Simultaneous ultra-low-volume application of adulticide (malathion) and larvicide (*Bacillus thuringiensis* H-14) for the control of dengue vectors

Lee HL, Antonietta PE, Seleena P and Chiang YF *Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.*

Abstract

The evaluation of the effectiveness of ultra-low-volume fogging with a mixture of bioinsecticide *Bacillus thuringiensis* H-14 and malathion for the control of dengue vectors was conducted in a residential area. The effectiveness was measured using four different indicators: larval mortality, adult mortality, *Bti* enumeration and droplet analysis. The fogging formulation which was a mixture of 9 parts VECTOBAC 12AS® and 1 part 96% technical grade malathion was dispersed using a Dynafog Maxi Pro4® ULV generator with a 18 horse power engine. The flowrate which was 0.25 L/min was able to effectively deliver sufficient *Bti* toxins and malathion up to a distance of 19.7 m (65 ft) from the generator. The amount discharged was also sufficient to effect complete residual activity in *Aedes* mosquito larvae for a minimum of 14 days post ULV. Malathion does not affect the activity of latvicidal toxin of *Bti* since 100 % larval mortality was obtained in the test samples. On the other hand, VECTOBAC 12AS® too did not exert any negative effect on malathion as 100 % adult mortality was obtained in the *Aedes* mosquito species. *Bti* fogged at a dosage of 0.25 L/min had good coverage of the target area and penetration of dwellings, these being attributed to satisfactory droplet profiles. Based on the results of this study, it can be concluded that VECTOBAC 12AS®, an aqueous suspension formulation of *Bti*, dispersed simultaneously with malathion, a chemical insecticide, using ULV fogging at a discharge rate of 0.25 L/min, was very effective in controlling *Aedes* mosquitoes.

Key words: *Bacillus thuringiensis* H-14; Malathion; Dengue vector control

Introduction

In Malaysia, mosquito-borne diseases especially dengue continue to cause public concern. In the absence of effective and specific treatment of dengue, the interruption of disease transmission by the vectors remain the most effective method of control. The effective control of the dengue vectors, *Aedes aegypti* and *Ae albopictus* relies on the proper use of adulticides and larvicides. In Malaysia, the adulticide of choice is malathion, an organophosphate, while the recommended larvicide is temephos (Vector Borne Diseases Control Programme, 1986). Malathion is often applied as a thermal or cold (ULV) fog by the health authorities and the use of temephos by the layman to treat household containers is encouraged. Such compartmentisation of adulticiding and larviciding is not effective, primarily due to the low usage of temephos (Lee, 1991) and thus the possibility of simultaneous application of adulticide and larvicide in the same operation has been proposed. An effective alternative larvicide is *Bacillus thuringiensis* H-14 (*Bti*). This microbial agent has been shown to be effective when dispersed by ULV fogging (Lee *et al.*, 1996).

The combined use of chemical insecticide and *Bti* has been reported. In Dominican Republic, Tidwell *et al.* (1994) evaluated *Bti* and permethrin using the Scorpion(20 ULV forced-generator for the emergency control of *Ae. aegypti*. The flow rates used were 788, 1,577 & 2,586 mL/ha in three separate tests. Seleena *et al.* (1995) also found that applications of a combination of *Bti* and adulticide was effective in controlling adult and larvae simultaneously using a flow rate of 1.6 L/min.

In this study attempts were made to evaluate the effectiveness of a mixture of *Bacillus thuringiensis H-14* and malathion using an ULV generator under real field conditions.

Materials and Methods

Study area

The trial was conducted in a housing estate consisting of single storey semi-detached houses in Pandamaran, Klang, about 50 km west of Kuala Lumpur. Ten houses were selected randomly for the trial. Two rows of houses facing opposite to one another were chosen as test houses. The length of each house was about 19.8 m (65 ft) from the gate to the backyard. Each house was divided into 3 sections: The 1st 12.1 m (40 ft) from the gate to the front door was labeled as outside; The inside of the house which was about 7.6 m (25 ft) long was divided into 2 sections.

ULV generator

A vehicle mounted Dynafog Maxipro4® generator was used to disperse *Bti*. The machine was operated at an

air pressute of 8 psi with an 18 horse power engine during the ULV fogging operation. The bacteria-malathion mixture was dispersed at a discharge rate of 0.25 L/min at a constant vehicle speed of <10 kph covering a distance of about 65 ft (19.8 m) with its nozzle pointing at 45° towards the test houses.

Insecticides used

A commercial aqueous *Bti* formulation VECTOBAC 12AS® supplied by Abbott Laboratories and containing 1,200 ITU/mg against *Ae. aegypti*. Its LC_{50} value against laboratory bred *Ae. aegypti* was 0.0027 mg/L (Lee & Seleena, unpublished data). Deodorised malathion 96 % technical grade (Fyfanon®) was used. The final fogging formulation was a mixture of 9 parts of VECTOBAC 12AS® and 1 part of malathion.

Mosquitoes

Late 3rd instar and early 4th instar larvae of laboratory breed *Ae. aegypti* and *Ae. albopictus* were used. Adult females (<7 days old), laboratory reared *Ae. aegypti, Ae. albopictus* and *Culex quinquefasciatus* were also used. The adult mosquitoes were transported to the study area two hours prior to fogging and transferred to the test cages several minutes before the fogging operation.

