MICRODROPLET APPLICATION OF MOSQUITOCIDAL *BACILLUS THURINGIENSIS* USING ULTRA-LOW-VOLUME GENERATOR FOR THE CONTROL OF MOSQUITOS

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Abstract. In an effort to develop a more effective technique in dispersing a microbial control agent, *Bacillus thuringiensis* (Bt), a truck- mounted ultra low volume (ULV) generator (Scorpion™) was used to disperse *B. thuringiensis israelensis* (Bti) and Bti with malathion. Complete larval and adult mortalities for all tested mosquito species within the first 70-80 feet from the ULV generator were achieved. Beyond that distance less than 50% mortality was achieved as insufficient sprayed particles reached the area. A minimum of 10^3 Bti colony forming units per ml is required to cause 100% larval mortality. The sprayed Bti larvicidal toxins were persistent in the test water 7 days post ULV. The effectiveness of *B. thuringiensis jegathesan* (Btj), a new mosquitocidal Bt serotype was also evaluated. Similar mortality results as Bti were achieved except that the Btj toxins underwent degradation in the test water, since less than 50% less in larval mortality was observed in 7 days post ULV samples. This ULV method has the potential to disperse Bt and malathion effectively for a simultaneous control of mosquito adults and larvae.

INTRODUCTION

Bacillus thuringiensis israelensis serotype (H-14) (Bti) is the most effective microbial control agent against mosquitos that is available to date. Bti has many advantages over chemical insecticides: it is enviromentally friendly, it is highly specific and toxic towards mosquitos without development of resistance in the insects. However its main limitation is that the toxin needs 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 pseudovishnui* (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 pre-requisite.

Microdroplet application of chemicals using a ultra-low-volume (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 larvicidal effect, 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 that will be able to control both adult and larval vector mosquito populations simultaneously. So in our study the effectiveness of dispersing a larvicidal insecticide, and an adulticidal insecticide, malathion, together using an ULV generator was also studied.

MATERIALS AND METHODS

An open air football field was selected as the study site. An ULV generator (Scorpion™) was used to disperse the formulations. The generator was mounted onto a truck and the flowmeter was adjusted to effect a flow rate of 1.6 l/minute. The truck moved at a slow constant speed (<10 kph) for a minute, covering a distance of about 150m, with its nozzle pointing towards the test samples.

The ULV trials were conducted on 3 different days, dispersing 3 different formulations:

i. **Trial 1** (27/10/94)

Formulation: A commercial aqueous formulation of Bti (BMP 144 2X ™, a Becker Microbial

Product) with 1,200 ITU/mg against *Ae. aegypti* larvae.

ii. **Trial 2** (28/10/94)

Formulation: A mixture of 9 parts of BMP 144 2X ™ with 1 part of 96% technical grade malathion.

iii. **Trial 3** (6/12/94)

Formulation: A laboratory aqueous preparation of *B. thuringiensis jegathesan* (H28a28c) (Btj), a new mosquitocidal *B. thuringiensis* serotype, with 1150 ITU/mg against *Ae. aegypti* larvae.

The effectiveness of ULV fogging was evaluated by measuring 4 different parameters, namely:

1. **Larval mortality**

Cups containing 50ml water with 15 fourth instar (L4) laboratory-bred *Ae. aegypti, Cx. quinquefasciatus* and *An. maculatus* larvae were placed at 10 feet intervals, beginning with 10 feet from the generator to 100 feet in a straight line. 30 minutes after ULV fogging the cups were removed from the test sites and brought to the laboratory. The larval mortality was scored 24 hours post ULV. All dead/alive larvae were subseqently removed from the cups. These cups with the test water were left at room temperature (28° - 32°C) with relative humidity of about 85%.

The persistency of the fogged *B. thuringiensis* (Bt) mosquitocidal toxins in the test water was determined by adding 10 fresh L4 larvae into their respective cups 7 days post ULV and scoring the larval mortality after overnight exposure.

