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RESEARCH ARTICLE

Residual efficacy of deltamethrin applied to foliage against Malaysian *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse)

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ABSTRACT

The spraying of insecticide on foliage to provide an insecticidal barrier may serve as part of the vector control measures to combat the increasing threat of *Aedes*-borne diseases. The effectiveness of insecticide barrier spraying was evaluated by assessing the residual efficacy of deltamethrin sprayed on foliage against Malaysian *Ae. aegypti* (L.) and *Ae. albopictus* (Skuse). In this semi-field study, landscape plants grown within the vicinity of the Institute for Medical Research (IMR), Malaysia, were treated with deltamethrin suspension concentrate (SC) with the dosage of 30 mg/m^2 and 50 mg/m^2 in three rounds of spraying. Deltamethrin residual activity on treated and untreated leaves was investigated using standard WHO cone bioassays. Wild *Aedes* populations at both deltamethrin-treated and untreated plant clusters were monitored by ovitrap surveillance. Ovitrap monitoring revealed that the mean number of *Ae. albopictus* larvae at deltamethrin-treated were significantly lower than the mean number of larvae of the same species at the untreated plant cluster. Cone bioassay results showed that the insecticide remained effective for up to 4 weeks (> 80% mortality), but the insecticide residual activity was affected by rainfall. These results suggest that insecticide barrier spraying is a promising tool and may be used along with other mosquito control tools such as indoor residual spray and space spraying to reduce the dengue burden.

Keywords: Barrier spraying; deltamethrin; suspension concentrate; mosquito control.

INTRODUCTION

Dengue is a major arthropod-borne disease transmitted by mosquitoes and presents a substantial threat for human populations, especially those residing in the tropical and subtropical regions. The causative agent of dengue, dengue viruses (DENV-1 – 4), are members of the *Flaviviridae* family, which are transmitted among humans through the primary vector *Aedes aegypti* and the secondary vector *Ae. albopictus*. For decades, vector control has remained the principal intervention against *Aedes* mosquitoes as a safe and efficacious dengue vaccine is not yet available. Recent vector control tools developed to reduce dengue burden include insecticide-, biological-, gene-, and mechanical-based tools (Lee *et al.*, 2015).

Vector control through the application of insecticides to localized areas of vegetation that creates a barrier against *Aedes* mosquitoes is a promising and under-exploited insecticide-based vector control tool. The insecticide barrier treatment could be applied to a small perimeter like the house backyards and large but limiting areas like the recreational parks. Besides serving as a barrier treatment in the residential or non-residential settings, the application of residual insecticide on foliage could potentially reduce adult mosquito abundance since resting, or sugar-feeding mosquitoes would come into contact with the foliage at sufficient time and subsequently pick up the lethal dose on the treated foliage. The small-scale and large-scale barrier has been reported to be effective against various species of mosquitoes such as *Ae. atlanticus* (Qualls *et al.*, 2012), *Ae. aegypti* (Fulcher *et al.*, 2015), *Ae. sollicitans* (Madden *et al.*, 1947), *Ae. taeniorhynchus* (Anderson *et al.*, 1991), *Anopheles quadrimaculatus* (Ludvik, 1950), and *Ae. albopictus* (Trout *et al.*, 2007).

Many studies have reported on the residual efficacy of insecticides on inert surfaces. For instance, indoor residual spraying (IRS) and outdoor residual spraying (ORS) measures insecticide efficacy on walls and ceilings (Oxborough *et al.*, 2014; Paredes-Esquivel *et al.*, 2016; Dunford *et al.*, 2018; Ab Hamid *et al.*, 2019). However, only a few studies, particularly in Malaysia, have investigated the residual efficacy of insecticide on foliage. Different classes of insecticides (carbamates, organochlorines, organophosphates, pyrethroids) have been previously tested for insecticide barrier spraying on foliage involving various plant species (Ludvik, 1950; Perich *et al.*, 1993; Xue, 2008; Fulcher *et al.*, 2015). An interesting study conducted by Cilek and Hallmon (2006) has tested the residual efficacy of two pyrethroids; deltamethrin and permethrin, against female *Ae. albopictus* and *Culex quinquefasciatus* in screened cage field tests. They found that deltamethrin outperformed permethrin in leaf bioassays, and deltamethrin lasted for up to four weeks (Cilek & Hallmon, 2006).

