

FIELD TRIALS TO DETERMINE THE EFFECTIVENESS OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* APPLICATION USING AN ULTRA LOW VOLUME GENERATOR FOR THE CONTROL OF *AEDES* MOSQUITOES

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ABSTRACT

Ultra low volume (ULV) fogging trials of mosquitocidal *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) together with malathion against *Aedes* mosquito larvae and adults were conducted in an open-air field, in housing estates and at a construction site. A commercial aqueous *Bti* formulation, VECTOBAC 12AS® (Abbott Laboratories) containing 1,200 ITU/mg against *Aedes aegypti*, and malathion 96% technical grade were used. ULV generators, viz. IGEBBA® and Dynafog Maxi Pro 4® were used to disperse these formulations at discharge rates ranging between 0.25 to 0.50 L/min. The effectiveness of the ULV fogging was evaluated by measuring 4 different parameters at various distances from the ULV generator: larval mortality, adult mortality, *Bti* count from the test samples and ULV droplet analysis. These trials have indicated that ULV fogging is effective in dispersing a mixture of malathion and *Bti*, to affect complete larval and adult mortality simultaneously. However, the mortality varied in relation to the distances from the ULV generator, depending on the structure and surroundings of the house. The fogged *Bti* particles were able to affect larval mortality for a duration of 14 d post ULV, indicating the persistency of the fogged *Bti* particles in the environment. These trials have shown that ULV fogging is effective in dispersing bacteria and malathion simultaneously; however, to ensure a successful fogging operation the flow rates have to be adjusted in accordance with the ULV generator used and the environment.

KEY WORDS: Ultra low volume fogging, *Bacillus thuringiensis* subsp. *israelensis*, malathion, *Aedes* mosquito control.

INTRODUCTION

In Malaysia, dengue fever and dengue haemorrhagic fever are *Aedes aegypti* and *Ae. albopictus* borne viral diseases. These *Aedes* vectors are indoor and outdoor container breeders, respectively. Malathion is dispersed by ULV and/or thermal fogging by the health authorities as an adulticidal control measure during outbreaks. Residents in the affected areas are encouraged to apply temephos (a chemical larvicide) in all water-storing containers.

Bacillus thuringiensis subsp. *israelensis* (*Bti*) is the most effective microbial control agent against mosquito larvae available to date. *Bti* has many advantages over chemical insecticides: it is environmentally friendly, and highly specific and toxic towards mosquitoes without development of resistance in the insects. However, its main limitation is that the toxin has to be ingested by the mosquito larvae, thus making the dispersal of *Bti* in the larval breeding sites very crucial and difficult, especially so when these sites are not easily detectable and/or inaccessible. Experience gained from field trials using *Bti* for the control of *Culex pseudo-vishnui* (Lee and Seleena, 1990), *Anopheles*

ingested by the mosquito larvae, thus making the dispersal of *Bti* in the larval breeding sites very crucial and difficult, especially so when these sites are not easily detectable and/or inaccessible. Experience gained from field trials using *Bti* for the control of *Culex pseudo-vishnui* (Lee and Seleena, 1990), *Anopheles maculatus* (Lee et al., 1994) and *Aedes albopictus* (Lee and Seleena, 1992) showed that for effective application close proximity with the larval breeding sites was a prerequisite.

Microdroplet application of chemicals using an ULV generator is a technique developed for the dispersion of chemical insecticides (Matthews, 1985). This technique, commonly used in Southeast Asian countries for the fogging of malathion, has enabled an efficient coverage of target areas with insecticides. Since ULV-applied malathion does not exhibit any *Ae. aegypti* larvicidal effect (Lee et al., 1987), the following study was conducted to determine the effectiveness of dispersing *Bti* through an ULV generator for the control of mosquito larvae.

An effective and efficient control of vector mosquito populations will be a system able to control both adult and larval vector mosquito populations simultaneously. Hence in our study the effectiveness of dispersing a larvicidal bioinsecticide, *Bti*, and an adulticidal insecticide, malathion, together, using an ULV generator, was also studied for the control of *Aedes* mosquitoes.

