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INDOOR THERMAL FOGGING AGAINST VECTOR MOSQUITOES WITH TWO *BACILLUS THURINGIENSIS ISRAELENSIS* FORMULATIONS, VECTOBAC ABG 6511 WATER-DISPERSIBLE GRANULES AND VECTOBAC 12AS® LIQUID

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ABSTRACT. Bioefficacy of thermal fogging application of 2 *Bacillus thuringiensis israelensis* formulations, Vectobac ABG 6511 water-dispersible granules (3,000 international toxic unit [ITU]/mg) and Vectobac 12AS® liquid (1,200 ITU/mg), was assessed for larvicidal activities against *Aedes aegypti, Aedes albopictus, Anopheles dirus,* and *Culex quinquefasciatus*. Portable Agrofog® AF35 sprayers were used to apply the 2 formulations indoors in residential premises on Penang Island, Malaysia. Vectobac ABG 6511 showed good larvicidal effect against all 4 mosquito species at 3 of the higher doses tested (2.91×10^9, 1.45×10^9, and 0.71×10^9 ITU/ha), with more than 96% mortality at 48 h after spraying. As a comparative formulation, Vectobac 12AS also showed good larvicidal activity against all 4 mosquito species at 2 of the higher doses tested (2.87×10^9 and 1.46×10^9 ITU/ha), with more than 92.5% mortality at 48 h after spraying. Larvae of *An. dirus* were significantly more susceptible to both water-based Vectobac formulations when compared to the other 3 mosquito species. Both microbial formulations showed better efficacy at higher doses. However, even at the lowest dose tested, Vectobac ABG 6511 and Vectobac 12AS (both at 0.36×10^9 ITU/ha) showed larvicidal properties, with more than 66% mortality at 48 h after spraying. Overall, for this bacterial agent, the water-dispersible granule formulation has better prospects than the liquid formulation for the control of larvae of vector mosquitoes.

KEY WORDS Thermal fogging, *Bacillus thuringiensis israelensis, Aedes aegypti, Aedes albopictus, Anopheles dirus, Culex quinquefasciatus*

INTRODUCTION

Control of vectorborne diseases, in particular dengue fever (DF) and dengue hemorrhagic fever (DHF), in Malaysia and other neighboring Southeast Asian countries follows World Health Organization (WHO) guidelines. House-to-house indoor thermal fogging sprays of diesel-based malathion (Cythion®; American Cyanimide, Princeton, NJ) are carried out when a DF or DHF outbreak occurs (Yap 1984; WHO 1995, 1997; Yap and Zairi 1999). Field evaluation of a few thermal fogging insecticide formulations against mosquitoes have been carried out in Malaysia (Yap et al. 1983, 1988, 1999, 2000, 2001) as well as in other tropical countries (Castle et al. 1999).

Microbial control of vector mosquitoes, such as by use of *Bacillus thuringiensis israelensis* (*Bti*), is a relatively recent development. Several types of formulations are used in Malaysia (Foo and Yap 1982, 1983; Lee and Seleena 1992; Lee et al. 1996, 1997a). The effectiveness of *Bti* has been demonstrated in the control of mosquitoes such as *Culex* species (Foo and Yap 1982, Balaraman et al. 1983, Fry-O'Brien and Mulla 1996), *Aedes* species (Van Essen and Hembree 1980, Foo and Yap 1982, Klowden and Bullar 1984), *Anopheles* species (Foo and Yap 1982, Pantuwatana and Yougvanitsed 1984), and *Mansonia* species (Foo and Yap 1982, 1983).

The advantages of use of a microbial agent, in particular the bacterial agent *Bti*, for indoor vector control are that it can be used in drinking water, it has adequate larvicidal activity, and it is nontoxic to nontarget organisms (WHO 1999).

Therefore, further development of such microbial agents is needed as an additional tool for the overall control program for vector mosquitoes. Recently, a new formulation of *Bti*, water-dispersible granules (WDGs; Vectobac ABG 6511), was evaluated as a larvicide against *Culex* species (Su and Mulla 1999). The objective of this study was to investigate the larvicidal efficacy of the WDG formulation granules in comparison with Vectobac 12AS® liquid, by using a portable thermal fogging sprayer in living premises on Penang Island, Malaysia.

MATERIALS AND METHODS

Mosquitoes: Laboratory-cultured, late 3rd- or early 4th-stage larvae of 4 mosquito species (*Aedes aegypti* (L.), *Aedes albopictus* (Skuse), *Culex quinquefasciatus* Say, and *Anopheles dirus* Peyton and Harrison) from the Vector Control Research Unit, Universiti Sains Malaysia, were used in this study.