Currently, data on the residual efficacy of insecticides applied to foliage as the insecticide barrier treatment in Malaysia is still lacking. Consequently, we investigated the residual efficacy of deltamethrin suspension concentrate (SC) insecticide sprayed on the foliage, which acted as the insecticide barrier treatment against Malaysian *Ae. aegypti* (L.) and *Ae. albopictus* (Skuse). Standard WHO cone bioassays were conducted on treated and untreated excised leaves of landscape plants grown within the Institute for Medical Research (IMR) compound, Kuala Lumpur. The influence of environmental factors on the insecticide residual efficacy was also investigated in this study.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Institute for Medical Research Committee (JPP-IMR) and National Institutes of Health Malaysia (JPP-NIH) and registered with the National Medical Research Register (NMRR-13-921-17915). Ethical approval was obtained from the Medical Research and Ethics Committee (MREC) (Ref No: KKM/NIHSEC/800-2/2/2JldP13905).

Meteorological data

A hand-held thermohygrometer Tinytag View 2 (TV-4500, Gemini Data Loggers Ltd, Sussex, UK) was used to measure both temperature (°C) and relative humidity (% RH) of the air. Rainfall was closely monitored using a rain gauge (RGR126N, Wireless Rain Gauge, Oregon Scientific, Oregon, USA) placed on the roof of the insectary of the Medical Entomology Unit, IMR.

Insecticide formulation and application

Deltamethrin SC (Suspend PolyZone®, active ingredient: 0.42 lb deltamethrin per gallon, Bayer Environmental Science, NC, USA) was used as the residual barrier treatment on the foliage. The insecticide was prepared at two dosages (30 mg/m^2 and 50 mg/m^2) based on the manufacturer's instructions. Three rounds of spraying were conducted, whereby each round comprised of the spraying conduct on the first day and subsequent 14 days of post-treatment. Each spraying was carried out in the morning using a hand compression sprayer (Hudson X-Pert, Chicago, IL, USA) with a flat fan nozzle (Teejet 8002, Spray Systems Co., Bessemer, AL, USA). Prior to insecticide application, the sprayer was calibrated as described in the application of residual sprays for vector control guidelines (WHO, 2007). Following the IRS application, the spraying procedure was performed and was carried out by trained staff with appropriate safety procedures as recommended by WHO (2007).

Site selection

The study was conducted at the IMR compound. Three low-lying landscape plant clusters of *Canna indica* and *Dracaena fragrans* representing three different treatments were deployed for this study which consisted of an untreated plant cluster as the control group, a treated plant cluster sprayed with 30 mg/m^2 of deltamethrin SC, and another treated plant cluster sprayed with 50 mg/m^2 of deltamethrin SC. The distance between each plant cluster is 80-100 m.

Monitoring of *Aedes* populations using ovitraps

Aedes populations in the surroundings of untreated and treated plant clusters were monitored using ovitraps. Ovitraps were made of 300 ml black plastic containers with a diameter of 7 cm and 9 cm in height and equipped with oviposition paddles made from hardboard (10 cm x 2.5 cm x 0.3 cm). All ovitraps were filled with three-quarters of tap water. One ovitrap was placed on the ground under the untreated or treated landscape plants of the respective plant clusters. Ovitraps were collected and replaced every 7 days. Exposed ovitraps were collected at the weekly interval. The contents of collected ovitrap (containing eggs and larvae) were reared in containers at 27 ± 2°C with 75 ± 10% relative humidity. The first and second instar larvae were fed with beef liver powder (Difco Laboratories, MD, USA), while the third and fourth instar larvae were fed with half-cooked liver chunks. Species identification was conducted at the third instar of larvae using the compound microscope. The total number of larvae for each species was also recorded and grouped accordingly into their respective treatments.