Bioassay procedures

Assessment of mortalities induced were by caged assays of adult mosquitoes and larvae. Twenty five sucrose-fed mosquitoes were placed in each cage constructed of fine cloth with a wire frame support. The caged mosquitoes and the paper cups with the magnesium oxide-coated slides amongst the cups were placed close to each other outdoors, in the porch and indoor, in the sitting room and kirchen.

To evaluate the effectiveness of *Bti* with malathion applied by ULV fogging, four different indicators were measured:

(a) Larval mortality - The most important parameter used to evaluate the effectiveness of *Bti* is to measure larval mortality 24 hours post ULV. Paper cups containing 50 mL sterile distilled water were placed randomly in the porch, and in the interior of houses. One set of cups was collected one hour after fogging while the other 2 sets were left behind to determine the persistence of *Bti* toxins applied under field conditions. In the laboratory *Ae. aegypti* and *Ae. albopictus* larvae were added into the cups and mortality was scored after overnight (18 hour) exposure. All dead and alive larvae were removed from the cups and fresh larvae were added 7d and 14d post ULV to determine the residual activity. The cups which were left behind in the field were brought back to the laboratory 7d and 14d post ULV. Similarly, 15 L_3 / L_4 larvae were added to these cups and mortality was scored after overnight exposure.

(b) Adult mortality - Since malathion was dispersed together with *Bti*, the adult mortality was measured. Cages of 25 sucrose- fed, (<7 days old) adult female *Ae. aegypti* and *Ae. albopictus* mosquitoes were placed outside and inside the houses at various distances from rhe ULV generator. One hour after fogging the adult mosquitoes were brought back to the laboratory. The mosquitoes were then transferred to paper cups and fed with sugar solution. The adult mortality was recorded 24 hours post ULV.

(c) *Bti* enumeration - Another parameter used in the evaluation is the colony-forming units enumeration per mL (cfu/mL). The amount of *Bti* in terms of colony forming units in the test samples was also measured. This was done by collecting water samples from the rest cups into sterile containers at intervals of 1 hour, 7 days and 14 days post ULV. The samples were plated onto a *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 chloramphenicol (1 mg/L). Then 0.3 mL of each water sample was inoculated onto this medium and the cfu/mL were enumerated after 24 hour incubation at 32°C. Corresponding serial dilutions were done for samples whose cfu were more than 300/plate. Two replicates were done for each water sample. The number of cfu/mL in water samples collected 24 hour post ULV indicated the coverage of ULV fogging, while cfu count for 7d and 14d post ULV water samples showed the persistency of *Bti* in the test samples.

(d) Droplet analysis - The distribution and size of sprayed particles were monitored through the use of MgO coated slides. These slides were prepared by burning a 25.4 cm (10 in) strip of magnesium ribbon on the underside of a cleaned glass slide to produce a uniform coat of MgO. These slides were placed horizontally with the coated surface upward amongst the cups holding the larvae. After 1 hour, the slides were collected and brought back to the laboratory. Droplet diameter was measured for an average of 60 droplets for each MgO coated slide using a calibrated micrometer. The data were analyzed using the ULV Droplet Analysis Programme of Sofield & Kent (1984).

Fogging operations

The formulation of a mixture of 9 parts VECTOBAC 12AS® and 1 part of 96 % malathion was dispersed at a flow rate of 0.25 L/min towards the test houses. Fogging was started at 0800 hr and the vehicle made 1 pass at each row of houses. There was a slight breeze (< 1 km/hour) during the trial and the weather was fair. Homeowners in the area were requested to leave their doors and windows open during the fogging operation and until one hour after fogging. This was to allow the

ULV fog to penetrate into the house. They were asked further to cooperate by leaving the test cups inside the house until 7d and 14d post ULV.

Cages of each species were kept 600 m away from the test houses as controls. These cages were brought back to the laboratory and mortality was recorded 24h post ULV. In the laboratory 2 sets of cups containing L_3 / L_4 larvae of *Ae. aegypti* and *Ae. albopictus* were also kept as controls.

Results

A combined VECTOBAC 12AS® and malathion fogged at a discharge rate of 0.25 L /min achieved complete larval mortality (laborarory samples) in 80% of the houses within 12.1 m (40 ft) from the generator. The mortality was maintained until 14d posr ULV. The other 20% of the houses had 80% mortality and this declined with time. The cfu count ranged from 0 - 800 cfu/mL. There was an increase in cfu count within 7d post ULV after which it decreased with rime. Likewise complete larval mortality was achieved for *Ae. albopictus* house samples in 60% of the houses within 12.1 m (40 ft) from the generator in which the mortality persisted until 14d post ULV. The mortality in rhe other 40% of the houses ranged from 60-95% as shown in Fig. 1.

Complete larval mortality for *Ae. aegypti* laboratory samples in 90% of the houses within 12.1 m (40 ft) from the generaror was achieved and this was maintained until 14d post ULV, except for two houses where rhe mortality decline by 30%. The cfu count ranged from 0 - 800 cfu/mL; there was an increase within 7d post ULV after which it decreased with time. On the other hand, *Ae. aegypti* house samples achieved complete larval mortality in 90% of the test samples within 12.1 m (40 ft) from the generator and this was maintained until 14d post ULV except in four houses where the mortality declined with rime (Fig. 2).