2. **Adult mortality**

For Trial 2 whereby malathion, the adulticidal insecticide was dispersed together with Bti, the adult mortality was measured. Cages of 50 sucrose-fed, < 7days old adult female *Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus* and *An. maculatus* mosquitos were placed at 10 feet intervals, beginning with 10 feet from the generator to 100 feet in a straight line. 30 minutes after ULV fogging the adult mosquitos were transferred to paper cups and fed with sugar solution. The adult mortality was recorded 24 hours post ULV.

3. ***B. thuringiensis* enumeration**

The amount of *B. thuringiensis* in terms of colony forming units/ml (CFU/ml) in the test sample was also measured. For this purpose water samples were collected in sterile containers at intervals of 30 minutes, 24 hours and 7 days post ULV. The samples were then plated onto *B. thuringiensis* selective media, (NYPC) containing nutrient agar (23 g/l) yeast (0.5 g/l $MnCl_2$ (6 mg/l, $CaCl_2$ (80 mg/l, $MgCl_2$ (70 mg/l), polymyxin B sulphate (0.1 g/l) and cloramphenicol (1 mg/l). The CFU/ml were enumerated after 24 hours incubation at 32°C.

The number of CFU/ml in water samples collected 30 minutes post ULV indicated the coverage of ULV fogging, while CFU count for 24 hours and 7 days post ULV water samples showed the persistency of Bt in the test samples.

4. **Droplet analysis**

The distribution and size of sprayed particles were monitored through the use of magnesium oxide (MgO) coated slides. A slide were 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 Program of Sofield and Kent (1984).

RESULTS AND DISCUSSION

The results for each trial was correlated between droplet analysis against distance from the ULV generator with larval and adult mortality and number of Bt colony forming units (CFU/ml) against distance from the ULV generator.

The most widely used parameter of droplet size is the volume median diameter (vmd) measured in micrometers (μm). 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). An uniform size of droplets, when the ratio of vmd to nmd is near to 1, is preferred for an efficient ULV spray (Matthews, 1985).

Trial 1 - Commercial aqueous Bti formulation (BMP 144 2X™)

The diameter of the sprayed particles was in the range of 100 to 150 μm and the ratio of vmd to nmd

was close to 1. The number of Bti droplets decreased with increasing distance from the ULV generator. Few droplets were observed on the MgO coated slides beyond 80 feet from the generator.

Complete larval mortality was achieved for all mosquito species within the first 80 feet from the ULV generator with a CFU/ml count ranging between 10^3 - 10^5 CFU/ml (Fig 1). It was observed from this trial that in order to achieve a 100% mortality in all test larvae a minimum of 10^3 CFU/ml was required. A CFU count less than 10^3 CFU/ml only achieved less than 50% mortality in the test larvae beyond 80 feet from the ULV generator. low CFU count beyond 80 feet was due to insufficient numbers of sprayed Bti particles reaching beyond 80 feet from the ULV generator as shown on the MgO coated slides.

The percentage larval mortality in 7 days post ULV samples remained the same as 24 hours post ULV samples indicating that Bti sprayed toxins are very persistent at room temperature of (28°-32°C) and relative humidity of about 85% and did not undergo any degradation in the water even a week after fogging (Fig 1).

There was an increase in CFU count by 10 to 100 fold in some 24 hours and 7 days post ULV test samples especially in samples collected beyond 80 feet from the generator but there was no increase in larval mortality (Fig 1). The increase in the CFU count could be due to the fogged Bti in the water growing into vegetative forms only and was unable to sporulate due to insufficient nutrients in the water, thus not producing the larvicidal toxins which are only synthesised during sporulation.

Trial 2 - Malathion (96% TG) and BMP 144 2X™

The droplet size ranged between 100 to 150 µm just as in Trial 1 but uniform droplet size was not obtained at all sites. Similar to Trial 1 the droplet density decreased with increasing distance from the ULV generator.