WHO cone bioassays

WHO cone bioassays were conducted on deltamethrin-treated excised leaves of *Canna indica* and *Dracaena fragrans* based on guidelines for laboratory and field-testing of long-lasting insecticidal nets (WHO, 2013) with a few modifications. The first bioassay was conducted on a day after the spraying and repeated on day 7 and day 14 of post-spraying (DPS). Similar sizes of untreated and treated leaves were excised, transported to the laboratory, and subsequently fixed on the filter papers. Sampled leaves taken from each cluster were of similar sizes. Standard WHO bioassay cones were firmly positioned onto the leaves using masking tape. Laboratory-reared *Ae. aegypti* and *Ae. albopictus* female adults (F4) from the insectary of the Medical Entomology Unit, IMR were utilized. For each species, ten sucrose-fed mosquitoes, aged 3-6 days, were carefully introduced into the bioassay cones using the battery-operated aspirator. The bioassay cone openings were then plugged with cotton balls and subsequently covered with a black cloth. The knockdown was observed for 30 min at a 5-minute interval time. After the exposure time, the mosquitoes were aspirated out and transferred to clean paper cups. The mosquitoes were held for 24 hours at the IMR laboratory and with access to a 10% (w/v) sugar solution on cotton balls. Mortality counts were taken at 24 hours post-exposure. Three replicates were conducted for each treatment. The categorizations of adult mosquitoes as knocked down or dead in cone bioassays were reported as described by WHOPES (2012).

Data analysis

All statistical analyses were conducted using IBM Statistical Package for Social Science software (SPSS) version 25.0 (IBM, 2017). We performed independent t-test analyses to compare the mean number of larvae between the different treatments for each *Aedes* species. Data for the WHO cone bioassays were pooled from the two plant species and presented as the knockdown percentage after 30 min (KD30) and mortality percentage after 24 hours, which were calculated independently for three separate spray rounds and treatments. Multivariate analysis of variance (MANOVA) for univariate analysis was performed to determine any significant differences in the mean percentages of mortality between the variables spray round, dosage, and *Aedes* species. ANOVA and independent t-test were performed to compare the mean percentages of mortality between the variables. All differences in analyses were considered statistically significant at $P < 0.05$.

RESULTS

The mean temperature and the mean relative humidity throughout this study period were 27.4 ± 0.2°C (19.4 – 34.3°C) and 71.8 ± 0.8% (33.7 – 96.7%), respectively. The study was conducted during the inter-monsoon period (September until October 2014), in which the west coast of Peninsular Malaysia usually receives heavy rainfall during this time (Jamaludin *et al.*, 2010). The average rainfall precipitation recorded by the rain gauge was 5.23 mm.

A total of 2142 larvae were collected from ovitrap surveillance conducted during the treatment period, with 82.3% of these larvae were *Ae. albopictus*. The highest mean number of *Ae. aegypti* larvae (6.58 ± 3.42) were recorded from the plant cluster treated with 50 mg/m^2 deltamethrin, followed by the untreated plant cluster (4.08 ± 2.32) and plant cluster sprayed with 30 mg/m^2 deltamethrin (3.96 ± 1.93) (Table 1). There was no significant difference in the number of *Ae. aegypti* larvae between all treatments ($P = 0.73$). For *Ae. albopictus*, untreated plants (control) had the highest mean number of larvae (38.73 ± 6.87) followed by plants sprayed with deltamethrin 50 mg/m^2 (14.88 ± 3.81) and 30 mg/m^2 (14.15 ± 4.13) (Table 1). The mean number of *Ae. albopictus* larvae between different treatments were statistically significant ($P = 0.001$).

The residual efficacy of deltamethrin sprayed on the foliage was evaluated against laboratory-reared *Ae. aegypti* and *Ae. albopictus* using standard WHO cone bioassays. Overall, the knockdown and mortality percentages among *Ae. aegypti* and *Ae. albopictus* populations exposed to leaves treated with 30 mg/m^2 and 50 mg/m^2 of deltamethrin were higher than *Ae. aegypti* and *Ae. albopictus* populations exposed to untreated leaves during the entire study (Figures 1 and 2). The knockdown percentage of *Ae. aegypti* exposed to deltamethrin-treated leaves ranged from 30% to 75.2%. However, except for the 14 DPS of the second spraying round, the knockdown percentage of *Ae. aegypti* increased during the second and third spraying round, which ranged from 88.3% to 100%. A similar trend was observed in the mortality percentage, whereby high mortality percentage was achieved during the second and third spraying rounds (98.3-100%) except for the 14 DPS of the second spray. Meanwhile, the knockdown percentage of *Ae. albopictus* exposed to deltamethrin-treated leaves was much lower as compared to *Ae. aegypti* (Figure 2). However, the mortality percentage of *Ae. albopictus* was similar to *Ae. aegypti* in which more than 80% mortality was achieved at 24 hours post-treatment.

