river water, and for 2.5 months in well water ($p < 0.05$). The VectoBac treated river water had an average of 1-3 pupae per week for 3 months versus the UTC with an average density of 40-57 pupae per week. The treated well water had an average of 1-9 pupae per week for 3 months, and the UTC had 23-38 pupae per week.

For the rain water, the pupae density in the VectoBac treated jars was not significantly different from the untreated jars, although the treated jars yielded 2.0 to 5.2 fold fewer pupae than in the untreated jars during the 3 months post-treatment. In the 1st month post-treatment, the treated rain water had an average of 12-22 pupae per week versus the UTC density of 61 pupae per week. In the 2nd month the pupae density decreased in all containers, and this was followed by an increase in

Table 2
Mean number of pupae collected from cement jars per week from 12 June to 6 September 2004 and successfully emerged into *Ae. aegypti* adults.

Water type	Treatment	Mean no. of <i>Ae. aegypti</i> pupae collected per week		
		1 st month post-treatment $\bar{x} \pm SE$ (p value) ^a	2 nd month post-treatment $\bar{x} \pm SE$ (p value) ^a	3 rd month post-treatment $\bar{x} \pm SE$ (p value) ^a
River water	VectoBac WG	2.0 ± 1.41 (p=0.023)	1.25 ± 0.95 (p=0.066)	1.75 ± 1.75 (p=0.008)
	VectoBac DT	2.5 ± 2.50 (p=0.025)	2.25 ± 1.65 (p=0.072)	1.75 ± 0.25 (p=0.008)
	UTC	45.50 ± 14.22	39.75 ± 17.14	56.75 ± 13.98
Well water	VectoBac WG	5.5 ± 5.17 (p=0.082)	0.5 ± 0.29 (p=0.013)	9.0 ± 2.86 (p=0.396)
	VectoBac DT	4.25 ± 4.25 (p=0.069)	5.00 ± 3.72 (p=0.051)	3.50 ± 1.94 (p=0.242)
	UTC	37.75 ± 14.56	23.25 ± 6.50	22.50 ± 14.51
Rain water	VectoBac WG	21.50 ± 19.84 (p=0.377)	6.25 ± 5.60 (p=0.626)	29.5 ± 29.5 (p=0.715)
	VectoBac DT	11.50 ± 7.24 (p=0.23)	1.50 ± 1.50 (p=0.095)	9.0 ± 8.03 (p=0.213)
	UTC	61.00 ± 36.35	9.75 ± 3.88	44.00 ± 23.82

^a T-test was done to determine the significant difference between the respective populations in treated and untreated jars with each *Bti* formulation.

the 3rd month post-treatment with an average of 9-30 pupae per week in the treated containers versus an average of 44 pupae per week in the untreated containers.

The efficacy of VectoBac WG to control *Ae. aegypti* was not significantly different from the efficacy of VectoBac DT in the 3 water types ($p > 0.05$).

DISCUSSION

Pre- and post-treatment entomological surveillance established that the principal dengue mosquito vector breeding in the potable cement water jars in the study village, Phum Thmei, was *Ae. aegypti*. While *Ae. albopictus* was not found during this study. Occasionally *Ae. aegypti* was found together with *An. vagus* and *Cx. quinquefasciatus* in the same container. River, well and rain water supported the colonization of immature *Ae. aegypti* cement jars.

The potential for *Bti* to control dengue vectors in potable waters has been evaluated in countries with widespread temephos resistance in the *Ae. aegypti* larval population (Lerdthusnee *et al*, 1996; Santos *et al*, 2001; Ponce *et al*, 2002; Vilarinhos and Monnerat, 2004; Benjamin *et al*, 2005). *Bti* formulations (tablets, aqueous suspensions and water dispersible granules) were evaluated in potable containers fabricated from varying materials except cement. Thus, a pilot study was conducted for 6 months (April-September 2004) in Phum Thmei, to determine the efficacy of *Bti* formulations, VectoBac WG and VectoBac DT, in controlling natural infestation of *Ae. aegypti* in cement jars holding river, well, and rain water. Pupae surveillance was used to measure the efficacy of the VectoBac treatments (Benjamin *et al*, 2005). VectoBac WG and VectoBac DT were similarly effective in controlling *Ae. aegypti* in cement containers ($p > 0.05$). The efficacy and persistency of the VectoBac treatments were evident in the 3 water types for 2.5-3 months. It was most

evident in the river water due to the least interference from the aquatic insect predators. The efficacy results observed in the Cambodian village with VectoBac WG and VectoBac DT are similar to the control period of 1 to 3 months achieved in other countries, without water replenishment.

VectoBac WG and VectoBac DT are easy-to-use formulations. They required no repackaging before being introduced into the containers. The amount of VectoBac used to treat the waters was 12.5 fold less in weight than the weight of Abate 1% SG. On introduction, the VectoBac settled to the bottom of the containers. Sedimentation of the product to the base of the containers made it possible for the *Bti* toxins to be readily available to *Ae. aegypti* larvae, which are constantly found grazing at the base and sides of the container. In the study period of 3 months, the treated water remained clear and there was no unpleasant odor.

VectoBac WG and VectoBac DT did not have any effect on the colonization of the aquatic predatory insect, a corixid bug *Micronecta* sp, a natural enemy of *Ae. aegypti* larvae. In Thailand, it is believed that *Micronecta* is able to persist and provide sustainable control of *Ae. aegypti* in water storage containers (Suphaphathom *et al*, 2002).

VectoBac WG, at recommended mosquito control dose rates, is nontoxic to mammals on ingestion, skin contact or inhalation. WHOPES recommends the use of VectoBac WG in containers breeding *Ae. aegypti* and *Ae. albopictus* larvae (WHO/CDS/WHOPES/2004). Since 2003, the National Dengue Control Program in Brazil has used VectoBac WG for routine treatment of drinking water reservoirs to control temephos resistant *Ae. aegypti* larvae. VectoBac WG is a larvicide that can be used to control dengue mosquito vectors in Cambodia. For VectoBac WG to be considered for the National Dengue Vector Control Program, an operational research study

needs to be conducted in Cambodia; the efficacy and persistency of VectoBac WG needs to be determined in communes (>1,000 households) where all the treated containers are subjected to daily water exchange.

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