MATERIALS AND METHODS

The trials were conducted using the commercial aqueous *Bti* formulation, VECTOBAC 12AS[®] (Abbott Laboratories) containing 1,200 ITU/mg against *Aedes aegypti*, and malathion 96% technical grade. Truck mounted ULV generators, viz. IGEBA[®] or Dynafog Maxi Pro 4[®] were used, as available, in the target sites to disperse the formulations. The flowmeter was adjusted to provide the desired flow rate. The truck moved at a slow constant speed (10 kph) covering the test site, with the generator's nozzle pointing towards the test samples.

Five different trials were conducted:

Trials 1 and 2

Trials 1 and 2 were conducted as preliminary trials in an open-air field measuring about 150 × 100 m, before embarking on the actual trials in housing estates and at a construction site. In trial 1 the bacteria was dispersed at a discharge rate of 0.3 L/min and in trial 2 at 0.5 L/min using the ULV generator IGEBA[®].

Trial 3

This trial was conducted in a double storey terrace housing estate in Petaling Jaya, about 35 km from Kuala Lumpur. Two rows of houses facing each other were chosen for this study. Each house is about 50 ft long from the gate to the backyard. To determine the effectiveness of the fogging in relation to the distance from the ULV generator, each house was divided into 4 sections: the first 15 ft from the gate to the front door was labelled as outside; the inside of the house, which is about 35 ft long, was divided into 3 sections. The ULV generator IGEBA[®] was used to disperse VECTOBAC 12AS[®]. The truck moved at a slow constant speed of 10 kph, with the nozzle pointing towards the porch of the house, dispensing the *Bti* at a flow rate of 0.5 L/min. There was a slight breeze during the trial and the weather was fair.

Trial 4

Trial 4 was conducted in a housing estate consisting of a single-storey semi-detached housing estate in Pandamaran, Klang, 50 km from Kuala Lumpur. Again two rows of houses were chosen for this study. Each house is about 65 ft long from the gate to the backyard. The house was divided into 3 sections: the first 40 ft from the gate to the front door; the inside of the house, which is about 25 ft long, was divided into 2 sections. The ULV generator Dynafog Maxi Pro 4[®] was used to disperse a mixture of 9 parts of VECTOBAC 12AS[®] with 1 part malathion 96% technical grade at a flow rate of 0.25 L/min. There was a slight breeze during the trial and the weather was fair.

Trial 5

Trial 5 was conducted in a partially completed multi-storey car park in Petaling Jaya. The ULV generator IGEBA[®] was used to disperse a mixture of VECTOBAC 12AS[®] and malathion 96% technical grade (9v:1v) at a flow rate of 0.5 L/min.

EVALUATION OF TRIALS

The effectiveness of each trial was evaluated by measuring 4 different parameters, namely: larval mortality, adult mortality, *Bti* quantification and ULV droplet analysis.

Larval mortality

In trials 1 and 2 the larval mortality was measured by placing cups containing 50 mL water with 15 fourth instar (L_4) lab-bred *Ae. aegypti* larvae at 10 ft intervals, beginning with 10 ft from the generator to 100 ft in a straight line. One hour after ULV fogging the cups were removed from the test sites and brought to the laboratory. The cups with the test water were left at room temperature (28–32°C). Larval mortality was scored 24 h post ULV. All dead/live larvae were subsequently removed from the cups.

The persistency of the fogged *Bti* mosquitocidal toxins in the test water was determined by adding 10 fresh L_4 larvae into the cups 7 d and 14 d post ULV and scoring the larval mortality after overnight exposure.

In trials 3 and 4, cups with 50 mL sterile distilled water were placed randomly in the porch, and in the interior of the house. One set of the cups was collected an hour after fogging, but 2 sets were left behind to determine the persistency of the fogged *Bti* toxins under field conditions. *Ae. aegypti* and *Ae. albopictus* larvae were added into the cups in the laboratory and the larval mortality was scored after overnight exposure. The cups which were left behind in the field were brought to the laboratory 7 and 14 d post ULV. To these cups also larvae were added and the mortality was scored after overnight exposure.