ANOVA analysis showed a significant difference between the means mortality rate ($F_{2,10} = 4.76$, $P = 0.010$) among the three spray rounds (Table 2). The second spray round produced the highest mean mortality rate (71.10 ± 0.46), followed by the first spray round (70.80 ± 0.43) and the third spray round (54.40 ± 0.41) (Table 2). In addition, there was also a statistically significant difference in the mean percentage of mortality ($P = 0.032$) between the *Aedes* species, in which *Ae. aegypti* displaying a higher mean percentage of

Table 1. Comparisons of the mean number of larvae between the wild, free-flying *Aedes* species in each treatment

Species	Dosage	Mean ± SE	95% CI	*P*
Ae. aegypti	Control	4.08 ± 2.32	(-0.70, 8.85)	0.73
	30 mg/m^2	3.96 ± 1.93	(-0.02, 7.94)	
	50 mg/m^2	6.58 ± 3.42	(-0.47, 13.62)	
Ae. albopictus	Control	38.73 ± 6.87	(24.57, 52.89)	0.001*
	30 mg/m^2	14.15 ± 4.13	(5.64, 22.67)	
	50 mg/m^2	14.88 ± 3.81	(7.04, 22.73)	

* = Statistically significant different ($P < 0.05$).

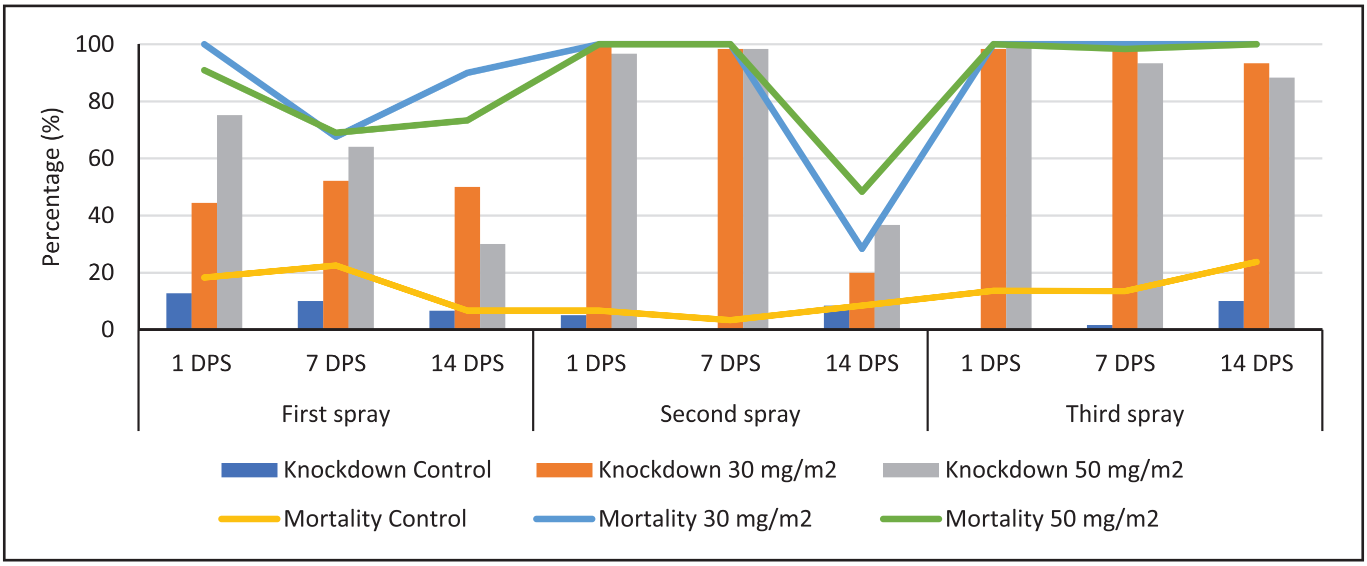

Figure 1. Knockdown percentage (KD30) and mortality percentage at 24 hours post-exposure of *Ae. aegypti* exposed to deltamethrin-treated leaves at 1, 7, and 14 DPS in three rounds of spraying.