There was complete larval mortality for *Ae. albopictus* laboratory samples in 20% of the houses within 12.1-15.2 m (40-50 ft) from generator (Fig. 3). The mortality was maintained until 14 d post ULV. The mortality in the other 80% of the houses ranged from 20 - 95% after which it declined with time. High residual activity against *Ae. albopictus* was observed in the 14d water samples. *Ae. albopictus* house samples within 12.1-15.2 m (40-50 ft) from the generaror also showed the same mortality as in *Ae. albopictus* laboratory samples. The mortality in the other 80% of the houses ranged from 40 - 85% after which it decreased with time. The cfu count was very low but ir increased from 7d to 14d post ULV.

Aedes aegypti laboratory samples within 12.1-15.2 m

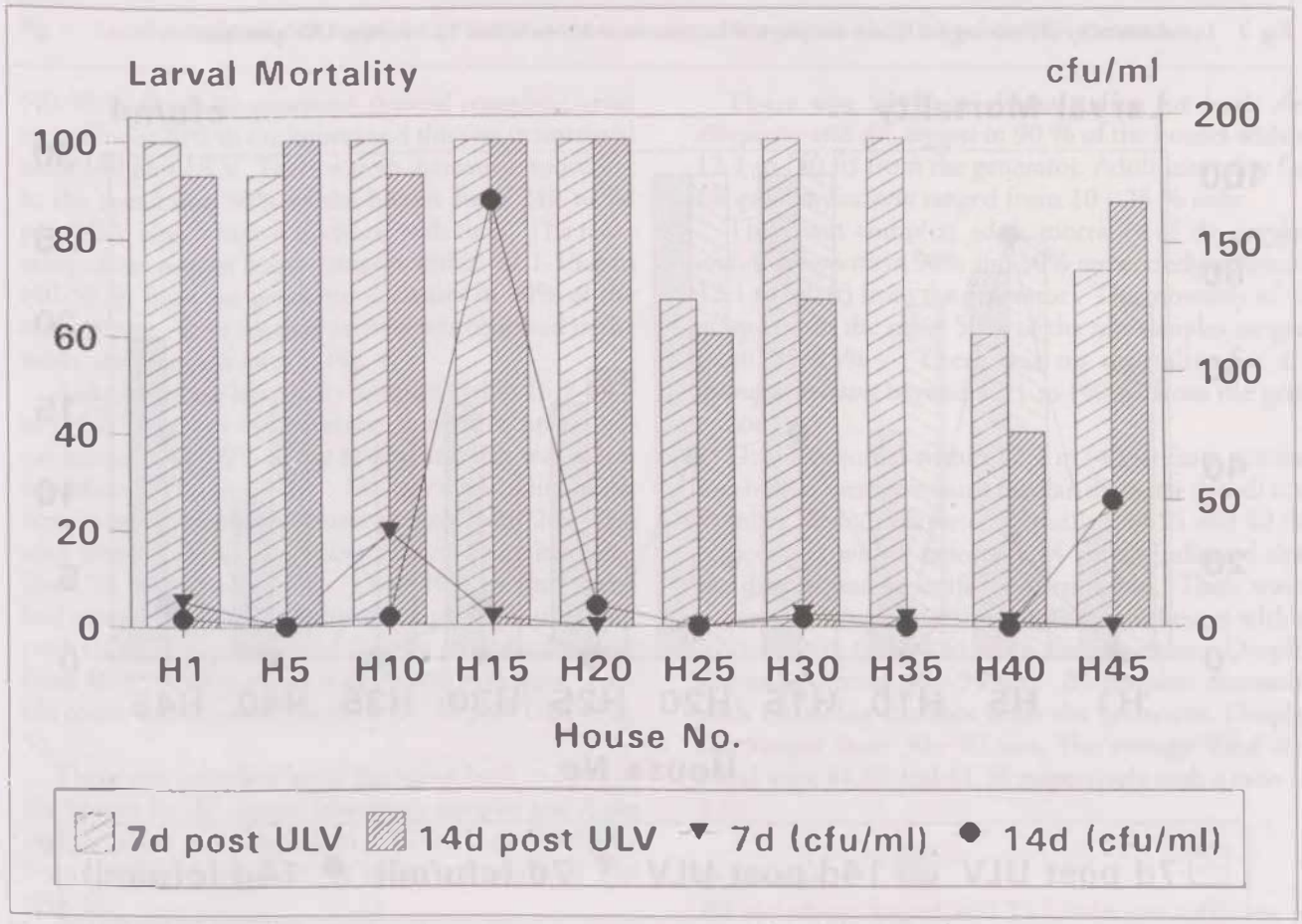

Fig. 1 Larval mortality of *Aedes albopictus* (house sample) and bacteria enumeration within 12.1 m from ULV generator.

