THERMALLY APPLIED *LYSINIBACILLUS SPHAERICUS* AND PYRETHROIDS AGAINST *CULEX SITIENS* WIEDEMANN AND *CULEX QUINQUEFASCIATUS* SAY IN MALAYSIA

HL Lee[1], L David[2], WA Nazni[1], H Rozilawati[1], H Nurulhusna[1], A Noor Afizah[1], R Rosilawati[1], A Roziah[1], CH Teh[1] and B Seleena[1]

[1]Medical Entomology Unit, Infectious Disease Research Centre, Institute for Medical Research, Kuala Lumpur; [2]Sarawak Vector Borne Disease Control Programme, Department of Health Services, Kuching, Malaysia

Abstract. The effectiveness of a thermally fogged *Lysinibacillus sphaericus* (Ls) formulation (VectoLex® WG) and a mixture of Ls and a pyrethroid formulation, Resigen® dispersed using a portable thermal fog generator against adults and larvae of *Culex sitiens* and *Culex quinquefasciatus* was evaluated in simulated field trials. The adult mortality, larval mortality and droplet profiles were used to measure the effectiveness of the control agents. Thermal fogging of Ls was possible without loss of effectiveness. However, the effective maximum dispersal distance was only up to 10 ft. A higher larval mortality was achieved at test point of 5 ft compared to 10 ft. The Ls dosage of 600-800 g/ha resulted in a higher larval mortality with longer residual effect compared to 500 g/ha. Resigen® alone was effective against adult *Cx. sitiens* and *Cx. quinquefasciatus* but did not exhibit larvicidal effect. Thermal application of a mixed formulation of Ls and pyrethroids was effective against both larvae and adults of *Cx. sitiens* and *Cx. quinquefasciatus*. The mixed formulation induced high larval and adult mortality. No antagonistic effect on the activity of the mixture of Ls and pyrethroids was observed. Simultaneous thermal fogging of a mixed formulation of Ls and pyrethroids exhibited synergistic effect that is applicable for the effective control of *Culex* species.

Keywords: *Culex sitiens*, *Culex quinquefasciatus*, *Lysinibacillus sphaericus*, biolarvicide, pyrethroid, thermal fogging

INTRODUCTION

In tropical countries, mosquito-borne diseases such as malaria, dengue, filariasis and Japanese encephalitis are still major public health problems. Japanese encephalitis is a *Culex* - borne disease and the leading cause of viral encephalitis in Asia. About 68,000 clinical cases of JE are estimated to occur annually in Asia (WHO, 2015). Two species of *Culex* (Diptera: Culicidae) mosquitoes; *Culex tritaeniorhyncus* and *Culex gelidus* are well known vectors of Japanese encephalitis (JE) in Southeast Asia. Vythilingam *et al* (1994) and Vythilingam *et al* (1995) reported for the first time

Correspondence: Dr Lee Han Lim, Medical Entomology Unit, Infectious Disease Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
Tel/Fax: +603 2616 2688
Email: leehl@imr.gov.my

in Malaysia the isolation of JE virus from *Culex bitaeniorhyncus* and *Culex sitiens*. One of the methods of controlling the *Culex* vectors relies mainly on the space application of chemical adulticides, especially the pyrethroids and organophosphates. However, ULV space application of a pyrethroid formulation and fenitrothion in Thailand could only control the *Culex* vectors for 4 days (Phanthumachinda, 1995). Hence for more effective control, the effectiveness of other control agent, such as the biolaravicide *Lysinibacillus sphaericus* should be evaluated.

Lysinibacillus sphaericus (Ls) is a microbial larvicidal agent specifically effective against all *Culex* mosquitoes. With the ability to kill 90% of the *Culex quinquefasciatus* larvae tested with just a concentration of 105 spores/ml, *Lysinibacillus sphaericus* (Strain 1593) has shown good potential as a mosquito biocontrol agent (Cheong and Yap, 1985; Lee *et al*, 1986). Yadav *et al* (1997) reported that Ls has good potential for use against disease vectors and mosquito breeding in polluted as well as clean water. An aqueous suspension formulation of Ls (Spicbiomoss®) can be used against *Cx. quinquefasciatus* in an integrated vector control management program (Mariappan *et al*, 1999). Siegel and Novak (1999) reported that the Ls microbial larvicide VectoLex® CG was effective when used in Illinois catch basins and tire dumps, while Su and Mulla (1999) found that the minimum effective dosage for Ls WDGs with 350-630 ITU/mg was 0.05-0.10 lb/acre, which yielded significant control of immature *Culex* mosquitoes for up to 14-20 days.

The effective use of larvicidal microbial control agents such as *Bacillus thuringiensis israelensis* (Bti) and Ls is very much subjected to effective application technologies for its dispersal. Lee *et al* (1996) conducted a series of ULV-Bti tests to determine the optimal effective dosage and found that high larval mortality was achieved at a discharge rate of 0.5 l/min. In another study, it was reported that Ls could be dispersed efficiently with cold-fogging sprayer (Kapa, 2000).

In the study, a thermal fogger was used to disperse a Ls formulation (VectoLex® WG) and a mixture of Ls and a pyrethroid formulation (Resigen®) which resulted in high larval and adult mortality. To date, thermally fogged Ls has not been reported, except Bti (Seleena *et al*, 2001). This paper reported for the first time thermally applied Ls alone and mixed with a pyrethroid formulation against *Cx. sitiens* and *Cx. quinquefasciatus* under simulated field conditions.