Insecticide formulations and equipment: Samples of the WDG formulation (Vectobac ABG 6511, 3,000 international toxic unit [ITU]/mg) and liquid formulation (Vectobac 12AS, 1,200 ITU/mg) of *Bti* obtained from Abbott Laboratories (now Valent Biosciences Corporation, Libertyville, IL) were used for the tests. Two portable thermal foggers (Agrofog® model AF 35, Agro Technic Pte Ltd., Singapore) were used for larvicide applications. The discharge rate of the machines with nozzle size

Table 1. Summary of fogging time, discharge rate for Agrofog thermal fog machine (model AF35) with nozzle size 0.8, and mean *Bacillus thuringiensis* H-14 sprayed per house for each bacterial formulation in assessed premises at Lorong Mahsuri, Bayan Baru, Penang Island, Malaysia. The dilution rates given in the column heads are the ratio of Vectobac to seasoned water.[1]

	Vectobac WDG (ABG 6511)				Vectobac 12AS®			
	1:20	1:40	1:80	1:160	1:8	1:16	1:32	1:64
Mean Vectobac formulation sprayed	968.6 g/ha	483.3 g/ha	237.5 g/ha	121.0 g/ha	2389.2 ml/ha	1215.5 ml/ha	597.3 ml/ha	303.9 ml/ha
Mean ITU per ha sprayed	2.91×10^9	1.45×10^9	0.71×10^9	0.36×10^9	2.87×10^9	1.46×10^9	0.72×10^9	0.36×10^9

[1] WDG, water-dispersible granules; ITU, international toxic unit.

of 0.8 were set at 245 ± 5 ml/min. All spraying activities were conducted between 1800 and 1930 h.

Fogging operations and bioefficacy assessment: The trials were carried out in single-story terrace residential houses in an urban settlement situated in Bayan Baru, a town on the southwestern coastal area on Penang Island, Malaysia. The performance of the WDG formulation at dilution rates of 1:20, 1:40, 1:80, and 1:160 and the liquid formulation at dilution rates of 1:8, 1:16, 1:32, and 1:64 were compared. The assessments for each of the species were carried out with 20 larvae placed in a cylindrical paper cup (top diameter 8 cm and height 10 cm) filled with 200 ml of seasoned tap water at each checkpoint. Two checkpoints, 1 in the living room and the other in the kitchen, were set for each tested house. The test site consisted of more than 20 lanes of residential terrace houses. A minimum of 10 similar-sized single-story concrete houses (5 with assessment cups) at alternate positions (with 1 house in between) from a single lane were chosen for the spraying of each of the formulation. Different lanes, at least 50 m apart, were chosen for the spraying of each formulation. Each of the selected houses was sprayed for a period of 56.6 ± 2.5 sec and an area of approximately 120 m^2 was covered. With the mean discharge rate of the machine at 245.0 ± 5.0 ml/min, the mean volume sprayed per house was 231.0 ± 3.0 ml.

The thermal fogging application of *Bti* formulations followed essentially the same technique used in the operational control of dengue vectors, namely, *Ae. aegypti* and *Ae. albopictus,* during a DF outbreak (WHO 1995, 1997). When conducting a house-to-house spray, a team of 2 persons is required. The 1st person operates the fogging machine and the 2nd person guides the 1st person and keeps track of spraying time. The team usually starts spraying from the back of the house (i.e., the kitchen area) and walks backwards until they reach the front of the house (i.e., the living room). The entire process of spraying from the back to the front of the house is done within a specified time. While spraying, the nozzle of the thermal fogging machine is pointed approximately 30° downwards and is swung constantly from side to side to ensure even coverage of the targeted house.

Larvae were brought back to the laboratory after 1 h of exposure and kept in clean paper cups. Mortality of larvae was recorded at 24 and 48 h after treatment. For comparative efficacy, the percentage of mortality values were subjected to an arcsine transformation followed by a comparison of means using the Duncan multiple range test (SAS Institute 1985). Temperature and percentage of relative humidity of indoor premises were recorded.

RESULTS AND DISCUSSION

Details of the sprays applied (dilutions, quantity, and ITUs applied) are shown in Table 1. The mean

Table 2. Mean percentage mortality against mosquito larvae of *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus,* and *Anopheles dirus* in both living rooms and kitchen areas from 5 assessed premises situated at Lorong Mahsuri, Bayan Baru, Penang Island, at 24 and 48 h after treatment by thermal fog spraying with various *Bacillus thuringiensis* H-14 formulations. No mortality was recorded in the control group.[1]