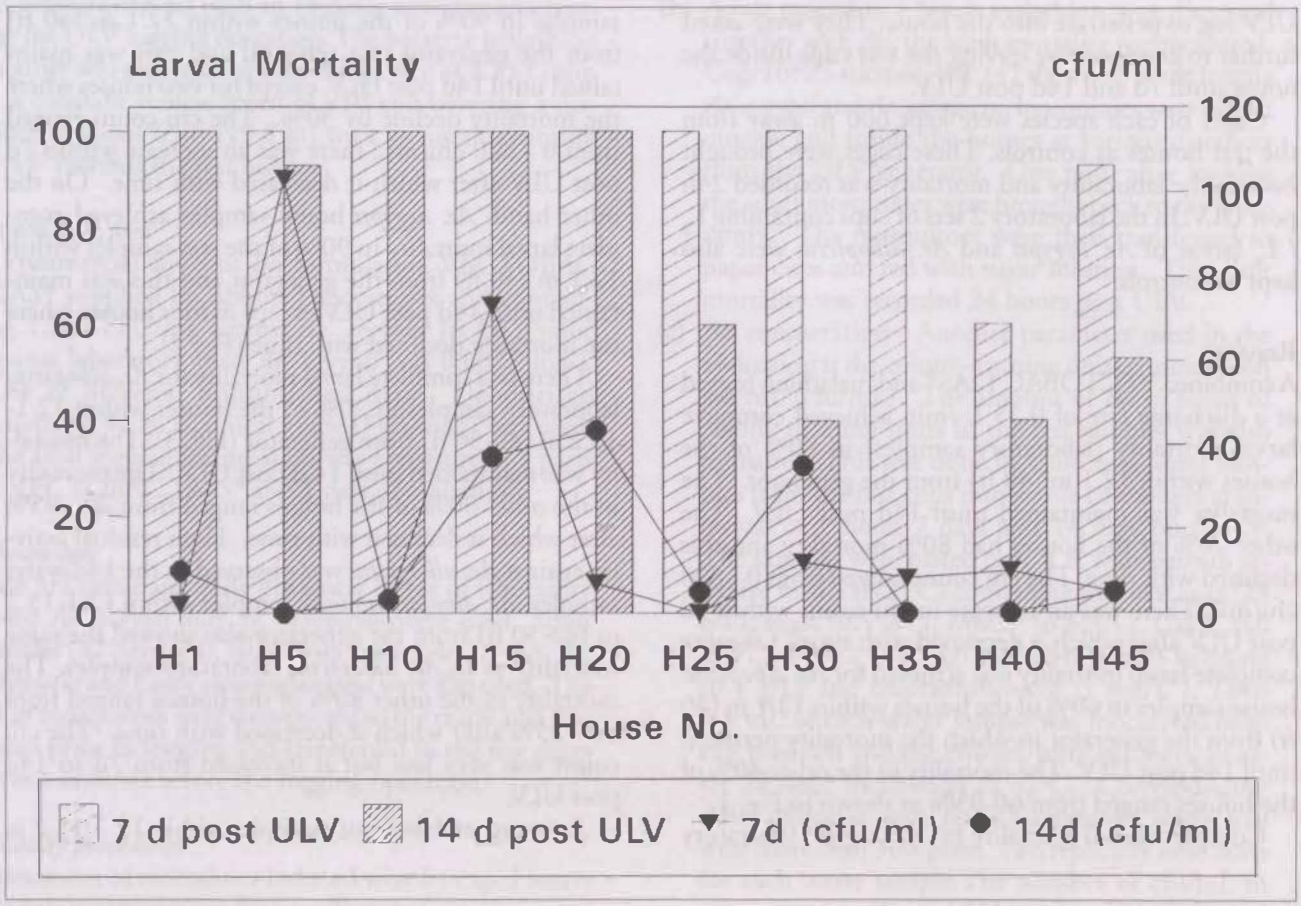

Fig. 2 Larval mortality of *Aedes aegypti* (house sample) and bacteria enumeration within 12.1 m from ULV generator.