MATERIALS AND METHODS

Test mosquitoes

Laboratory-bred strain of *Cx. sitiens* and *Cx. quinquefasciatus* maintained in the insectarium of the Institute for Medical Research, Kuala Lumpur was used in the study. Late 3rd instar and early 4th instar (L3/L4) instar larvae were used to measure larval mortality 30 minutes post-treatment and to determine the residual activity of control agents in the test water 30 minutes, and 7 and 14 days post-treatment. Sucrose-fed adult females, 2-7 days old, were used to determine the adult mortality.

Lysinibacillus sphaericus formulation

A commercial wettable granule (WG) formulation of *Lysinibacillus sphaericus*, VectoLex® WG (Abbott Laboratories; Johor, Malaysia) containing 650 ITU/mg against *Cx. quinquefasciatus*, and has a LC_{50} value of 0.01 mg/l against laboratory bred *Cx. quinquefasciatus* larvae was used for the trial. The manufacturer's recommen-

dation for the outdoor application rate is 500 g/ha. In this trial, various dosages were tested.

Insecticide

A commercial pyrethroid formulation Resigen® [a.i.: S-bioallethrin, 0.8 %w/v; permethrin (25/75), 18.7% w/w; piperonyl butoxide, 16.8% w/v and inert ingredients, 63.7% w/v)] was used for the trial. The dosage used was based on the manufacturer's recommendations for outdoor thermal fog application, that is, 1:100 mixture.

Thermal fog generator

The Agrofog AF 35, IGEBA® thermal fogger (IGEBA Geraetebau; Weitnau, Germany) was used to disperse the insecticide formulations.

Test site

The field trials were conducted in an open area (50 ft x 50 ft). To determine the effectiveness of the spraying in relation to the distance from the fogging machine, the field was divided into two locations of 5 ft and 10 ft apart which were designated as test points.

Test formulation (Table 1)

a) VectoLex® WG (Ls) only was thermally applied in trial D1, D2, D3, D4 and D5 at application dosage of 95.6 g, 460 g, 500 g, 630 g and 844 g/ha, respectively; b) Resigen® (1:100) emulsifiable concentrate only was fogged in trial D6; c) A mixture of VectoLex WG® (625 g/ha) and Resigen® (1:100) was fogged in trial D7.

In all the trials, the operator walking parallel to the cages at a pre-designated distance with the nozzle pointing toward the field dispersed the formulation towards the test site.

Evaluation of trial

The effectiveness of each trial was evaluated using three different parameters: larval mortality; adult mortality and droplet profile analysis.

Larval mortality. In all the seven trials, three sets of paper cups each containing 200 ml seasoned tap water (for *Cx. quinquefasciatus* larvae) and seasoned tap water with 1% sodium chloride (for *Cx. sitiens* larvae) were placed at the designated test points. The cups were left exposed for 30 minutes post-fogging. All the cups were collected and brought back to the laboratory. The first set of cups was tested for larva mortality 30 minutes post-exposure. Twenty larvae (L3/L4) of *Cx. sitiens* or *Cx. quinquefasciatus* were added into each cup. The control sets of cups were left in the laboratory. The larval mortality was scored after 30 minutes, 24 hours, and 48 hours post-exposure. The 2nd and 3rd sets of cups were kept at room temperature in the laboratory and were tested for the residual activity of control agents at 7 and 14 days post-fogging.

Adult mortality. In trial D7, the adult females of each species were transferred into cages and hung onto poles at the designated test points at 5 ft and 10 ft. The control for adults in every trial were placed in cages and kept in the insectarium. Thirty minutes after fogging, all adults were transferred into paper cups and given 10% sucrose solution in a cotton pad. Adult mortality was observed for the interval of 2 hours, 5 hours, and 24 hours post-fogging.

Droplet profile analysis. Magnesium oxide (MgO)-coated glass slides were used to measure the droplet size and density of the fogged particles. The slides were examined under a light microscope (100 x) fitted with an ocular micrometer to measure the diameter of the impinged droplets. A total of 30 droplets per slide were measured. The data were analysed using

Table 1
Field evaluation of VectorLex® WG and Resigen® using a portable thermal fog generator.

Trial no.	Test formulation	Discharge rate	Dosage (a.i/ha)
D1	VectoLex® WG (20.0 g) dissolved in 2,000 ml H_2O (water)	220 ml in 66 secs	95.6 g a.i/ha
D2	VectoLex® WG (20.0 g) dissolved in 2,000 ml water	230 ml in 60 secs	460.0 g a.i/ha
D3	VectoLex® WG (20.0 g) dissolved in 2,000 ml water	250 ml in 60 secs	500.0 g a.i/ha
D4	VectoLex® WG (25.2 g) dissolved in 2,000 ml water	250 ml in 60 secs	630.0 g a.i/ha
D5	VectoLex® WG (36.7 g) dissolved in 2,000 ml water	230 ml in 60 secs	844.0 g a.i/ha
D6	Resigen® (15.4 ml) mixed in 2,000 ml water	300 ml in 60 secs	462.0 ml a.i/ha
D7	VectoLex® WG (28.7 g) and Resigen® (15.4 ml); dissolved and mixed in 2,004.6 ml water	220 ml in 60 secs	VectoLex® WG: 625.0 g a.i/ha Resigen®: 335.5 ml a.i/ha

the droplet analysis program of Sofield and Kent (1984). The droplets with large volume are represented by the volume median diameter (vmd) while the small volume droplets represented by the number median diameter (nmd).

