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Evaluation of Sumithion L-40 against *Aedes aegypti* (L.) and *Aedes albopictus* Skuse

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Abstract. Space spraying of chemical insecticides is still an important mean of controlling *Aedes* mosquitoes and dengue transmission. For this purpose, the bioefficacy of space-sprayed chemical insecticide should be evaluated from time to time. A simulation field trial was conducted outdoor in an open field and indoor in unoccupied flat units in Kuala Lumpur, to evaluate the adulticidal and larvicidal effects of Sumithion L-40, a ULV formulation of fenitrothion. A thermal fogger with a discharge rate of 240ml/min was used to disperse Sumithion L-40 at 3 different dosages (350 ml/ha, 500 ml/ha, 750 ml/ha) against lab-bred larvae and adult female *Aedes aegypti* and *Aedes albopictus*. An average of more than 80% adult mortality was achieved for outdoor space spray, and 100% adult mortality for indoor space spray, in all tested dosages. Outdoor larvicidal effect was noted up to 14 days and 7 days at a dosage of 500 and 750 ml/ha for *Ae. aegypti* and *Ae. albopictus*, respectively. Indoor larvicidal effect was up to 21 days (500 ml/ha) and 14 days (750 ml/ha), respectively, after spraying with larval mortality > 50% against *Ae. aegypti*. This study concluded that the effective dosage of Sumithion L-40 thermally applied against adult *Ae. aegypti* and *Ae. albopictus* indoor and outdoor is 500 and 750 ml/ha. Based on these dosages, effective indoor spray volume is 0.4 – 0.6 ml/m^3. Additional indoor and outdoor larvicidal effect will be observed at these application dosages, in addition to adult mortality.

INTRODUCTION

Dengue is a serious public health disease in Malaysia since the first nationwide dengue outbreak in 1973; the most prevalent vectors for dengue are *Aedes aegypti* and *Aedes albopictus* (Lee *et al.*, 1997). In the continued absence of specific treatment and effective vaccine against dengue virus, dengue control relies on suppressing *Aedes* populations and subsequent interruption of disease transmission through the use of insecticides, especially during outbreaks (Esu *et al.*, 2010).

The organophosphate fenitrothion was applied in space spraying for dengue and malaria control since 1970s (Samutrapongse & Pant, 1973; Pant *et al.*, 1974; Fontaine *et al.*, 1978). In the context of Malaysian dengue vector control, fenitrothion was used in operational dengue control since 1980s and several studies had been conducted to evaluate the efficacy of fenitrothion against *Aedes* sp. in dengue endemic area by using ULV machine (Lam & Tham, 1988; Lee *et al.*, 1997; Sulaiman *et al.*, 1999). Since the last evaluation was done in 1999, a re-evaluation of fenitrothion deemed necessary. Thus, in this study we evaluated the efficacy of thermally-applied Sumithion L-40 against *Ae. aegypti* and *Ae. albopictus* adults and determined the optimum dosage of Sumithion L-40 for effective *Aedes* control.

MATERIALS AND METHODS

Mosquitoes

Sugar-fed adult females (aged 2-5 d) and late third and early fourth instar (L3/L4) larvae of *Ae. aegypti* and *Ae. albopictus* were obtained from the insectarium of Institute for Medical Research (IMR), Malaysia.

Outdoor fogging

An area of 0.15 ha (1500 m^2) in an open field at Jalan Fletcher, Kuala Lumpur (3°10'22.00 N, 101°42'32.28 E) was selected for the outdoor trial. A 60 m straight transect was designated in the middle of the site as the spray line to be followed by the fogger. T-shaped poles were set upon at distances of 5, 10, 15, 20 and 25 m from the spray line on a perpendicular transect. Three cages (9 x 24 cm) each with 25 *Ae. aegypti* females and 3 cages each with 25 *Ae. albopictus* females were placed 1.5 m above ground on each pole. Three plastic cups each containing 25 *Ae. aegypti* larvae, while another 3 plastic cups with *Ae. albopictus* larvae were placed below the cage on the ground. Magnesium oxide-coated slides were placed on the pole at every distance mentioned above.