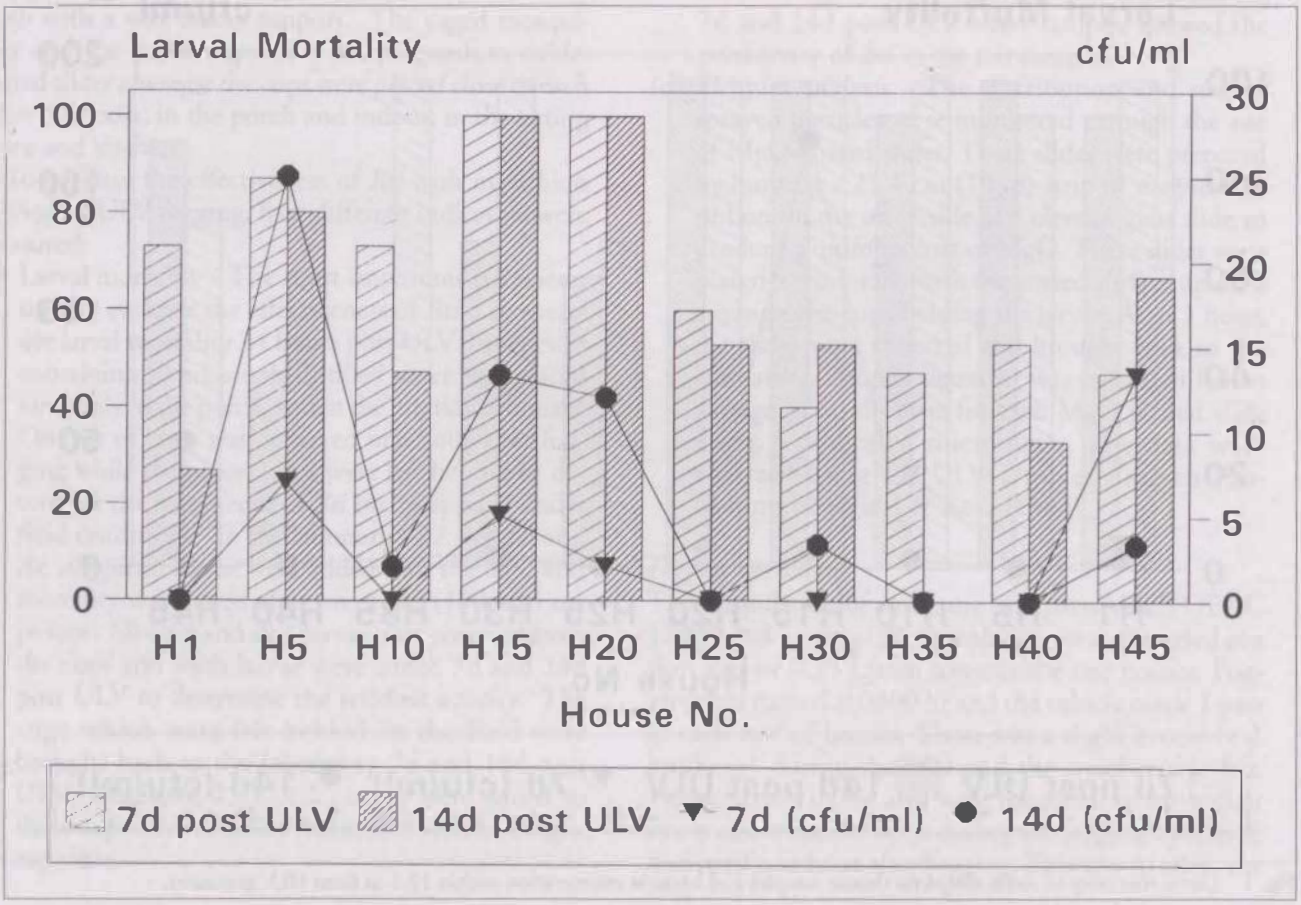

Fig. 3 Larval mortality of *Aedes albopictus* (house sample) and bacteria enumeration within 12.1-15.2 m from ULV generator.

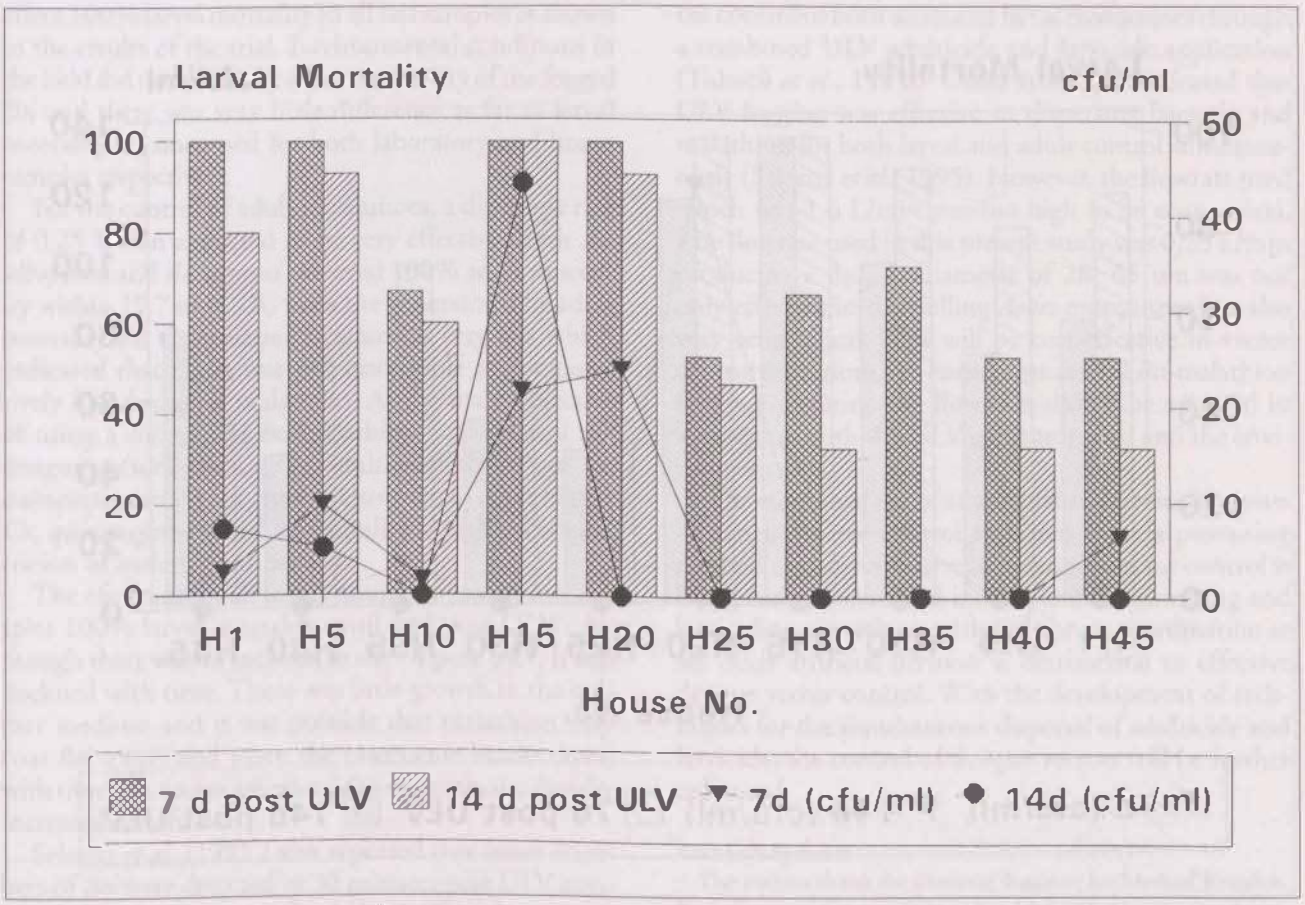

Fig. 4 Larval mortality of *Aedes aegypti* (house sample) and bacteria enumeration within 12.1-15.2 m from ULV generator.