Bioassay of pre- thermally applied VectoLex® WG

To prepare a stock solution, 37.0 g of VectoLex® WG were mixed well in 2,000 ml of tap water in a clean container. Ten milliliters of the 2,000 ml stock solution were transferred into a tube (pre-thermal sample) for the pre-thermal fogging bioassay. The other 1,990 ml was fogged and a sample was collected in a container at the end of the nozzle. Ten milliliters of the post-thermal sample were transferred into a tube and bioassayed.

For the bioassay, disposable 150 ml paper cups were used. For each test concentration, at least 3 replicates were used. A total of 25 *Cx. quinquefasciatus* larvae (late 3rd instar) were introduced into each cup. Two additional cups containing 150 ml distilled water were used as control replicates. The larva mortality was recorded after 24 hours and 48 hours exposure by counting the live larvae. The mortality data were pooled and analysed using Probit Analysis Program of Raymond (1985) to obtain the lethal concentration value.

RESULTS

Bioassay of VectoLex® WG (Ls)

The 24-hour LC_{50} value for the pre-thermal Ls sample was 0.017 mg/l (0.013 < LC < 0.022) and after 48-hours exposure, the LC_{50} value for the pre-thermal and sample was 0.0095 mg/l (0.0072 < LC < 0.013).

Trial D1 (Fig 1)

The droplets from the dispersed particles were detected on the MgO glass slides placed at test point of 10 ft. There was no droplet detected on the MgO glass slides placed at test point of 20 ft and 30 ft in D1, showing that the maximum dispersal distance is only up to 10 ft. The vmd and nmd of the dispersed particles was 74.55 μm and 41.71 μm, respectively

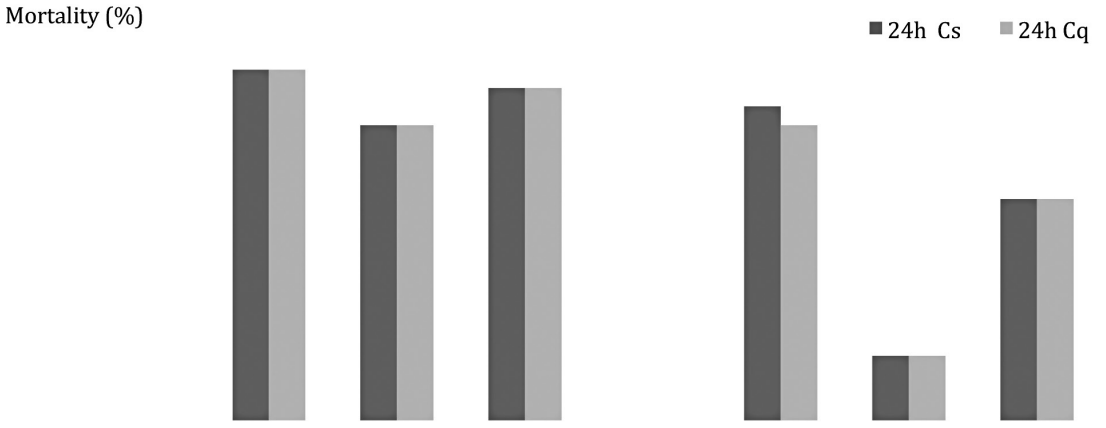

Fig 1–Mean 24 and 48 hours post-fogging larval mortality (%) of *Culex sitiens* exposed to thermally applied VectoLex WG® at 95.6 g/ha.

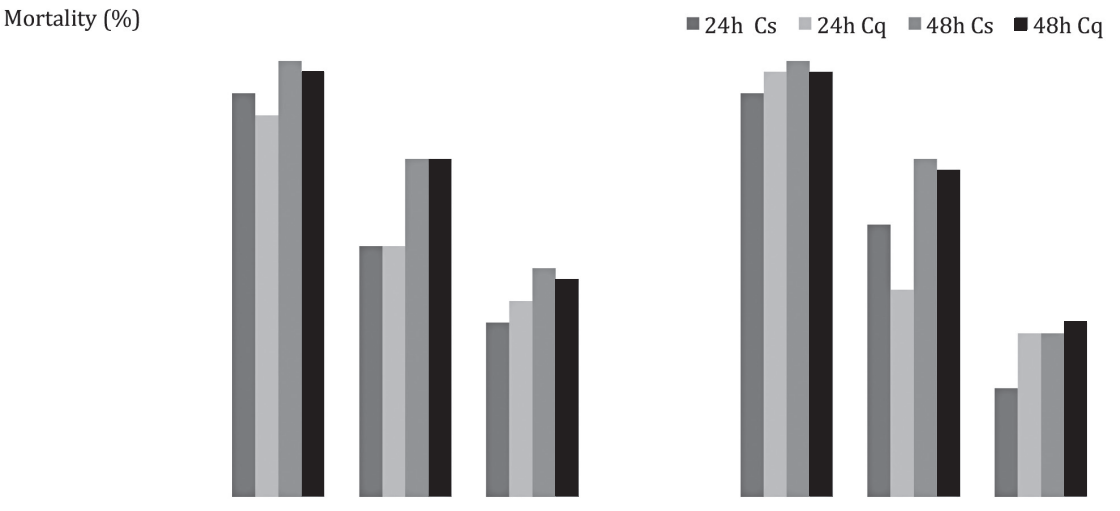

Fig 2–Mean 24 and 48 hours post-foging larval mortality (%) of *Culex sitiens* (Cs) and *Culex quinquefasciatus* (Cq) exposed to thermally applied VectoLex® WG at 460 g/ha.

with a ratio of 1:1.79, indicating that the dispersed particles were quite uniform.