		Aedes aegypti		*Aedes albopictus*		*Culex quinquefasciatus*		*Anopheles dirus*	
Formulations[2]	Location	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Vectobac WDG (ABG 6511) diluted in 20-fold water (2.91 × 10^9 ITU/ha)	Living room	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	98.0 ± 1.0^{a}	99.0 ± 1.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	Kitchen	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	Total	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	99.0 ± 1.0^{a}	99.5 ± 1.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Vectobac WDG (ABG 6511) diluted in 40-fold water (1.45 × 10^9 ITU/ha)	Living room	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	98.0 ± 1.0^{a}	98.0 ± 1.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	Kitchen	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	98.0 ± 1.0^{a}	99.0 ± 1.0^{a}	82.0 ± 6.4^{b}	100.0 ± 0.0^{a}
	Total	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	98.0 ± 1.0^{a}	98.5 ± 1.0^{a}	91.0 ± 3.2^{b}	100.0 ± 0.0^{a}
Vectobac WDG (ABG 6511) diluted in 80-fold water (0.71 × 10^9 ITU/ha)	Living room	52.0 ± 1.0^{d}	100.0 ± 0.0^{a}	67.0 ± 18.4^{c}	98.0 ± 1.0^{a}	97.0 ± 1.0^{a}	100.0 ± 0.0^{a}	84.0 ± 4.8^{b}	97.0 ± 2.2^{a}
	Kitchen	50.0 ± 1.0^{d}	100.0 ± 0.0^{a}	65.0 ± 14.6^{c}	98.0 ± 1.0^{a}	95.0 ± 1.0^{a}	100.0 ± 0.0^{a}	82.0 ± 3.2^{b}	95.0 ± 1.4^{a}
	Total	51.0 ± 1.0^{d}	100.0 ± 0.0^{a}	66.0 ± 16.5^{c}	98.0 ± 1.0^{a}	96.0 ± 1.0^{a}	100.0 ± 0.0^{a}	83.0 ± 4.0^{b}	96.0 ± 1.8^{a}
Vectobac WDG (ABG 6511) diluted in 160-fold water (0.36 × 10^9 ITU/ha)	Living room	53.0 ± 13.6^{c}	61.0 ± 15.4^{c}	62.0 ± 17.4^{c}	77.0 ± 6.4^{c}	71.0 ± 3.2^{c}	87.0 ± 2.6^{b}	94.0 ± 2.2^{a}	100.0 ± 0.0^{a}
	Kitchen	70.0 ± 14.2^{b}	79.0 ± 13.6^{b}	47.0 ± 22.4^{d}	61.0 ± 12.4^{c}	70.0 ± 4.6^{c}	77.0 ± 4.6^{b}	96.0 ± 1.8^{a}	98.0 ± 1.2^{a}
	Total	61.5 ± 13.9^{c}	70.0 ± 14.5^{c}	54.5 ± 19.9^{d}	69.0 ± 9.4^{c}	70.5 ± 3.9^{c}	82.0 ± 3.6^{b}	95.0 ± 2.0^{a}	99.0 ± 0.6^{a}
Vectobac 12AS® diluted in 8-fold water (2.87 × 10^9 ITU/ha)	Living room	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	99.0 ± 1.0^{a}	99.0 ± 1.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	Kitchen	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	Total	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	99.5 ± 0.5^{a}	99.5 ± 0.5^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Vectobac 12AS diluted in 16-fold water (1.46 × 10^9 ITU/ha)	Living room	93.0 ± 1.0^{a}	97.0 ± 2.2^{a}	94.0 ± 2.0^{a}	96.0 ± 2.0^{a}	80.0 ± 6.4^{b}	90.0 ± 3.6^{a}	96.0 ± 4.6^{a}	100.0 ± 0.0^{a}
	Kitchen	92.0 ± 1.0^{a}	95.0 ± 3.2^{a}	96.0 ± 1.0^{a}	99.0 ± 1.0^{a}	82.0 ± 8.2^{b}	95.0 ± 5.4^{a}	89.0 ± 4.2^{b}	99.0 ± 1.0^{a}
	Total	92.5 ± 1.0^{a}	95.5 ± 2.7^{a}	95.0 ± 1.5^{a}	97.5 ± 1.5^{a}	81.0 ± 7.3^{b}	92.5 ± 4.5^{a}	92.5 ± 4.4^{b}	99.5 ± 0.5^{a}
Vectobac 12AS diluted in 32-fold water (0.72 × 10^9 ITU/ha)	Living room	62.0 ± 14.2^{c}	62.0 ± 15.6^{c}	66.0 ± 16.8^{c}	76.0 ± 6.6^{b}	65.0 ± 12.4^{c}	77.0 ± 5.5^{b}	83.0 ± 8.4^{b}	99.0 ± 0.8^{a}
	Kitchen	79.0 ± 13.2^{c}	94.0 ± 5.2^{a}	83.0 ± 4.2^{b}	84.0 ± 4.2^{b}	71.0 ± 11.2^{c}	80.0 ± 4.3^{b}	86.0 ± 6.6^{b}	100.0 ± 0.0^{a}
	Total	70.5 ± 13.7^{c}	78.0 ± 10.4^{b}	75.0 ± 10.5^{c}	80.0 ± 5.4^{b}	68.0 ± 11.8^{c}	78.5 ± 4.9^{b}	84.5 ± 7.5^{b}	99.5 ± 0.4^{a}
Vectobac 12AS diluted in 64-fold water (0.36 × 10^9 ITU/ha)	Living room	60.0 ± 13.4^{c}	70.0 ± 13.8^{b}	69.0 ± 16.8^{c}	71.0 ± 8.2^{b}	34.0 ± 22.6^{e}	50.0 ± 12.2^{d}	55.0 ± 14.2^{d}	68.0 ± 12.6^{c}
	Kitchen	59.0 ± 25.2^{c}	62.0 ± 24.2^{c}	73.0 ± 14.6^{c}	83.0 ± 4.2^{b}	74.0 ± 16.2^{c}	82.0 ± 6.4^{b}	62.0 ± 9.8^{d}	90.0 ± 1.2^{b}
	Total	59.5 ± 19.3^{c}	66.0 ± 19.0^{c}	71.0 ± 14.7^{c}	77.0 ± 6.2^{b}	54.0 ± 19.4^{d}	66.0 ± 9.3^{c}	59.5 ± 12.0^{d}	79.0 ± 6.9^{b}