(40-50 ft) from the generator showed complete larval mortality in 20% of the houses and this was maintained until 14d post ULV. There was an increase in mortality in the remaining 80% of the houses from 24h to 7d post ULV after which it declined with time. The mortality of *Ae. aegypti* house samples within 12.1-15.2 m (40-50 ft) from the generator decreases in 90% of the test samples. Low residual activity was observed in the water samples as shown in Fig. 4.

Aedes albopictus laboratory samples within 15.2-19.7 m (50-65 ft) from the generator showed complete larval mortality in 20% of the houses and this was maintained until 14d post ULV. The larval mortality in the remaining 80% of the houses ranged from 20- 90% after which it decline with time. The cfu count increased from 7d to 14d post ULV. Only 10% of the houses had complete larval mortality which persisted until 14d post ULV. The mortality of the rest of houses ranged from 40-95% after which it decreased with time. Low cfu count was observed from 7d to 14d post ULV (Fig. 5).

There was complete larval mortality both in 30% of the houses for *Ae. aegypti* laboratory samples and *Aedes aegypti* house samples within 15.2-19.7 m (50-65 ft) from the generator. The cfu count was relatively low (Fig. 6).

There was 100% adult mortality for both *Ae. albopictus* and *Ae. aegypti* in 90 % of the houses within 12.1 m (40 ft) from the generator. Adult mortality for *Cx quinquefasciatus* ranged from 10 - 25 % only.

There was complete adult mortality of *Ae. aegypti and Ae albopictus* in 90% and 50% respectively of houses 12.1 m (40 ft) from the generator. The mortality of *Ae albopictus* in the other 50% of the test samples ranged from 85-95% . There was no mortality for *Cx quinquefasciatus* beyond 12.1 m (40 ft) from the generator.

Droplet profiles within 12.1 m (40 ft) from generator showed average volume median diameter (Vmd) and number median diameter (Nmd) of 48.55 and 42.48 respectively with a ratio of 1.14 which indicated that the droplet had an uniform distribution. There was a decrease in droplet density in 60% of the houses within 12.1-15.2 m (40-50 ft) from the generator. Droplet size ranged from 34 - 94 um. *Bti* droplets decreased with increasing distance from the generator. Droplet size ranged from 30 - 97 um. The average Vmd and Nmd were 44.60 and 41.56 respectively with a ratio of 1.07.