The larval mortality in *Cx. sitiens* was 75-90 % after exposure period of 48 hours in the 30 minutes post-fogging at 10 ft. The percentage dropped to 35-55 %, 7 days and 20-25 %, 14 days post-fogging.

Trial D2 (Fig 2)

The test point was reduced to 5 ft and 10 ft, because of absence of droplets beyond 10 ft based on trial D1. At test point of 5 ft, the vmd and nmd of the dispersed particles was 105.12 μm and 49.33 μm, respectively with a ratio of 2.13 indicating that the dispersed particles were not uniformly distributed. At 10 ft, the vmd and nmd was 71.02 μm and 50.45 μm, respectively with the ratio of 1.41 indicated that the dispersed particles were more uniform size.

Complete larval mortality in *Cx. sitiens*

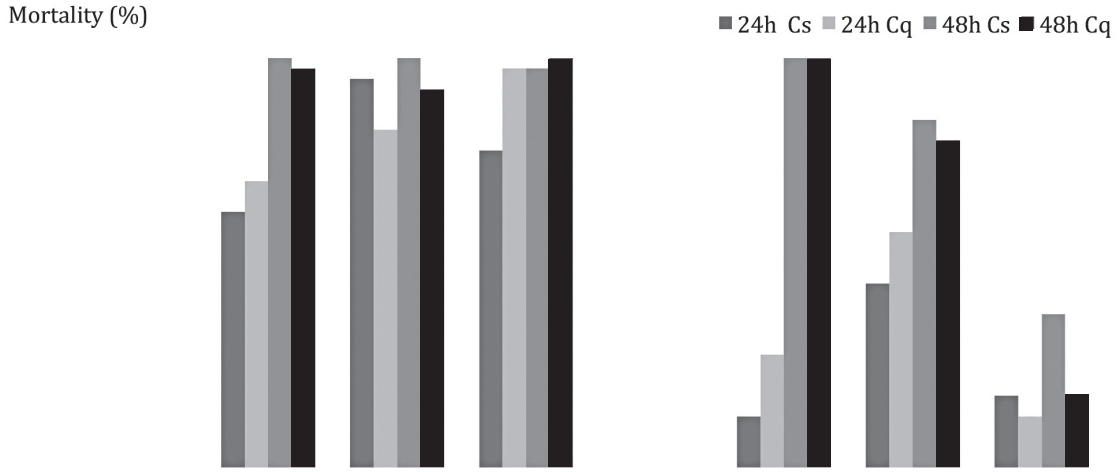

Fig 3–Mean 24 and 48 hours post-foging larval mortality (%) of *Culex sitiens* (Cs) and *Culex quinquefasciatus* (Cq) exposed to thermally applied VectoLex® WG at 500 g/ha.

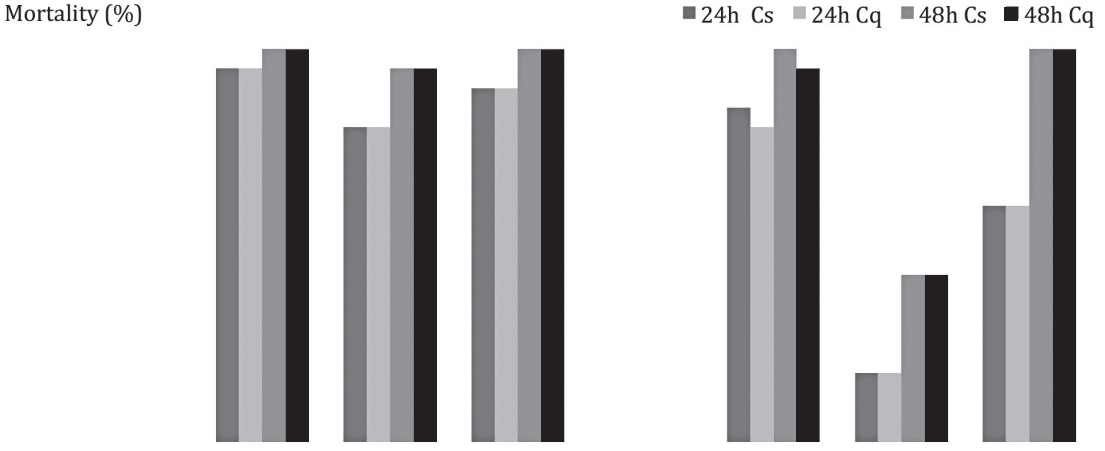

Fig 4–Mean 24 and 48 hours post-foging larval mortality (%) of *Culex sitiens* (Cs) and *Culex quinquefasciatus* (Cq) exposed to thermally applied VectoLex® WG at 630 g/ha.

was achieved after 48-hour exposure in the 30 minutes post-fogging for both test points at 5 ft and 10 ft, while in *Cx. quinquefasciatus* the larval mortality ranged from 90-100%. There is a significant difference ($p<0.05$) in number of larval killed between duration of post-fogging and also between *Cx. sitiens* and *Cx. quinquefasciatus*.

Trial D3 (Fig 3)

At test point of 5 ft, the vmd and nmd was 47.25 μm and 39.45 μm, respectively with a ratio of 1.20. At test point of 10 ft, the vmd and nmd was 45.16 μm and 36.19 μm, respectively with a ratio of 1.25. The ratios indicated that the dispersed particles were of uniform size.

Complete larval mortality was achieved in *Cx. sitiens* after 48 hours exposure in the 30 minutes post-fogging. In *Cx. quinquefasciatus*, the larval mortality was 90-100%.