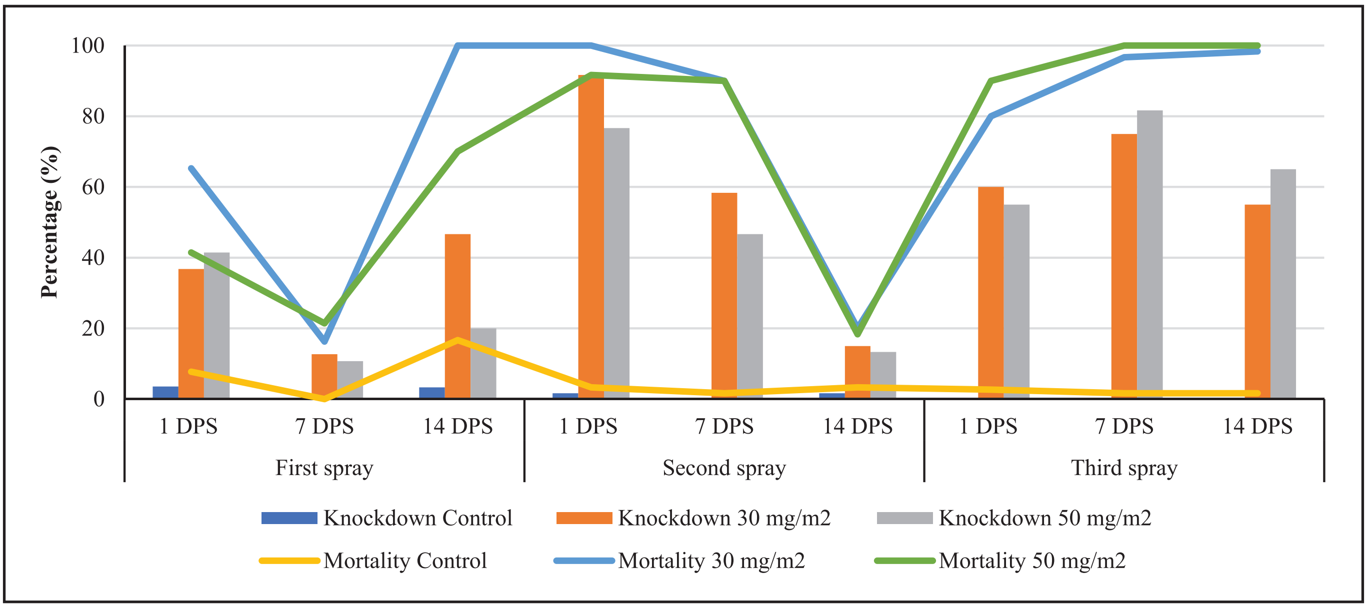

Figure 2. Knockdown percentage (KD30) and mortality percentage at 24 hours post-exposure of *Ae. albopictus* exposed to deltamethrin-treated leaves at 1, 7, and 14 DPS in three rounds of spraying.

Table 2. Summary of statistical analysis results of the mortality percentage of laboratory-reared *Ae. aegypti* and *Ae. albopictus*

Variables	Sub-variables/ Analysis test	Mean ± SE (95% CI)
Spray round	Spray round 1	70.80 ± 0.43 (62.20, 79.50)
	Spray round 2	71.10 ± 0.46 (62.00, 80.20)
	Spray round 3	54.40 ± 0.41 (46.20, 62.70)
	ANOVA	Df = 2 F = 4.76 *P* = 0.010*
Species	*Ae. aegypti*	70.90 ± 0.33 (64.50, 77.40)
	Ae. albopictus	60.00 ± 0.39 (52.30, 67.70)
	Independent t-test	Df = 1 F = 4.68 *P* = 0.032*
Dosage	30 mg/m^2	65.60 ± 0.36 (58.60, 72.70)
	50 mg/m^2	65.30 ± 0.37 (58.00, 72.50)
	Independent t-test	Df = 1 F = 0.01 *P* = 0.94
Spray round x Species	Univariate MANOVA	Df = 2 F = 0.08 *P* = 0.92
Spray round x Dosage	Univariate MANOVA	Df = 2 F = 0.22 *P* = 0.80
Species x Dosage	Univariate MANOVA	Df = 1 F = 0.26 *P* = 0.61
Spray round x Species x Dosage	Univariate MANOVA	Df = 2 F = 0.20 *P* = 0.82

