LABORATORY AND SEMI-FIELD EVALUATION OF MOSQUITO DUNKS® AGAINST *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* LARVAE (DIPTERA: CULICIDAE)

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Abstract. Laboratory bioassays and semi-field studies were conducted on the efficacy and longevity of Mosquito Dunks® (7,000 ITU/mg *Bti*) in order to determine the concentration-response relationship and the effectiveness on the potency of the *Bti* product against *Aedes* mosquito species based on the WHO protocol standard methods and to determine the longevity of release for this product against *Ae. aegypti* mosquito larvae in water storage containers. This bio-potency study with the late 3rd instar larvae of *Ae. aegypti* and *Ae. albopictus* was carried out according to WHO standard protocols. The six concentrations of the *Bti* product used in each test were replicated 4 times with 25 mosquito larvae. Probit analysis was then used to determine the LC_{50} and LC_{95} which was equated with dosages of 1.02 and 1.86 ppm for *Ae. aegypti*; and 0.39 and 0.84 ppm for *Ae. albopictus*, which reveals a potency of 382.95 and 303.74 ITU/mg, respectively. The semi-field evaluation of this product in 200-liter earthen jars against 3rd instar larvae of *Ae. aegypti* showed satisfactory control of greater than 80% at 11 weeks post-treatment.

INTRODUCTION

Currently, dengue fever is considered to be the most important arboviral disease of humans in terms of its public health impact (Gubler, 1989). The disease incidence and distribution have steadily increased with more than 2 to 3 billion people at risk of infection, and an estimated 20 million dengue cases annually (WHO, 1997). *Aedes aegypti* (Linnaeus) and *Ae. albopictus* (Skuse) serve as the primary and secondary vectors, respectively. Unlike the mosquitoes that cause malaria, dengue mosquitoes bite during the day. Although the viruses are related, antibodies obtained after infection with one serotype are not cross-protective for the other serotypes (Beaty and Marquardt, 1996). At present, dengue control measures include the use of chemicals for larvicide and space spraying, personal protection, health education and source reduction with the aim of immediate removal of infected mosquitoes. Insecticide use still remains a major component of any control strategy, especially during an outbreak. Chemicals frequently used are those belonging to the organophosphate and pyrethroid classes of insecticides (WHO, 1997). With the current trends in dengue incidence worldwide and without an effective vaccine or treatment, it is expected that the widespread use of insecticides will continue. This practice will likely lead to the selection of resistant strains, rendering current insecticides less effective, leading to the need to identify replacement control strategies. *Bacillus thuringiensis* var. *israelensis* (*Bti*) can be used to prevent these vectors from breeding (Marin/Sonoma Mosquito and Vector Control District, 2005). Controlled semi-field studies show that *Bti* can be effective for about 7 to 12 weeks (Mulla *et al*, 2004; Vilarinhos and Monnerat, 2004) in undisturbed conditions. We conducted laboratory investigations to determine the concentration-response

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relationship and the effectiveness of a *Bti* briquette against *Aedes* mosquito species based on WHO protocol standard methods and determined the longevity of the slow-release version of this product against the *Aedes aegypti* mosquito larvae in water storage containers.

MATERIALS AND METHODS

Laboratory study

Laboratory bioassays, based on the bioassay method for the titration of *Bti* preparations with IPS82 standard (World Health Organization Collaborating Center for Entomopathogenic *Bacillus*) were used to determine the effectiveness of the Mosquito Dunks® briquette [(AI 7,000 ITU *Aedes aegypti*/mg), Summit Chemical, Baltimore, MD, USA] against laboratory reared *Ae. aegypti* and *Ae. albopictus* larvae. The lyophilized powder of the reference standard IPS 82 (*Bti* strain 1884, standard titrating 15,000 ITU *Aedes aegypti*/mg) was used to compare with the *Bti* product. *Ae. aegypti* (strain BKK1) and *Ae. albopictus* late 3rd instar larvae were provided by the insectary, Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS). The tested larvae were starved for 24 hours before and during the test to minimize variability due to nutritional and metabolic conditions. *Bti* briquettes were ground and sieved several times before the bioassay test. In the assays, larvae were introduced into plastic cups containing 150 ml of test concentration. Bottled water was used for testing, as it is chlorine- free. Each bioassay involved 6 concentrations, 4 replications, and 25 larvae. A total of 600 larvae were tested with the standard IPS 82 and the *Bti* product and a total of 100 larvae for the control. A numbers of range-finding bioassays with widely spread exposure concentrations were conducted. Based on these tests, the results were used to determine a narrow range of concentrations for a more precise bioassay. The numbers of surviving larvae were counted at 24 hours and 48 hours. Death of mosquito larvae was determined as a complete lack of movement, even with gentle prodding with a probe. When pupation occured, the pupae were removed and their numbers excluded from the calculations. If more than 5% of the larvae pupated, the test was invalidated because larvae do not ingest 24 hours before pupation. Many larvae may have survived because they were too old.