Trial D4 (Fig 4)

At test point of 5 ft, the vmd and nmd of the dispersed particles was 60.91 μm and 42.31 μm, respectively with the ratio

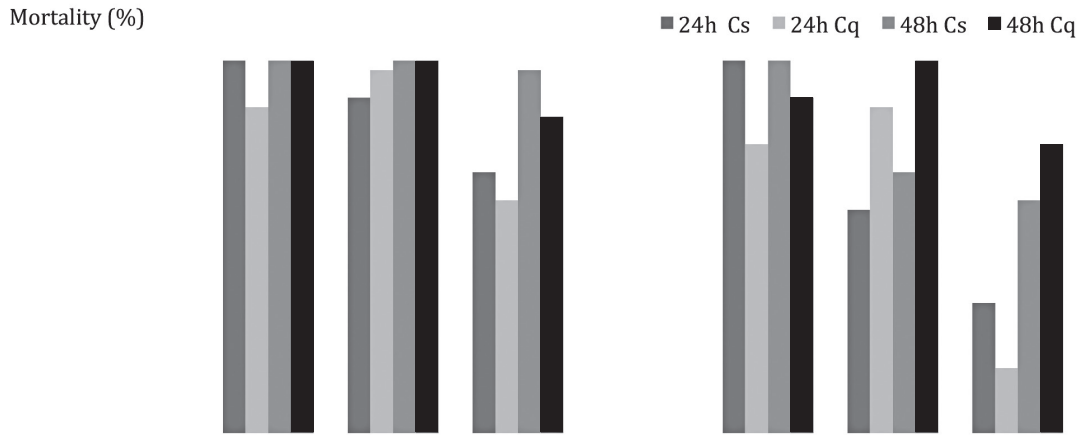

Fig 5–Mean 24 and 48 hours post-fogging larval mortality (%) of *Culex sitiens* (Cs) and *Culex quinqusfasciatus* (Cq) exposed to thermally applied VectoLex® WG at 844 g/ha.

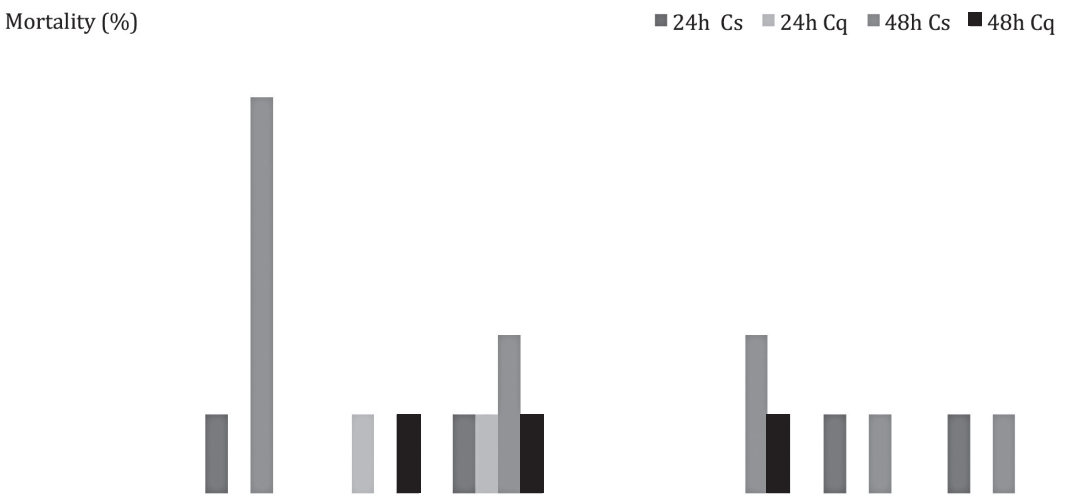

Fig 6–Mean 24 and 48 hours larval mortality of *Culex sitiens* (Cs) and *Culex quinquefasciatus* (Cq) exposed to thermally applied pyrethroid formulation (Resigen®) at 462 ml/ha.

of 1.43. At test point of 10 ft, the vmd and nmd of the dispersed particles was 55.77 μm and 42.31 μm, respectively with the ratio of 1.32. These ratios indicated that the dispersed particles were of uniform size.

Complete maximum larval mortality was achieved after 48 hours in both *Cx. sitiens* and *Cx. quinquefasciatus* at the test point of 5 ft in the 30 minutes post-fogging. The larval mortality after 48 hours ranged from 90-100% in the 7 days and 14 days post-fogging. When compared with trial D2, there was no significant difference ($p>0.05$) in number of larva killed in the 30 minutes and 7 days. However there is a significant difference ($p<0.05$) in number of larva killed 14 days post-fogging in *Cx. sitiens*.

Trial D5 (Fig 5)