In trial 5 that was conducted at the multi-storey car park, cups with 50 mL sterile distilled water were placed at various points in 2 of the floors: basement 1 and basement 2. An hour after fogging these cups were collected, *Ae. aegypti* larvae were added and the mortality was scored after overnight exposure. Prior to and after ULV fogging, 50 mL water samples were collected from the stagnant water pools from the basement floor, shafts and shallow ditches along the basement walls. To these samples, too, larvae were added and the mortality was scored after overnight exposure.

Adult mortality

In trial 4 in which malathion, the adulticidal insecticide was dispersed together with *Bti*, the adult mortality was measured. Cages of 25 sucrose-fed, <7 d old adult female *Ae. aegypti* and *Ae. albopictus* mosquitoes were placed in the interior and at the exterior of the houses (various distances from the ULV generator). An hour after ULV fogging the adult mosquitoes were transferred to paper cups and fed with sugar solution. The adult mortality was recorded 24 h post ULV.

In trial 5 adult mortality was not measured, as adult mosquitoes were not available at the time of the trial.

Bti quantification

The amount of *Bti* in terms of colony forming units/mL (cfu/mL) in the test samples was also measured. For this purpose water samples were collected in sterile containers at intervals of 1 h, 7 d and 14 d post ULV. The samples were then plated onto *B. thuringiensis* selective media (NYPC), containing nutrient agar (23 g/L), yeast (0.5 g/L), MnCl₂ (6 mg/L), CaCl₂ (80 mg/L), MgCl₂ (70 mg/L), polymyxin B sulphate (0.1 g/L) and chloramphenicol (1 mg/L). The cfu/mL were enumerated after a 24 h incubation at 32°C. The number of cfu/mL in water samples collected 1 h post ULV indicated the coverage of ULV fogging, while cfu count for 7 and 14 d post ULV water samples showed the persistency of *Bti* in the test samples.

Droplet analysis

The distribution and size of sprayed particles were monitored through the use of magnesium oxide (MgO) coated slides. A slide was placed amidst the cups holding the larvae. Droplet diameter was measured for an average of 60 droplets for each MgO coated slide using a calibrated micrometer. The data was analysed using the ULV Droplet Analysis Programme of Sofield and Kent (1984). The mostly widely used parameter of droplet size is the volume median diameter (vmd). In any spray the droplets are divided into 2 equal parts by volume. Droplets with large volume are represented by vmd whilst the small volume droplets are represented by number median diameter (nmd). A uniform size of droplets, when the ratio of vmd to nmd is near 1, is preferred for an efficient ULV spray (Mount, 1985).

RESULTS

Trials 1 and 2 — open-air field

In trial 1 when the flow rate was 0.3 L/min, only a small number of droplets was observed on the MgO coated slides. The vmd and nmd of the fogged particles were 43.53 µm and 11.39 µm, respectively, viz. a ratio of 3.82. In trial 2 for the flow rate of 0.5 L/min more droplets were observed on the slide and the vmd and nmd were 57.56 µm and 24.97 µm respectively, i.e., a ratio of 2.3.

VECTOBAC 12AS[®] fogged at a discharge rate of 0.3 L/min, achieved a 70–100% mortality for *Ae. aegypti* larvae within 100 ft from the ULV generator in 24 h post ULV samples but the larval mortality was reduced by 50% in the 7 d post ULV samples and further declined in the 14 d post ULV test samples. Correspondingly, the *Bti* count for 7 and 14 d test samples also declined by 10² cfu/mL in comparison to 1 h post ULV samples.

In trial 2, VECTOBAC 12AS[®] discharged at 0.5 L/min achieved a 100% mortality for

Ae. aegypti larvae within 100 ft from the ULV generator, 24 h post ULV. The number of *Bti* particles fogged was sufficient to achieve a 80–100% larval mortality in 7 d post ULV samples. A higher *Bti* count (10^6 cfu/mL) was observed in the test samples compared to trial 1.