Indoor fogging

The indoor trial was conducted in three unoccupied flat units at ground level. Each unit measured 8.4 (L) x 5.3 (W) x 2.8 m (H) [area width: 45 m^2; volume: 125 m^3] and comprised a bedroom, kitchen and sitting room. One screen mesh cage (9 x 24 cm) containing 25 adults each were placed against the wall 1.5 m above the ground in the sitting room, kitchen, bedroom and outside near the door. Magnesium oxide-coated slides were placed against the wall next to the cages. One plastic cup each holding 25 larvae was placed below the cage on the ground.

Spraying was conducted in the evening after sunset. The weather was fair during the course of the test and average wind velocity was < 1.0 m/s. The ambient temperature was 25±1°C and relative humidity was 80±5% during the trial.

Spray Equipment

A thermal fogging machine, Dynafog Falcon™ 4000 with 240 ml/min discharge rate nozzle was used to disperse the insecticide. The discharge rate was pre-calibrated in a separate spraying exercise. The operator conducted the fogging by moving along the spray line at a standard pace.

Insecticide formulation and treatment design

The Sumithion L-40 formulation, an ultra low volume (ULV) liquid, contained the active ingredients of: 40.0% w/w of fenitrothion and 1.0% w/w of tetramethrin. Three dosages were tested in this study: 350, 500 and 750 ml/ha, for both indoor and outdoor. Spraying time for each trial was adjusted to the machine flow rate at 240 ml/min. Details are shown in Table 1 below.

Table 1. Treatment design

Test no.	Treated area	Area Width (m^2)	Dosage (ml/ha)	Active ingredients/ treated area (gai/m^2)	Dilution rate (Sumithion L-40: diesel)	Spraying volume (ml)	Spraying time
1	Indoor	50	350	0.7	1:39	70	17.5s
2	Indoor	50	500	1.0	1:39	100	25s
3	Indoor	50	750	1.5	1:39	150	37.5s
4	Outdoor	1500	350	21	1:9	525	2.18 min
5	Outdoor	1500	500	30	1:9	750	3.12 min
6	Outdoor	1500	750	45	1:9	1125	4.70 min

Evaluation of fogging

Adult mosquitoes were transferred into clean paper cups and provided with a cotton pad soaked with 10% sugar solution after fogging. The knock down rate of adult mosquitoes was recorded every minute for the first 10 minutes followed by 15, 20, 25 and 30 min post-fogging. Plastic cups containing mosquito larvae were transported to the lab and mortality counts conducted after a holding period of 24 hours. The residual larvicidal effect was evaluated weekly up to 4 weeks. Twenty five L3/L4 larvae of both species were introduced into the plastic cups and mortality was recorded after 24 hours.

Droplet analysis

The MgO slides were examined under the compound microscope with the aid of a video camera to measure the droplets size and to count the density of droplets deposited on the slides. The data was analysed using a software to obtain the Volume Median Diameter (VMD) and Number Median Diameter (NMD), as described by Solfield & Kent (1984).

Data analysis

The data were pooled and analyzed using T-test and Dunnett T3 (ANOVA) for multiple comparisons (SPSS11.5).

RESULTS

In outdoor trial, the 24-h post-spraying mortality of *Ae. aegypti* and *Ae. albopictus* is shown in Tables 2 & 3, respectively. The results generally showed inconsistent mortality at different distance, especially at 20 m and 25 m, indicating that the drift resulted from the unexpected direction change of the wind during fogging was not consistent.