The ratio of vmd to nmd at test point

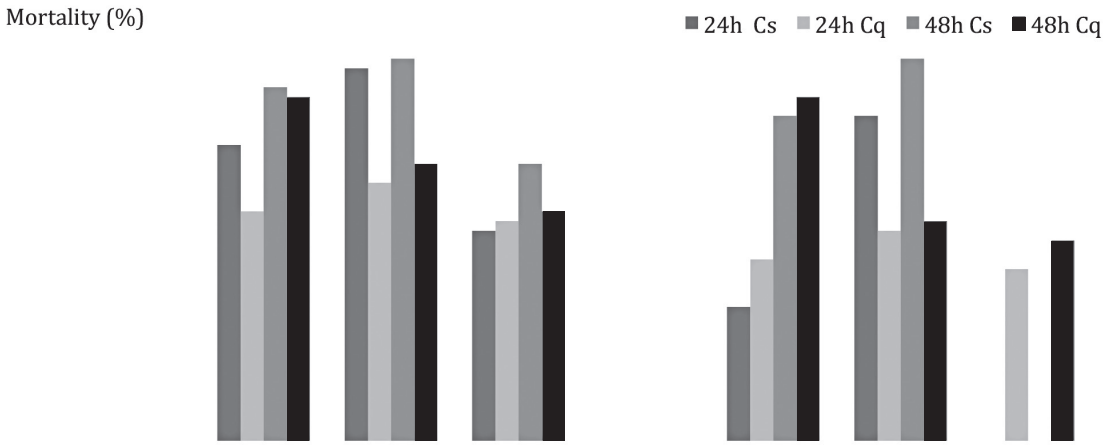

Fig 7–Mean 24 and 48 hours post-fogging larval mortality (%) of *Culex sitiens* (Cs) and *Culex quinqusfasciatus* (Cq) exposed to thermally applied VectoLex® WG at 625 g/ha and Resigen® at 335.5 ml/ha.

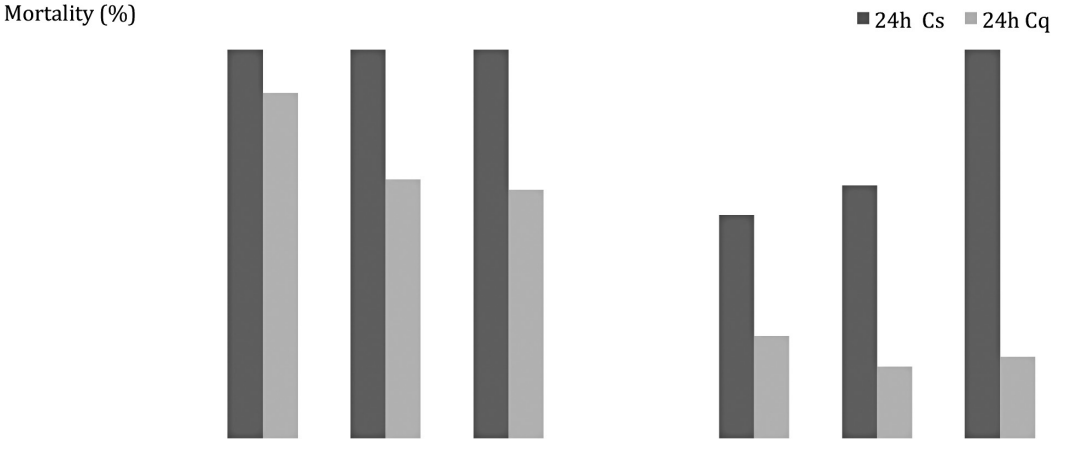

Fig 8–Mean post-fogging adult mortality (%) of *Culex sitiens* (Cs) and *Culex quinqusfasciatus* (Cq) exposed to thermally applied VectoLex® WG at 625 g/ha and Resigen® at 335.5 ml /ha.

of 5 ft and 10 ft was 1.40 and 1.30, respectively. These ratios indicated that the dispersed particles were of uniform size.

At test point of 5 ft, complete larval mortality was achieved after 48-hour exposure for both *Cx. sitiens* and *Cx. quinquefasciatus*. At test point of 10 ft, the larval mortality ranged from 80-100% after 48 hours in the 14 days post-fogging.

When compared with trial D2, there was no significant difference ($p>0.05$) in number of larva killed in *Cx. sitiens* after 24-hour exposure in the 30 minutes, 7th days and 14th days but there is a significant difference ($p<0.05$) after 48 hours of exposure. However in *Cx. quinquefasciatus*, there was no significant difference ($p>0.05$) in number of larva killed after 24 and 48-hours exposure in 30 minutes and 14 days post-fogging but there is a significant difference in 7 days post-fogging.

When compared with trial D4, there

is no significant difference (p>0.05) in number of larva killed in both *Cx. sitiens* and *Cx. quinquefasciatus* in the 30 minutes, 7 days, and 14 days after post-fogging.

Trial D6 (Fig 6): Thermal fogging of Resigen®

At test point of 5 ft, the vmd and nmd of the dispersed particles was 53.15 μm and 43.14 μm, respectively with a ratio of 1.23. At test point of 10 ft the vmd and nmd was 48.22 μm and 40.70 μm, respectively with a ratio of 1.18. These ratios indicated that the dispersed particles were of uniform size.

At the test point of 5 ft, complete adult mortality was achieved after 24 hours in *Cx. sitiens*. However, the mortality ranged from 80-100% in *Cx. quinquefasciatus*. A mortality of below 10% was achieved after 24 hours for the test point of 10 ft for both *Cx. sitiens* and *Cx. quinquefasciatus*.

Minimal larval mortality was achieved in both *Cx. sitiens* and *Cx. quinquefasciatus* in the 30 minutes, 7 days, and 14 days post-fogging. When compared with trial D5, there is a significant difference (p<0.05) in number of larva killed in both *Cx. sitiens* and *Cx. quinquefasciatus* in the 30 minutes, 7 days, and 14 days post-fogging.

Trial D7 (Figs 7 and 8) : Thermal fogging of a mixed formulation of VectoLex® WG and Resigen®

At test point of 5 ft, the vmd and nmd of the dispersed particles was 51.79 μm and 39.13 μm, respectively with a ratio of 1.32. At test point of 10 ft, the vmd and nmd of the dispersed particles was 35.67 μm and 31.98 μm, respectively, with a ratio of 1.12. These ratios indicated that the dispersed particles were of uniform size.