Complete larval mortality was achieved for all test larvae within 10 to 70 feet from the ULV generator with a CFU count ranging between 10^1 to 10^3 CFU/ml. Beyond 70 feet less than 50% larval mortality was observed with 10^0 to 10^2 CFU/ml (Fig 2). The post CFU count for all sites in this trial was 100 fold less than in Trial 1 but there was a 100 fold increase in all the 24 hours and 7 days post ULV test samples. Therefore the amount of Bti in the waters after 24 hours was equivalent to that of 30 minutes post ULV samples in Trial 1. Lesser numbers of Bti detected in 30 minutes post ULV samples in Trial 2 could be due to the presence of malathion which inhibited the growth of Bti on the NYPC media. As malathion breaks down over time, it has less inhibitionary effect on Bti's growth. This phenomenon of Bti's growth inhibition due to malathion was tested in the laboratory, whereby a mixture of BMP 144 2x and malathion (9v : 1v) and a mixture of BMP 144 2x and water (9v : 1v) were plated onto NYPC media 30 minutes after mixing. These same mixtures were plated on NYPC media a week after leaving them at room temperature. The CFU count obtained for the mixture of Bti in water were the

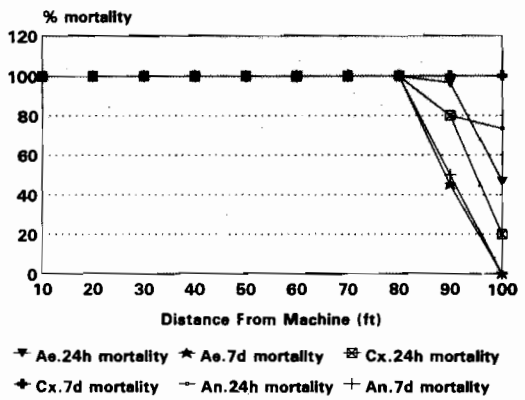

Fig 1–Trial 1: Effect of Bti (BMP 144 2X™) on mosquito lavae.

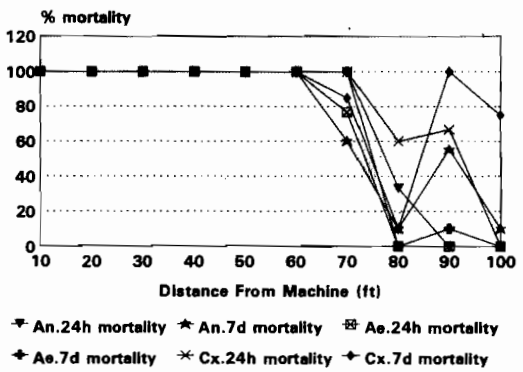

Fig 2–Trial 2: Effect of Bti (BMP 144 2X™) and malathion on mosquito larvae.

same as for 30 minutes and 1 week after mixing. But for the mixture of Bti in malathion, the 1 week Bti count was 2 fold more than the 30 minutes count. Hence malathion does inhibit the growth of Bti but it does not affect the activity of larvicidal toxin since 100% larval mortality was obtained in 24 hours and 7 days test samples similar to Trial 1.

BMP 144 2x too did not have any negative effect on malathion, as 100% adult mortality was obtained in all mosquito species within the first 60 feet from the ULV generator (Fig 3). Beyond 60 feet about 50% adult mortality was achieved due to fewer sprayed particles settling beyond 70 feet as shown on the MgO coated slides.

Trial 3 *B. thuringiensis jegathesan* (Btj) formulation

In this trial a laboratory preparation of Btj was used. 14.4g of Btj harvested from a 24 hours nutrient borth (Oxoid) culture was suspended in 1.0 l sterile distilled water and fogged.

The droplet diameter ranged between 150 to 250 µm, *ie* 1.5 times greater than the 2 previous trials. Larger size droplets was due to less viscous Btj formulation that was used, which hampered complete atomization before the Btj droplets were dispersed (mount, 1985). The larger size droplets coupled with a strong breeze blowing the sprayed droplets backwards and not towards the target area accounted for an inefficient ULV fogging. Insufficient Btj droplets were seen on the MgO coated slides beyond 50 feet from the ULV generator.