From trials 1 and 2 it was observed that VECTOBAC 12AS[®] can be dispersed efficiently by using an ULV generator, at discharge rates of 0.3 or 0.5 L/min. At these discharge rates an efficient coverage within 100 ft from the ULV generator in an open-air field was achieved. In comparison, the number of fogged *Bti* particles at 0.3 L/min was less than at 0.5 L/min and it was only efficient to achieve a 70–100% larval mortality among the *Aedes* larvae within 24 h post ULV. As the fogged VECTOBAC 12AS[®] *Bti* toxins undergo degradation with time, the numbers fogged at 0.3 L/min were not sufficient for residual activity against *Aedes* larvae. But the number of *Bti* particles fogged at 0.5 L/min was sufficient to bring about a complete mortality in 24 h post ULV samples and the numbers were just sufficient for 7 d post ULV residual activity in *Aedes* larvae. Thus, it was decided that for further trials a flow rate of 0.5 L/min would be used for the ULV generator IGEBAC[®] for microdroplet application of *Bti*.

Trial 3 — housing estate, Petaling Jaya

The diameter of the sprayed particles was in the range of 40–60 μm and the ratio of the vmd to nmd was close to 1.0, indicating that the dispersed droplets were of uniform size.

A 100% larval mortality for *Ae. aegypti* and *Ae. albopictus* was achieved in 60% of the test houses from the cups that were left outside the house. This complete mortality was maintained for the next 14 d after fogging. Less than 100% mortality was observed in the other 40% of the houses. The mortality in these houses was further reduced within the next 14 d post ULV. For the indoor test samples the maximum mortality achieved was only 35% and even this was solely for samples that had been placed within the first 5 ft of the indoors; beyond that, no mortality was observed. The mortality of the 7 d and 14 d post ULV indoor test samples further declined, indicating the deterioration of the fogged *Bti* toxins.

The larval mortality was correlated with droplet density and *Bti* cfu analysis. Droplet density and cfu count decreased with increasing distance from the ULV generator. The exterior of the house had a higher droplet density and cfu count than the interiors. This was due to very few *Bti* fogged particles reaching the inside of the house, thus causing a near negligible mortality in the interior of the house. This housing estate had more trees and plants in the exterior of the house than the housing estate fogged in trial 4. This vegetation presented a barrier, preventing sufficient *Bti* particles from reaching the target samples inside the house.

Trial 4 — housing estate, Pandamaran, Klang

The diameter of the sprayed particles was in the range of 45–48 μm and the ratio of the vmd and nmd was close to 1.0, indicating that the dispersed droplets were of uniform size.

Complete larval mortality was achieved for *Ae. aegypti* in 90% of the test houses from the samples that were left outside the house. This complete mortality was maintained for the next 14 d after fogging, except in 2 houses, near which the mortality declined by 30%. Complete mortality was also achieved in 30% of the test samples that were placed beyond 40 ft from the ULV generator and was maintained for the next 14 d post ULV. Mortality of the other 70% of the samples placed beyond 40 ft from the ULV generator ranged between 20 to 70% in 24 h post ULV, but the mortality of these samples increased by 30 to 80% in 7 d post ULV and then declined by 20% in 14 d post ULV.

Complete larval mortality was achieved also for *Ae. albopictus* in 80% of the cups that were left outside the house. Complete mortality in these was maintained for the next 14 d after fogging. The other 20% of the houses had 80% mortality and it declined with time. Complete mortality was also achieved in 25% of the test samples that were placed beyond 40 ft from the ULV generator and was maintained for the next 14 d post ULV. Mortality of the other 75% of the samples placed beyond 40 ft from the ULV generator ranged between 20 to 70% at 24 h post ULV, but the mortality of these samples increased by 30 to 80% in 7 d post ULV and then declined by 20% in 14 d post ULV.

The cfu count decreased with increasing distance from the ULV generator and this explains the small percentage of the test samples beyond 40 ft from the ULV generator having complete mortality.