The larval mortality of 80-100% was achieved after 48-hour exposure in the 30 minutes and 7 days after post-fogging. When compared with trial D2 and trial D5, there was no significant difference (p>0.05) in number of larva killed after 48-hour exposure in the 30 minutes, 7 days, and 14 days post fogging in both *Cx. sitiens* and *Cx. quinquefasciatus*.

At test point of 5 ft, complete adult mortality was achieved after 24 hours in *Cx. sitiens*. However in *Cx. quinquefasciatus* the mortality ranged from 60-80%.

DISCUSSION

In this study a microbial insecticide VectoLex® WG was tested alone or mixed with a chemical insecticide formulation, Resigen® for the control of *Culex sitiens,* and *Culex quinquefasciatus*. A portable thermal-fog generator, Agrofog AF35 IGEBA® was used to disperse the formulations. The ratio of vmd to nmd of the sprayed particles between the formulations used in the trials indicated that the portable thermal fog generator, Agrofog AF35 IGEBA® effectively dispersed the formulations to uniform size droplets. The VectoLex® WG and Resigen® was effectively sprayed without any clogging. Thus, the hot gas generated in the thermal fogger atomised the formulation with negligible deterioration of the Ls formulation. However, the maximum distance of the dispersed particles was only up to 10 ft. Beyond that point, there was no droplets detected on the MgO glass slides. Thermal fogger employs high heat to vaporise and disperse the control agent without use of pressure and as such, the spray distance is limited.

The formulation, VectoLex® WG was bioassayed using laboratory bred *Cx. quinquefasciatus* larvae (late 3rd instar). The bioassay was conducted for the pre-thermal fogging and the results indicated that VectoLex® WG was highly larvicidal to *Cx. quinquefasciatus*. Heat from the thermal

fogger did not reduce the larvicidal activity of the VectoLex® WG formulation. Seleena *et al* (2001) also mentioned that a thermal fog generator that employs the resonant pulse principle to generate hot gas at high velocity could be used to efficiently space spray Bti. They concluded that water used to dilute Bti formulation effectively protected Bti from deterioration and similar protection would have occurred in thermal fogging of Ls. Thermal fogging of Resigen® alone was effective against adults of *Cx. sitiens* and *Cx. quinquefasciatus;* however a higher mortality was achieved for *Cx. sitiens*, indicating that *Cx. sitiens* was more susceptible to pyrethroids compared to *Cx. quinquefasciatus*. A higher dosage of Resigen® should be able to induce higher mortality in both species. Resigen® has no larvicidal effect, but it was noticed that there was a knocked-down effect after 2-3 hours exposure on the larvae of both *Cx. sitiens* and *Cx. quinquefasciatus* without mortality.

Thermal fogging of VectoLex® WG was effective against larval stages of both *Cx. sitiens* and *Cx. quinquefasciatus*. The portable thermal fog generator effectively dispersed the formulation without affecting the Ls larvicidal toxins. The larvicidal toxins of VectoLex® WG at 500-800 g/ha was very stable and able to effect high larval mortality for a duration of 14 days post-treatment. As a comparison, Seleena *et al* (2001) reported that the larvicidal toxin of VectoBac 12AS® (Bti) also lasts for a duration of 14 days post-treatment against *Aedes aegypti* larvae. Mulligan *et al* (1980) reported that persistence of Ls increased with dosage and this was also observed in this study.

Thermal fogging of a mixed formulation of pyrethroids and Ls (Resigen® and VectoLex® WG) was significantly effective against both *Cx. sitiens* and *Cx. quinquefasciatus*. The mixture formulation induced higher mortality in both larvae and adult stage. There is no antagonistic effect against each other between the two formulations. Seleena *et al* (2001) reported that a Bti formulation could be dispersed efficiently using a similar thermal fog generator, Agrofog AF 35. In term of formulation stability, the wettable granule formulation of Ls (VectoLex® WG) is more stable compared to aqueous formulation of Bti (Vectobac 12AS®).

The concept of simultaneous adulticiding and larviciding has been evaluated in the control of disease-carrying vectors. Seleena *et al* (2001) reported that a thermal fog generator that employed the resonant pulse principle to generate hot gas (over 200°C) at high velocity can be used to efficiently space spray Bti. In the study, a similar fog generator, Agrofog AF 35 IGEBA® (IGEBA Geraetebau; Weitnau, Germany) was used to disperse a 50x diluted Bti formulation and a similar pyrethroid formulation, Aqua-Resigen®. The 50 folds diluted VectoBac 12AS® formulation caused a 95% mortality among the tested *Ae. aegypti* larvae in the 15 minutes post-treatment samples at 48 hours exposure and the residual effects lasted for 14 days. High adult and larval mortality was achieved on fogging a mixture of the 50 fold diluted VectoBac 12AS® formulation at a dosage of 300 ml/ha together with Aqua-Resigen®. The portable thermal fog generator can be used efficiently to disperse larvicides such as Bti and a water-based adulticide simultaneously in an area inaccessible to vehicle-mounted sprayers by road (Seleena *et al*, 2001).

Previous study by Seleena and Lee (1998), Seleena *et al* (1999), and Kapa (2000) have shown that microbial control agent (Bti and Ls) and chemical insecticides (*eg*, water-based formulation of

pyrethroid insecticide) were not antagonistic to each other and they concluded that larviciding and adulticiding activities could be incorporated into a single vector control operation. Seleena *et al* (2004) reported that complete larval mortality was achieved on spraying a mixture of VectoLex® WDG and Fendona® SC (alpha cypermethrin) in a field evaluation against malaria vector in Ranau, Sabah. These data suggested that the larvicidal toxins of VectoLex® WDG are stable and are not degraded by chemical adulticide such as alpha cypermethrin.