Because of the nature of thermal fogging, i.e. the effective range of the fog, only adult mortality at the distance of 5 m & 10 m was combined and averaged (Table 4). The

Table 2. Post-treatment 24 hour mortality (%) of adult *Aedes aegypti* treated with different dosages of Sumithion L-40 after outdoor space spray

Dosage (ml/ha)	% Mortality (Mean ± S.D.)				
	5m (n=3)	10m (n=3)	15m (n=3)	20m (n=3)	25m (n=3)
350	$90.7 \pm 7.42^{a,A}$	$80.0 \pm 9.23^{a,A}$	$96.0 \pm 2.31^{a,A}$	$94.7 \pm 5.33^{a,A}$	$77.3 \pm 7.06^{a,A}$
500	$100 \pm 0.0^{a,A}$	$100.0 \pm 0.00^{a,A}$	$88.0 \pm 12.0^{a,A}$	$58.7 \pm 21.3^{a,A}$	$85.3 \pm 8.11^{a,A}$
750	$100 \pm 0.0^{a,A}$	$88.0 \pm 12.0^{a,A}$	$88.0 \pm 12.0^{a,A}$	$5.33 \pm 5.33^{b,B}$	$80.0 \pm 8.33^{a,A}$

Knockdown/mortality for the control mosquitoes was less than 10%.
^{ab}Within the column, mean followed by different letters are statistically significant using Dunnett T3 test ($P < 0.05$).
^{AB}Within the row, mean followed by different letters are statistically significant using Dunnett T3 test ($P < 0.05$).

Table 3. Post-treatment 24 hour mortality (%) of adult *Aedes albopictus* treated with different dosages of Sumithion L-40 after outdoor space spray

Dosage (ml/ha)	% Mortality (Mean ± S.D.)				
	5m (n=3)	10m (n=3)	15m (n=3)	20m (n=3)	25m (n=3)
350	$98.7 \pm 1.33^{a,A}$	$70.7 \pm 17.3^{a,A}$	$60.0 \pm 4.00^{a,A}$	$64.0 \pm 12.2^{a,A}$	$45.3 \pm 5.33^{ab,B}$
500	$100.0 \pm 0.0^{a,A}$	$100.0 \pm 0.0^{a,A}$	$89.3 \pm 4.81^{a,A}$	$18.7 \pm 6.67^{a,B}$	$9.3 \pm 4.81^{a,B}$
750	$100.0 \pm 0.0^{a,A}$	$100.0 \pm 0.0^{a,A}$	$53.3 \pm 17.0^{a,A}$	$48.0 \pm 18.9^{a,A}$	$81.3 \pm 12.7^{b,A}$

Knockdown/mortality for the control mosquitoes was less than 10%.
^{ab}Within the column, mean followed by different letters are statistically significant using Dunnett T3 ($P < 0.05$).
^{AB}Within the row, mean followed by different letters are statistically significant using Dunnett T3 ($P < 0.05$).

Table 4. Mean post-treatment 24 hour mortality of adult *Aedes aegypti* and *Aedes albopictus* treated with different dosages of Sumithion L-40 at 5 m & 10 m after outdoor space spray

Dosage (ml/ha)	No. Cages	Mortality (Mean ± S.D)	
		Aedes aegypti	*Aedes albopictus*
350	6	85.3 ± 5.81^{a}	84.7 ± 9.98^{a}
500	6	100 ± 0.0^{a}	100 ± 0.0^{a}
750	6	94.0 ± 6.00^{a}	100 ± 0.0^{a}

^{ab}Within the column, mean followed by different letters are statistically significant using T-test (P < 0.05).

Table 5. Post-treatment 24 hours mortality (%) of adult *Aedes aegypti* after treatment with different dosage of Sumithion L-40 after indoor space spray

Dosage (ml/ha)	No. cages	% Mortality (Mean ± S.D)
350	12	100.0 ± 0.0
500	12	100.0 ± 0.0
750	12	100.0 ± 0.0

Knockdown/mortality for the control mosquitoes was less than 10%

mortality of adult *Ae. aegypti* at 500 ml/ha and 750 ml/ha was >90%.