Complete adult mortality was obtained for *Ae. aegypti* and *Ae. albopictus* which were placed in the exterior and interior of the houses within a maximum distance of 65 ft from the ULV generator.

Trial 5 — multi-storey car park

The droplet analysis data indicated an average nmd of 33.5 μm and vmd of 36.3 μm with a ratio of about 1, indicating uniform distribution of the droplets.

A 100% larval mortality was obtained for *Ae. aegypti* in all the 28 cups that were placed at various points in basement 1 and basement 2. Complete mortality was maintained for the next 14 d post ULV.

As to the 50 mL water samples that were collected from the stagnant water pools from the basement floor, shafts and shallow ditches along the basement walls — no mortality was observed in the samples collected prior to fogging, but complete mortality was obtained for all the samples that were collected post ULV fogging and was maintained for the next 14 d post ULV.

DISCUSSION

The five trials that were carried out indicated that VECTOBAC 12AS[®], an aqueous suspension formulation of *Bti*, is adequate for effective dispersion in mosquito larval breeding sites using ULV techniques. With the sufficient amounts reaching the target sites, VECTOBAC 12AS[®] was able to achieve complete mortality, especially for the container-breeding *Aedes* mosquito larvae. Even with some degradation of its toxins in the field, VECTOBAC 12AS[®] was still able to ensure sufficient residual activity for a duration of 14 d post ULV.

From trials 4 and 5 it can be concluded that VECTOBAC 12AS[®] can be dispersed simultaneously with malathion, with neither *Bti* nor malathion having any adverse effects on the other's mosquitocidal activity. In trial 4, compared to the 24 h post ULV samples an increase in larval mortality was observed in 70% of the 7 d post ULV samples that had been placed beyond 40 ft from the ULV generator. This indicated that malathion in the formulation may have delayed the larvicidal activity of *Bti*. This phenomenon was tested in the laboratory with 2 different formulations being bioassayed against *Ae. aegypti* larvae: formulation 1 — a mixture of *Bti* and water (9v:1v); formulation 2 — a mixture of *Bti* and malathion (9v:1v). These formulations were bioassayed 0 h, 7 d and 14 d post preparation. The 0 h post preparation formulation 1 gave an LC50 of 0.0003 mg/L, while formulation 2 gave an LC50 of 0.0005

mg/L. The 7d and 14 d post preparation formulations 1 and 2 both gave an LC50 of 0.0003 mg/L. The laboratory and field results thus indicated that malathion delays the larvicidal activity of *Bti*. The delay could be due to malathion being an oily organic solution coating the *Bti*, thus making the fogged *Bti* particles unavailable for larvicidal activity in the larval midgut. But with time, as malathion breaks down/uncoats itself from *Bti*, the fogged *Bti* particles were made available without any loss of toxicity. VECTOBAC 12AS[®], too, did not exert any negative effect on malathion, as 100% adult mortality was obtained in the *Aedes* mosquito species.

Two different ULV generators, viz., IGEBA[®] and Dynafog Maxi Pro 4[®] were used in the five trials; IGEBA[®] operates at an air pressure of 4.5 psi with a 9 horse power engine, while Dynafog Maxi Pro 4[®] operates at an air pressure of 8.0 psi with an 18 horse power engine. The Dynafog Maxi Pro 4[®] with its 4 nozzles was able to effectively deliver sufficient *Bti* toxins and malathion to a distance of 65 ft at a flow rate of 0.25 L/min as shown in trial 4. The amounts discharged were also sufficient to give complete residual activity against container-breeding *Aedes* larvae for a minimum of 14 d post ULV. As IGEBA[®] operates only at 9 hp, it was only able to achieve this efficiency in trials 2, 3 and 5 at 0.5 L/min, a flow rate that was twice that discharged by Dynafog Maxi Pro 4[®].

These trials have thus shown that ULV fogging is effective in dispersing *Bti* and malathion simultaneously for the control of *Ae. aegypti* and *Ae. albopictus* mosquito populations, but to ensure a successful larviciding and adulticiding fogging operation, the flow rates have to be adjusted in accordance with the ULV generator used and the nature of the target site.

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