Semi-field study

A semi-field evaluation was conducted at the research station of the National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health in Bang Bua Thong district, Nonthaburi Province, Thailand beginning in December 2004 for 12 weeks. Eight earthen jars (200 l/jar) were filled with water from the domestic water supply. Twenty-five laboratory-reared 3rd instar larvae of *Ae. aegypti* (from the NIH's insectary) were added to each jar. A quarter piece of Mosquito Dunks® (approximately 65 g) was then added to each jar. There were 4 treatment and 4 control jars. New batches of larvae were added weekly to the jars over a period of 12 weeks. Alive remaining mosquitoes were counted 1 week after each treatment. To count without disturbing the larvae, and to assess of adult emergence, pupal skins were recorded from the jars with a syringe and a small fish net were used for this semi-field experiment. Pupal skins floating on the water surface inside the jar wall were removed without disturbing the water. Pupal skins were placed in the water in white plastic trays and counted (Mulla *et al*, 2004). All tested jars were placed in a shaded test area and covered with Celocrete sheets. Covers were in place continuously, except during the addition of larvae and during assessment of efficacy once a week for 2-3 hours each time. Placement of covers prevented UV light and windborne debris from entering and other mosquitoes from laying eggs inside the test jars.

Analysis of data

Tests with control mortality greater than 10%, or any pupation greater than 5%, were discarded. A probit model was used to analyze the mortality of *Ae. aegypti* larvae as a function of *Bti* concentration. Probit regression analysis which the slope, LC_{50}, LC_{95} and their 95% confidential intervals (CI) were obtained. The toxicity (ITU/mg) of the *Bti* product was determined according to the following formula:

$$\text{Potency (}Bti\text{ product)} = \frac{LC_{50}\text{ (standard)}}{LC_{50}\text{ (}Bti\text{ product)}} \times \text{Potency (standard)}$$

The survival rate (%) and mortality rate (%) were calculated on the basis of the number of pupal skins (indicating adult emergence) compared to the initial number of larvae added. Mortality was not considered in the calculations, since it generally was very low and would not change the results (Mulla *et al*, 2004).

RESULTS

In the laboratory bioassay study, significantly different LC_{50} and LC_{95} values were determined for the *Bti* product tested against both the *Aedes* species (Table 1). *Ae. aegypti* tolerated higher *Bti* concentrations compared to *Ae. albopictus*. The LC_{50} and LC_{95} for *Ae. aegypti* were both 1.86 ppm, giving a potency of 382.95 ITU/mg. The LC_{50} and LC_{95} for *Ae. albopictus* were 0.39 and 0.84 ppm, respectively giving a potency of 303.74 ITU/mg.

During the 12-week semi-field study, we observed 100% adult emergence and 100% larval mortality in the first and second weeks post-treatment. Evaluation of the first batch added on the day of treatment, and the second and third batches added on weeks 1 and 2, respectively, showed 100% mortality (absence of pupal skins). There were some live larvae after week 3. The emergence rate of 7% was seen for weeks 4 and 5. At week 8, the survival rate started to increase. The emergence rates in weeks 10, 11 and 12 were 16, 20 and 23%, respectively, revealing declining efficacy (Table 2). This semi-field study shows that this larvicidal formulation provides long-lasting control of *Ae. aegypti* in water-storage jars under experimental conditions at a quarter of a briquette per 200 liters of water. It also showed a mortality rate of about 90% for 9 weeks. Although the mortality rate declined after weeks 8 post-treatment, satisfactory control of greater than 80% was observed for about 11 weeks. However, water replenishment may cause a reduction in *Bti* efficacy.

DISCUSSION

The *Bti* formulation in this experiment was in the form of a solid briquette of *Bti*, additives and cork, which floated on the water surface. The lethal concentration values obtained from this tested product may differ from other *Bti* formulations, as shown in the Brown *et al* (2001). They evaluated a liquid formulation of *Bti* against *Aedes* mosquito larvae. The liquid product tested on the 3rd instar larvae of *Ae. aegypti* were Cybate, Teknar and VectoBac 12 AS, which showed LC_{50} and LC_{95} values of 0.42, 0.72 l/ha and 0.14, 0.46 l/ha and 0.20, 0.40 l/ha, respectively. They revealed that these differences in

Table 1
Twenty-four-hour probit analysis of Mosquito Dunks® against late 3rd instar-larvae of *Ae. aegypti* and *Ae. albopictus*.