Discussion

Bti-malathion fogged at 0.25 L/min was sufficient to

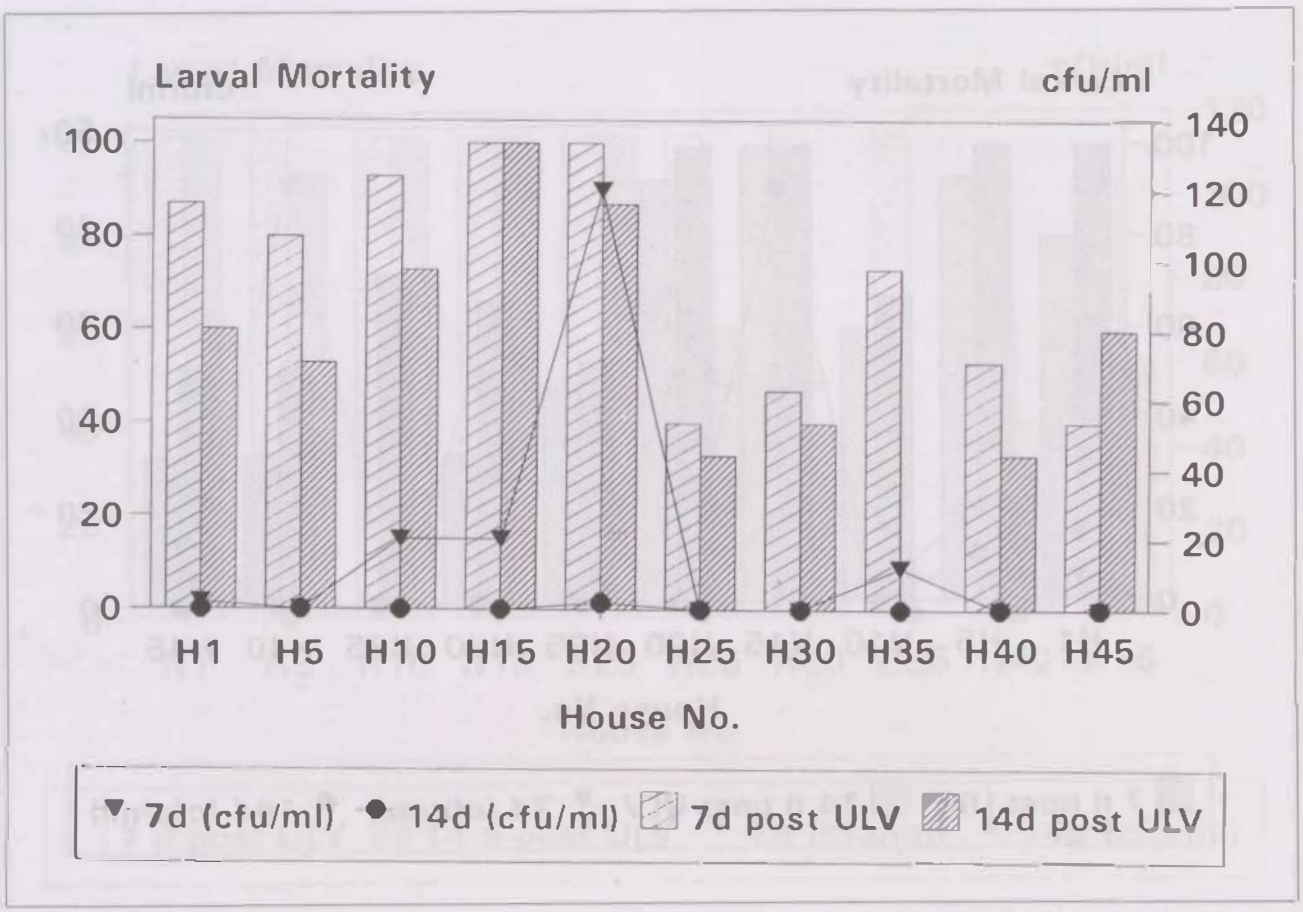

Fig. 5 Larval mortality of *Aedes albopictus* (house sample) and bacteria enumeration within 15.2-19.7 m from ULV generator.

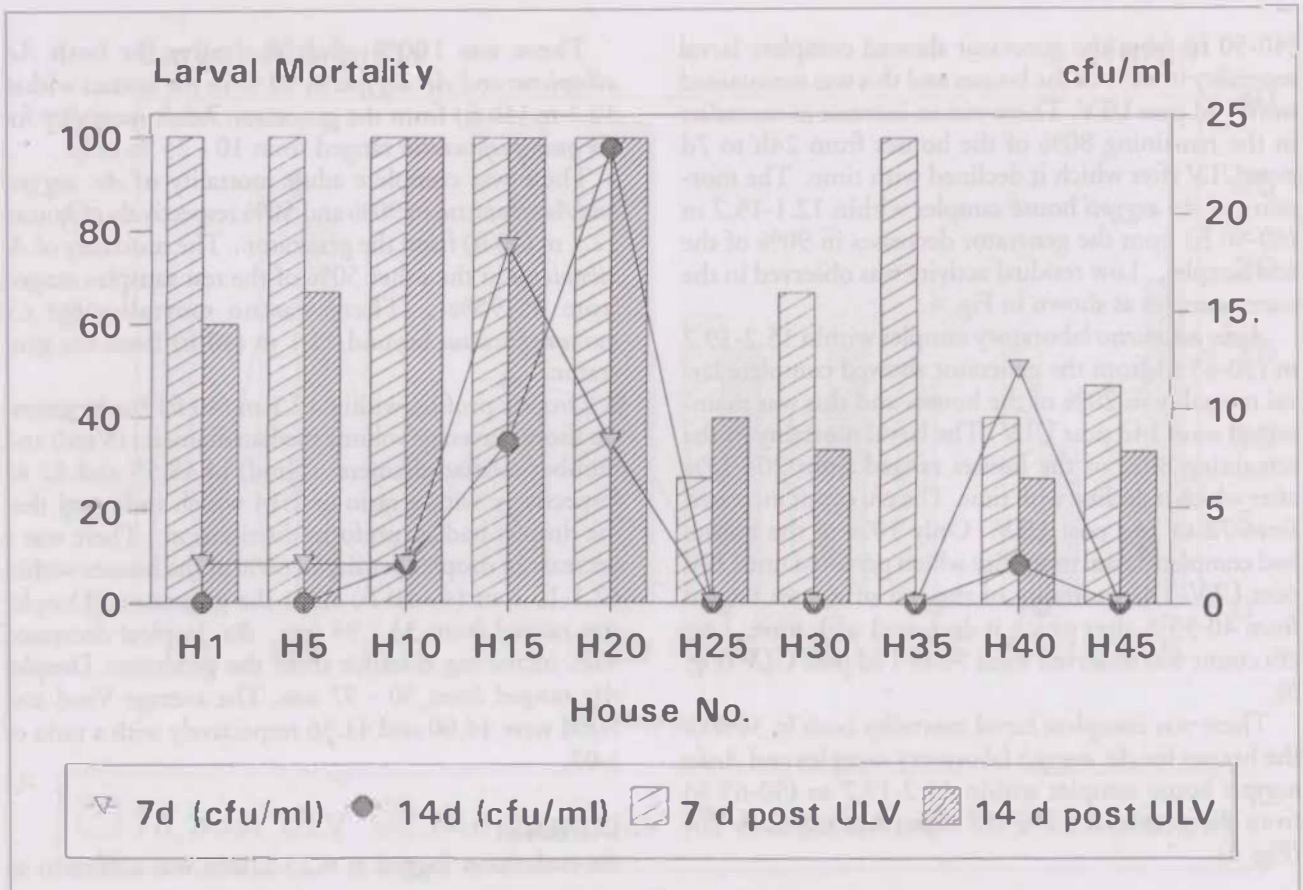

Fig. 6 Larval mortality of *Aedes aegypti* (house sample) and bacteria enumeration within 15.2-19.7 m from ULV generator.

effect 100% larval mortality in all test samples as shown in the results of the trial. Environmental conditions in the field did not adversely affect the activity of the fogged *Bti* and there was very little difference as far as larval mortality is concerned for both laboratory and house samples respectively.