[1] Mean percentages mortality followed by the same letters within the same columns are not significantly different ($P < 0.05$, Duncan multiple range test).

[2] WDG, water-dispersible granules; ITU, international toxic unit.

temperature (27 ± 1.2°C) and relative humidity (90 ± 3.5%) that were recorded reflect the normal hot and humid indoor tropical environment. Vectobac ABG 6511 showed excellent larvicidal effects against all 4 mosquito species at 3 of the doses tested (2.91 × 10^9, 1.45 × 10^9, and 0.71 × 10^9 ITU/ha), with more than 96% mortality at 48 h after spraying. As a comparative formulation, Vectobac 12AS showed good larvicidal activity against all 4 mosquito species at 2 of the higher doses tested (2.87 × 10^9 and 1.46 × 10^9 ITU/ha), with more than 92.5% mortality at 48 h after spraying. However, Vectobac 12AS at 0.36 × 10^9 ITU/ha was less effective against *Cx. quinquefasciatus* at 24 h after spraying (Table 2).

Larvae of *An. dirus* were significantly more susceptible than the other test species to both water-based Vectobac ABG 6511 and Vectobac 12AS at lower doses (0.36 × 10^9 ITU/ha). Overall, results indicate that Vectobac ABG 6511 and Vectobac 12AS at higher doses (1.45–2.91 × 10^9 and 1.46–2.87 × 10^9 ITU/ha) are highly effective larvicides against all 4 mosquito species (Table 2). Moreover, even at the lowest dose tested, Vectobac ABG 6511 (0.36 × 10^9 ITU/ha) and Vectobac 12AS (0.36 × 10^9 ITU/ha) showed some larvicidal properties, with more than 66% mortality at 48 h after spraying. The results in both of the spraying localities (kitchen and living room) were similar for both Vectobac formulations against all 4 mosquito species tested.

In conclusion, Vectobac ABG 6511 provided significantly better larvicidal activity when compared with Vectobac 12AS at similar doses (in terms of ITU/ha). This suggests that the new granule formulation is more effective as a larvicide against all 4 vector mosquito species tested and should be considered as an additional tool for vector control. Furthermore, the WDG formulation is a more suitable formulation for vector control in a tropical environment because of its easy shipment and longer shelf-life when compared with liquid formulations.

In vector mosquito control programs, simultaneous control of adult and larval stages is preferable so as to reduce the overall vector mosquito population and subsequently reduce or disrupt disease transmission. In the case of dengue vector control, such an approach is essential because of transovarial transmission of dengue virus in *Ae. aegypti* and *Ae. albopictus* (Lee et al. 1997b).

In outdoor ultra-low–volume spray, tests recently have been carried out on the combination of chemical adulticides such as Pesguard™ 102 (active ingredient [AI]: *d*-allethrin and *d*-phenothrin, both at 5% weight/weight [w/w]), Aqua Resigen® (AI: *s*-bioallethrin 0.14%, permethrin 10.11%, and piperonyl butoxide 9.96% w/w/w), and malathion with *Bti* as larvicide so as to achieve both adult and larva control with 1 spray (Lee et al. 1997b; Yap et al. 1997a, 1997b). The possibility of achieving both larvicidal and adulticidal activity by integrating chemical and microbial agents with indoor thermal fogging spray should also be investigated.

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