In indoor space spray, complete adult mortality of *Ae. aegypti* was observed in all the tested dosages (Table 5). The result (Table 6) on residual effect against larvae of *Ae. aegypti* suggested that a dosage of 350 ml/ha was effective from 0-7 days after spraying. However, the mortality was low (<13% mortality) in all cases. Likewise, a dosage of 500 ml/ha and 750 ml/ha was effective against the larvae for 14 days.

Table 7 showed the residual effect of Sumithion L-40 against larvae of *Ae. albopictus*. There was very low larval mortality at 350 ml/ha (<2%). The residual effect for 500 ml/ha and 750 ml/ha were effective against the larvae up to 7 days, with larvicidal activity >40%.

Sumithion L-40 showed a high residual effect against larvae of *Ae. aegypti* indoor (Table 8). All the 3 dosages exhibited larvicidal activity up to 28 days post-spraying, achieving >50% until day 21 at dosage 500 – 750 ml/ha.

The droplet analysis (Tables 9 & 10) showed irregular distribution of droplet sizes as indicated by the ratio of VMD:NMD. In most instances, the ratio was below 2.

DISCUSSION

Pyrethroids (PYs) and organophosphates (OPs) are the insecticides approved by WHO for space spraying. In Malaysia, the commonly used PYs are permethrin, deltamethrin, lambda-cyhalothrin and cyphenothrin; while OPs are fenitrothion, malathion and pirimiphos-methyl.

Adult mortality rate was observed to decline with increasing distance from spray line for outdoor spray application (Tables 2 & 3). Deposits of droplet drift are highest near the spray line, decreased rapidly as the distance increased from spray line (Bretthauer, 2011) as drift occurred downwind of the application site (Burt &

Table 6. Residual effect of Sumithion L-40 against larvae of *Aedes aegypti* for a period of 28 days after outdoor space spray

Dosage (ml/ha)	No. cups	% Mortality (Mean ± S.D)				
		24 hours	Day 7	Day 14	Day 21	Day 28
350	6	$12.0 \pm 4.13^{a, A}$	$12.7 \pm 6.23^{a, A}$	$4.00 \pm 2.07^{a, A}$	$0.67 \pm 0.67^{a, A}$	$1.33 \pm 0.84^{a, A}$
500	6	$52.0 \pm 15.7^{ab, ABC}$	$85.3 \pm 2.67^{b, B}$	$26.7 \pm 4.22^{b, C}$	$0.67 \pm 0.67^{a, A}$	$0.0 \pm 0.0^{a, A}$
750	6	$82.0 \pm 7.78^{b, A}$	$86.7 \pm 5.97^{b, A}$	$61.3 \pm 12.8^{b, A}$	$6.0 \pm 4.10^{b, B}$	$0.0 \pm 0.0^{a, B}$

Mortality for the control larvae was less than 10%.
^{ab}Within the column, mean followed by different letters are statistically significant using Dunnett T3 test (P < 0.05).
^{ABC}Within the row, mean followed by different letters are statistically significant using Dunnett T3 test (P < 0.05).

Table 7. Residual effect of Sumithion L-40 against larvae of *Aedes albopictus* for a period of 28 days after outdoor space spray

Dosage (ml/ha)	No. cups	% Mortality (Mean ± S.D)				
		24 hours	Day 7	Day 14	Day 21	Day 28
350	6	2.00± 0.89^{a,A}	0.67± 0.67^{a,A}	0.00± 0.00^{a,A}	0.00± 0.00^{a,A}	0.00± 0.00^{a,A}
500	6	23.3 ± 13.3^{ab,AB}	40.0 ± 4.26^{b,B}	0.00± 0.00^{a,A}	0.00± 0.00^{a,A}	0.00± 0.00^{a,A}
750	6	39.3 ± 9.82^{b,AB}	50.7 ± 9.45^{b,B}	9.33± 3.96^{a,A}	2.00± 2.00^{b,A}	1.33± 0.84^{a,A}

Mortality for the control larvae was less than 10%.
^{ab}Within the column, mean followed by different letters are statistically significant using Dunnett T3 test ($P < 0.05$).
^{AB}Within the row, mean followed by different letters are statistically significant using Dunnett T3 test ($P < 0.05$).