In term of applicability, the concept of thermal fogging of a biological-based insecticide formulation such as Ls and Bti together with chemical insecticide especially pyrethroid is an effective approach in vector control since this method is highly effective against both adult and larval stage of the target insect. In vector control strategy, controlling the immature stages (larval) is as important as adult stage especially during disease outbreak. Therefore, simultaneous application of adulticide and larvicide should be adopted as an effective strategy in vector control.

ACKNOWLEDGEMENTS

The authors thank the Director General of Health, Malaysia for permission to publish and Director, Institute for Medical Research, Kuala Lumpur for support. Thanks are also due to Mr Chiang Yee Fook, Mr Mustami Talib and Mr Subramaniam, Medical Entomology Unit, IMR for their assistance. This project was partially supported by the SEAMEO-TROPMED National Centre, Malaysia.

REFERENCES

Cheong WC, Yap HH. Bioassays of *Bacillus sphaericus* (Strain 1593) against mosquitoes of public health important in Malaysia. *Southeast Asian J Trop Med Public Health* 1985; 16: 54-8.

Kapa O. Field evaluation of a mixture of Malaysian isolate of *Bacillus sphaericus* and a pyrethroid dispersed by cold fogging for the control of *Culex* vector. Kuala Lumpur: SEAMEO TROPMED IMR Malaysia, 2000. 37 pp. Dissertation.

Lee HL, Chan, ST, Cheong WH. Laboratory bioassays of *Bacillus sphaericus* 1593, 2297 and 2362 against mosquitoes of public health importance in Malaysia. *Trop Biomed* 1986; 3: 161-8.

Lee HL, Ernesto RG Jr, Khadri MS, Seleena P. Ultra-low-volume application of *Bacillus thuringiensis* serotype H-14 for the control of mosquitoes. *J Am Mosq Control Assoc* 1996; 12: 651-5.

Mariappan T, Amalraj DD, Doss PS, *et al*. Field evaluation of Spicbiomoss, a biolarvicidal formulation of *Bacillus sphaericus* against immature of *Culex quinquefasciatus* & *Anopheles* species. *Indian J Med Res* 1999; 110: 128-32.

Mulligan FS, Schaefer CH, Wilder WH. Efficacy and persistence of *Bacillus sphaericus* and *Bacillus thuringiensis* H-14 against mosquitoes under laboratory and field conditions. *J Econ Entomol* 1980; 73: 684-8.

Phanthumachinda B. Ecology and biology of Japanese encephalitis vectors. *Southeast Asian J Trop Med Pub Health* 1995; 26: 11-6.

Raymond M. Log-probit analysis basic program of microcomputer. *Cahiers ORSTOM Entomol Med Parasitol* 1985; 23: 117-21.

Seleena P, Lee HL. Field trials to determine the effectiveness of *Bacillus thuringiensis* ssp. *israelensis* application using an ultra low volume (ULV) generator for the control of *Aedes* mosquitoes. *Israel J Entomol* 1998; 32: 25-31.

Seleena P, Lee HL, Chiang YF. Compatibility of *Bacillus thuringensis* serovar *israelensis* and chemical insecticides for the control of

Aedes mosquitoes. *J Vect Ecol* 1999; 24: 216-23.

Seleena P, Lee HL, Chiang YF. Thermal application of *Bacillus thuringiensis* serovar *israelensis* for dengue vector control. *J Vector Ecol* 2001; 26: 110-3.

Seleena P, Lee HL, Chooi KH, Junaidith S. Space spraying of bacterial and chemical insecticides against *Anopheles balabacensis* Baisas for the control of malaria in Sabah, East Malaysia. *Southeast Asian J Trop Med Public Health* 2004; 35: 68-78.

Siegel JP, Novak RJ. Duration of activity of the microbial larvicide VectoLex CG (*Lysinibacillus sphaericus*) in Illinois catch basins and waste tires. *J Am Mosq Contr Assoc* 1999; 15: 366-70.

Sofield RK, Kent R. A basic programme for the analysis of ULV insecticide droplets. *Mosq News* 1984; 44: 73-5.

Su T, Mulla MS. Field evaluation of new water-dispersible granular formulations of *Bacillus thuringiensis* ssp. *israelensis* and *Bacillus sphaericus* against *Culex* mosquitoes in microcosms. *J Am Mosq Contr Assoc* 1999; 15: 356-65.

Vythilingam I, Oda K, Tsuchie H, Mahadevan S, Vijayamalar B. Isolation of Japanese encephalitis virus from *Culex sitiens* mosquitoes in Selangor, Malaysia. *J Am Mosq Contr Assoc* 1994; 10: 228-9.

Vythilingam I, Oda K, Chew *et al*. Isolation of Japanese encephalitis virus from mosquitoes collected in Sabak Bernam, Selangor, Malaysia in 1992. *J Am Mosq Contr Assoc* 1995; 11: 94-8.

World Health Organisation (WHO). Japanese encephalitis. *WHO Fact Sheet* Dec 2015; 386. [Cited 2016 Jun 3]. Available from: http://www.who.int/mediacentre/factsheets/fs386/en/

Yadav RS, Sharma VP, Upadhyay AK. Field trial of *Bacillus sphaericus* strain B-101 (serotype H5a, 5b) against filariasis and Japanese encephalitis vectors in India. *J Am Mosq Contr Assoc* 1997; 13: 158-63.