Bti product	Mosquito species	LC_{50} (ppm) (95% CI)	LC_{95} (ppm) (95% CI)	Slope (SE)	P^1
Mosquito Dunks®	*Ae. aegypti*	1.018 (0.976,1.066)	1.864 (1.518,2.360)	6.267 (0.66)	0.9
	Ae. albopictus	0.395 (0.332,0.471)	0.845 (0.551,1.314)	4.982 (0.85)	0.99
IPS 82	*Ae. aegypti*	0.026 (0.022,0.03)	0.114 (0.074,0.178)	2.545 (0.26)	0.99
	Ae. albopictus	0.008 (0.006,0.01)	0.029 (0.018,0.049)	2.891 (0.44)	0.98

P^1 refers to the probability corresponding to maximum likelihood chi-square statistics for goodness of fit of the model

Table 2
The survival and mortality rates of *Ae. aegypti* larvae after treated with *Bti* product for 12 weeks.

Bti product	Weeks post-treat	Adult emergence from pupal skin count post-treatment (weeks)	
		Survival rate (%)	Mortality rate (%)
Mosquito Dunks®	1	0	100
	2	0	100
	3	3	97
	4	7	93
	5	7	93
	6	10	90
	7	8	92
	8	13	87
	9	10	90
	10	16	84
	11	20	80
	12	23	77
Control	1	95	5
	2	98	2
	3	96	4
	4	94	6
	5	97	3
	6	98	2
	7	98	2
	8	94	6
	9	91	9
	10	94	6
	11	90	10
	12	92	8

efficacy were related to formulation characteristics. The *Bti* toxins per milligram vary between products. For our bioassay, the *Bti* briquette formulation contained both bacterial spores and associated toxins, which are crystals made of protein. The high lethal concentration values occurred probably because the product was ground into fine particles for serial dilution in the laboratory bioassay against IPS82 bacterial standard strain. Grinding and disposal of the cork may have resulted in the loss of toxin crystals, thereby diminishing the potency. The high LC_{50} and LC_{95} values in this bioassay reflect changes in the amount of toxin.

Mulla *et al* (2004) showed the longevity of VectoBac tablets (*Bti* 5%) with 3 regimens of water over a period of 20 weeks at a dose of 1 tablet (0.37 g) per 50 liters of water. The water regimens were full jars, half-full jars, and full jars emptied half way and refilled weekly. At week 12 post-treatment, VectoBac tablets provided excellent control for 98% inhibition of emergence (IE) in the full jars, 96% IE in the half-full jars, and 75% IE in the full jars emptied half way and had water refilled weekly. VectoBac tablets (full jars) showed good control for 16 weeks. A semi-field bioassay in Brazil by Lima *et al* (2005) showed a low persistence of *Bti* product in different weather conditions. They tested 0.2 g of VectoBac WDG per 100 liters of water against the late 3rd instar larvae of *Ae. aegypti* using various types of containers placed in a shaded area: plastic, iron, concreate, and asbestos. They found that during periods of higher temperature (21.5-39.3ºC), 70-100% mortality was observed for 1 week, which then declined abruptly thereafter in all types of containers. They also revealed that low persistence of *Bti* was obtained with-

out water replacement. An even lower residual effect of this formulation is expected in house storage conditions, where water is used, then refilled during rainfall.

Heavy use of insecticide in some tropical countries for vector control can enhance resistance in the *Ae. aegypti* population. Tests of different formulations and concentrations of Mosquito Dunks® reveal that this can be used as an alternate option for controlling *Aedes* mosquitoes. The Briquette formulation is an alternative used to overcome the lack of persistence, as it can be used in fast-flowing or turbulent waters, which is one of the major limitations of the *Bti* formulations. It is essential to evaluate this product in dengue vector control in different municipalities.

ACKNOWLEDGEMENTS

We would like to thank Mr Wichai Kongngamsuk, Division of Medical Entomology, Department of Medical Sciences, National Institute of Health, Ministry of Public Health of Thailand for providing the IPS-82 international reference standard *Bti* for this study and for his valuable comments and guidance on the laboratory techniques.

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