Table 8: Residual effect of Sumithion L-40 against larvae of *Aedes aegypti* for a period of 28 days after indoor space spray

Dosage (ml/ha)	No. cups	% Mortality (Mean ± S.D)				
		24 hours	Day 7	Day 14	Day 21	Day 28
350	12	59.3 ± 44.5^{a,ABC}	94.3 ± 3.36^{a,B}	71.3 ± 12.2^{a,B}	22.3± 5.01^{a,CD}	5.33± 2.27^{a,D}
500	12	93.3 ± 4.91^{ab,A}	76.0± 9.52^{a,A}	80.3 ± 10.5^{a,A}	69.7± 8.59^{b,A}	33.3 ± 7.62^{b,B}
750	12	96.3 ± 2.48^{b,A}	95.3 ± 2.50^{a,A}	93.0 ± 4.24^{a,A}	51.7± 9.84^{ab,B}	24.7 ± 6.80^{b,B}

Mortality for the control larvae was less than 10%.
^{ab}Within the column, mean followed by different letters are statistically significant using Dunnett T3 test test ($P < 0.05$).
^{ABCD}Within the row, mean followed by different letters are statistically significant using Dunnett T3 test ($P < 0.05$).

Table 9. Overall droplet analysis in outdoor application

Dosage (ml/ha)	Total no. droplets analyzed	VMD (µm)	NMD (µm)	Ratio
350	11	27.38	20.78	1.32
500	92	41.97	11.51	3.65
750	350	21.97	11.84	1.86

Table 10. Overall droplet analysis in indoor application

Dosage (ml/ha)	Total no. droplets analyzed	VMD (µm)	NMD (µm)	Ratio
350	345	31.25	16.60	1.88
500	360	32.79	22.14	1.48
750	350	54.58	23.23	2.35

smith, 1974). A wind direction of 15° and 30° off-perpendicular spray line increases distance by 3.5% and 15.4%, respectively (WHO, 2009).

Adult mortality was inconsistent at distance of 20 and 25 m, with higher mortality at 25 m than 20 and 15m for dosage of 500 and 750 ml/ha (Tables 2 & 3). These variations could be due to inconsistent and unexpected change in wind direction during spraying. Wind velocity recorded during spraying was an average of < 1.0m/s. It was noticed that there was a shift in wind direction half-way while the spray man was walking along the spray line, thereby causing sprayed droplets to drift to shorter distance with off-target deposition. This explains decreased mortality at distance of 20m for 500 and 750 ml/ha compared to 25 m.

Change of wind direction affects the homogeneity of droplets deposition as wind speed and direction play an important role in drifting (Bretthauer, 2011). As suggested by WHO (2009), an overall adult mortality of at least 90% is considered effective. Due to this natural and unpredictable condition and WHO guideline, 5 m and 10 m was taken as effective spray distance. Adult mortality did not differ significantly for both *Ae. aegypti* and *Ae. albopictus* at dosage of 350, 500 & 750 ml/ha (Table 4).

Magnesium Oxide (MgO) slide served as a monitoring tool for quality of spraying. Penetration of droplets, droplet density at each distance and optimum distance of the spray can be determined by examining the slides under microscope (Lee, 1995). Gravity will move droplets down and wind governs longitudinal distance, while majority will evaporate in the atmosphere, especially during outdoor spraying (WHO, 2003). This explained the condition of very minimal droplets deposited in MgO slides in outdoor application (Table 9) compared to indoor application (Table 10).