This trial was only evaluated with *An. maculatus* and *Mansonia uniformis* larvae as other larvae were not available during this trial. Complete larval mortality was achieved for *An. maculatus* larvae within the first 30 feet from the ULV generator with a CFU count of 10^2 to 10^3 CFU/ml. After 40 feet less than 50% mortality with a count of 0 to 10^1 CFU/ml was observed (Fig 4). As for *Mn. uniformis* larvae only a 20 to 60% mortality was achieved throughout the target area (Fig 4).

There was an increase in the CFU count by 10 fold in the 24 hours and 7 days post ULV test samples but a decrease in the larval mortality by 50% in all the 7 days post ULV test samples was observed (Fig 4). This clearly shows that there was no production of the Btj larvicidal toxins in the test water just as in Trials 1 and 2. In contrast to fogged Bti toxins, the fogged Btj toxins underwent degradation to give a 50% less mortality in 7 days post ULV test samples compared to 24 hours post ULV test samples.

Btj is a new serotype (Seleena *et al*, 1995) with 5 novel larvicidal toxins that display little or no immunological relationship with Bti toxins (Delecluse *et al*, 1994). New microbial control agents with novel toxins are recommended for use in the control of mosquito larvae so as to potentiate the use of larvicidal bacteria and also to reduce the risk of developing insect resistance to these larvicides. The toxicity of Btj used in this trial was about 1,000 times lower than the Bti formulation that was used in Trial's and 2 (Table 1). Nevertheless a 100% larval mortality was achieved for *An. maculatus* with a CFU count of just 10^2 to 10^3 CFU/

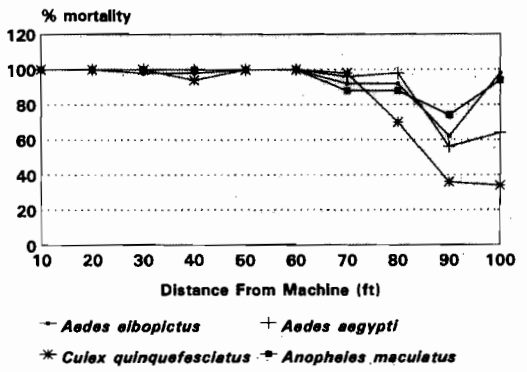

Fig 3–Trial 2: Effect of Bti (BMP 144 2X™) and malathion on mosquito adults.

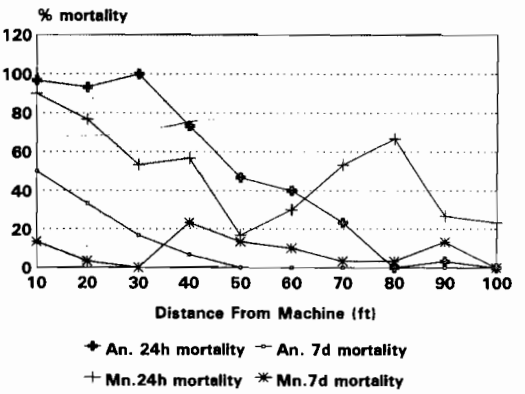

Fig 4–Trial 3: Effect of Bti on mosquito lavae.

ml, thus making Btj with its novel toxins a potential future candidate to be used for mosquito control using the microdroplet application technique.

Table 1

Larval toxicity of *Bacillus thuringiensis* to *Aedes aegypti* exposed for 24 hours.

	LC 50 (mg/l) (95% CL)	Regression
BMP 144 2X ™	0.0027 (0.0025 - 0.0029)	y = 4.96x - 31.81
B. thuringiensis serovar *jegathesan*	2.06 (1.80 - 2.30)	y = 2.95x - 25.41

From this study it can be concluded that ULV fogging is effective in dispersing bacteria and malathion for both larval and mosquito control, simultaneously. The flow rate used in this study (1.6 l/minute) producing a droplet diameter of 100 to 150 μm, was not economical as the droplets were too big to have an efficient coverage beyond 70-80 feet from the ULV generator. Droplet diameter with an optimum vmd and nmd of 50 to 100 μm will be able to effectively cover a wider area and control both mosquito adults and larvae simultaneously. This can be accomplished by using a lower flow rate.

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