For the control of adult mosquitoes, a discharge rate of 0.25 L/min appeared to be very effective. Both *Ae. albopictus* and *Ae. aegypti* achieved 100% adult mortality within 19.7 m (65 ft) from the generator. The adult mortality for *Cx. quinquefasciatus* was very low which indicated that *Culex* was not susceptible to comparatively low dosage of malathion. As the main objective of using a mixture of *Bti*-malathion is to control the dengue vectors, the lack of adulticidal effects on *Cx. quinquefasciatus* is not crucial. However, to effect higher *Cx. quinquefasciatus* adult mortality, a higher concentration of malathion is needed.

The cfu count in all tested samples was very low despite 100% larval mortality until 14d post ULV. Although there was an increase in the 7d post ULV, it still declined with time. There was little growth in the culture medium and it was possible that malathion may coat *Bti* spores and when the insecticide breaks down with time, the spores are released and germinate, thereby increased the cfu.

Seleena *et al.* (1995) also reported that lesser numbers of *Bti* were detected in 30 minutes post ULV samples due to the presence of malathion. This phenomena of *Bti* growth inhibition by malathion was rested in the laborarory (Seleena, unpublished data). Although malathion affects the growth of *Bti*, it does not interfere with the activity of the larvicidal toxin of *Bti* since 100% larval mortality was obtained in the test samples. On the other hand, VECTOBAC 12AS® too did not exert any negative effect on malathion as 100% *Aedes* adult mortality was obtained.

The results of droplet analysis showed that the droplet profile was very satisfactory using Dynafog Maxipro4® generator. This machine operates at an air pressure of 8.0 psi with an 18 horse power engine. Dynafog Maxipro4® with its 4 nozzles was able to effectively deliver sufficient *Bti* toxins and malathion to a distance of 19.7 m (65 ft) from the generator. The amount discharged was also sufficient to give complete residual activity in container breeding *Aedes* larvae for a minimum of 14 days post ULV. This agreed with the hypothesis of Lee *et al.* (1996) that a flowrate of 0.3 L/min and 0.5 L/min had a good penetration of dwellings which indicated that *Bti* ULV fogging is an effective method of controlling container breeding mosquitoes. In another trial, the authors found that after fogging, exposed water cups set up in the building and stagnant water collected was larvicidal to *Ae. aegypti* larvae with complete residual activity up to 2 weeks (Seleena, unpublished data).

These rests demonstrated the excellent potential for the conrrol of both adult and larval mosquitoes through a combined ULV adulticide and larvicide application (Tidwell *et al.*, 1994). Other trials also indicated that ULV fogging was effective in dispersing bacteria and malathion for both larval and adult control simultaneously (Seleena *et al.*, 1995). However, the flowrate used which was 1.6 L/min was too high to be economical. The flowrate used in this present study was 0.25 L/min producing a droplet diameter of 28- 65 um was not only effective in controlling *Aedes* mosquitoes but also very economical. This will be cost-effective in vector conrrol operations. To ensure a successful *Bti*-malathion fogging operation the flowrates should be adjusted in accordance with the ULV generator used and the environment.

The combined use of adulticidal and larvicidal agents in dengue vector control appeared to be a promising method of control. Currently, dengue vector control is being compartmentised into separate adulticiding and larviciding operations, with little or no coordination at all. Such artificial division is detrimental to effective dengue vector control. With the development of rechniques for the simultaneous dispersal of adulticide and larvicide, the control of dengue vectors will be further enhanced.

Acknowledgments

The authors thank the Director, Institute for Medical Research, Kuala Lumpur for permission to publish. Thanks are also due to Halex (M) Co. and Abbott Laboratories (M) Co. for providing samples of VECTOBAC®; Syarikat Perniagaan M.S. for the supply of Fyfanon® (malathion) and the Klang Municipality for the use of the ULV generator.

References

Lee HL (1991). A nationwide resurvey of the factors affecting the breeding of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) in urban towns of peninsular Malaysia- 1988-1989. *Tropical Biomedicine* 8, 185-189.

Lee HL, Enersto RG Jr, Khadri MS & Seleena P (1996). Ultra-low-volume application of *Bacillus thuringiensis* serotype H-14 for the conrrol of mosquitoes. *Journal of the American Mosquito Control Association* (in press).

Seleena P, Lee HL, Rohani A, Nazni WA & Khadri MS (1995). Microdroplet application of mosquitocidal *Bacillus thuringiensis* using ultra-low-volume generator for the control of mosquitoes. *Southeast Asian Journal of Tropical Medicine and Public Health* (in press).

Solield RK & Kent R (1984). A basic program for the analysis of ULV insecticide droplets. *Mosquito News* 44, 73-75.

Tidwell MA, Williams DC, Gwinn TA, Peña CJ, Tedders SH, Gonzalvez GE & Mekuria Y (1994). Emergency control of *Aedes aegypti* in the Dominican Republic using the Scorpion' 20 ULV forced-air generator. *Journal of the American Mosquito Control Association* 10, 403-406.

Vector Borne Diseases Control Programme (1986). *Guidelines for the prevention and control of dengue fever/dengue haemorrhagic fever.* Ministry of Health, Malaysia. 158 pp.

Received 24 November 1996; revised 14 January 1997; accepted for publication 31 March 1997.