Indoor application showed 100% mortality for all test dosages despite the high ratio of VMD/NMD indoor application (1.48 – 2.35) showing uneven distribution of large and small droplets. Similar results were obtained from Lee *et al.* (1997) who obtained complete mortality for adult *Ae. aegypti* and *Culex quinquefasciatus*, with the ratio ranging from 1.40 – 2.01 in indoor application. For outdoor application, the ratio VMD/NMD ranged from 1.32 – 3.65, which was higher than indoor application, particularly for 500 ml/ha due to change in wind direction. Nevertheless, these results revealed acceptable range of large and small droplets size under real life situation.

Sumithion L-40 consists of two active ingredients; fenitrothion and tetramethrin. Fenitrothion inhibits acetylcholinesterase causing accumulation of acetylcholine at the synapse of neuron, resulting in rapid muscle contractions which lead to paralysis and death (Ware & Withacre, 2004). Tetramethrin is a synthetic pyrethroid that acts on the sodium channel; basically provides high knockdown effect on adult mosquitoes (Nishizawa, 1971). Combination of fenitrothion and tetramethrin provides quick knockdown and residual effect (McDonald, 1983).

In this study, indoor and outdoor residual larvicidal effect was observed to last 14 and 21 days, respectively, against *Aedes* sp. at dosage of 500 and 750 mL/ha. Lee *et al.* (1997) reported that at a dosage of 1L/ha, larvicidal activity for Sumithion L-40 could last up to 4 weeks. This finding was similar to Lam & Tham (1988). Lee *et al.* (1997) and Sulaiman *et al.* (1999) reported larvicidal activity of ULV-applied fenitrothion in field trial while Vythilingam (1988) had shown fenitrothion at a dosage of 2.5% a.i. exhibited both adulticidal and larvicidal activity against *Ae. aegypti* larvae by thermal fogging application.

WHO (2009) suggested that in field testing, an overall adult mortality of at least 90% is considered effective. Therefore, in indoor spraying, 350ml/ha was sufficient to give 100% mortality. However, in order to have a better residual larviciding activity, 500 and 750 ml/ha proved to be better compared to 350ml/ha. In this case, the dosage of 500 and 750 ml/ha applied by thermal fogging was the effective dosage that gave >90% mortality against *Aedes* sp. in indoor and outdoor.

The dosage of 500 and 750 ml/ha is equivalent to 1.0 and 1.5 g ai per room, which is equivalent to 0.02 – 0.03 ml Sumithion L-40 per m^3 room space. Both dosages were shown to induce complete mortality with larvicidal residual effect lasting up to 21 days. Based on the dilution ratio of 1:20 (with diesel, as recommended in the label), a spray volume of 0.4 – 0.6 ml/m^3 is sufficient to provide 100% mortality against *Aedes* sp.

In a study conducted by Pant *et al.* (1974) in the northern suburb of Bangkok, using fenitrothion formulation with 85% active ingredients applied at 0.1 mL/m^3 by using portable ULV mist blower, 100% adult mortality and 79.5 – 97.5% larvae mortality was obtained. Two cycles of treatment effectively suppressed *Aedes* population for up to 12 months. Based on our findings, the effective dosage rate is 0.02 – 0.03 ml/m^3, which is much lower in comparison to Pant's study. Application at higher dosage will certainly give better efficacy but it may cause adverse effects to the applicator and environment.

Sumithion L-40 provides both adulticidal and larvicidal effects in a single spray. Control of adult mosquitoes and immature stages are equally crucial in disrupting transmission cycle of dengue due to transovarial transmission of *Aedes* sp. (Lee & Rohani, 2005).

In conclusion, the effective dosage for Sumithion L-40 thermally applied against adult *Ae. aegypti* and *Ae. albopictus* is 500 and 750 ml/ha for indoor and outdoor. Based on these calculations, indoor effective spray volume is 0.4 – 0.6 ml/m^3.

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