* = Statistically significant different ($P < 0.05$).
x = Correlation.

mortality (70.90 ± 0.33) in comparison to *Ae. albopictus* (60.00 ± 0.39) (Table 2). There was no significant difference ($P = 0.94$) between the two different deltamethrin dosages used (Table 2). Independent t-test analysis showed that the deltamethrin 30 mg/m^2-treated plants exhibited only a marginally higher mean rate of mortality (65.60 ± 0.36) compared to the plants sprayed with 50 mg/m^2 (65.30 ± 0.37) (Table 2). Univariate MANOVA showed that interactions between spray round and species, spray round and dosage, species, and dosage, as well as between spray round, species, and dosage were not significant at 0.05 level of significance (Table 2).

DISCUSSION

The number of *Ae. albopictus* larvae collected via ovitraps placed under deltamethrin-treated plant clusters were significantly lower in comparison with the number of *Ae. albopictus* larvae collected from ovitraps deployed under the untreated plant cluster. This result suggested that the deltamethrin-treated plant cluster attracted fewer *Aedes* adults to lay eggs, indicating that the barrier spraying is working to a certain extent. The large difference in the number of larvae collected between *Ae. albopictus* and *Ae. aegypti* was most likely due to differences in their resting behavior. *Ae. albopictus* is an exophilic species while *Ae. aegypti* is an endophilic species (Estrada-Franco & Craig, 1995; Chadee, 2013; Dzul-Manzanilla *et al.*, 2017). *Ae. albopictus* also feeds outdoors and is mainly found in vegetation areas (Hawley, 1988; Bonizzoni *et al.*, 2013). This species used to be found in rural areas, but many reports have suggested the adaptation of *Ae. albopictus* to the urban and suburban environments in which breeding of *Ae. albopictus* has been demonstrated in artificial containers like tires and plastic containers and also in natural sites like tree holes (Hawley, 1988; Higa, 2011). The reason for the shift is unclear, but the increasing number of gardens or parks with vegetation and home gardens grown by house residents could have contributed to this adaptation of *Ae. albopictus* (Higa, 2011). In contrast, *Ae. aegypti* is predominantly abundant indoors and is endophagy (Chadee, 2013; Dzul-Manzanilla *et al.*, 2017). A study in Panama City found that only a low percentage of resting *Ae. aegypti* (0.3% females, 0% males) were found in the garden, whereas a higher percentage of *Ae. aegypti* was recovered from bedrooms (43.1% females, 34.3% males) (Perich *et al.*, 2000). Thus, the placement of ovitraps under untreated and deltamethrin-treated plant clusters in this study is limited for the monitoring of *Ae. albopictus* population only.

Rainfall seemed to cause a noticeable effect on the residual reduction of deltamethrin on both treated plants. The decline in knockdown and mortality percentages on 14 DPS of the second round of spraying was due to heavy rain at the study site from 10 DPS until 14 DPS, in which the highest amount of rainfall (49 mm) was recorded on 10 DPS. The amount of rainfall and intensity directly influences the extent of pesticide removal from plant surfaces (Ware, 1987). Suspend PolyZone® used in this study is formulated as SC and contains a proprietary polymer layer that protects the active ingredient of deltamethrin, making it more durable and resistant against weathering. We believe that Suspend PolyZone® has longer residual efficacy and greater foliar persistence than other insecticides, but to a certain extent, it still could not withstand heavy and frequent rainfall, as demonstrated in this study. Interestingly, although rainfall appeared to cause residual reduction of deltamethrin on treated leaves, we detected gradual buildups of deltamethrin on the leaf surfaces from spray round 1 to spray round 3. This was depicted from the general lower knockdown and mortality percentages during the first spray round for both *Aedes* species and increased going from the second and third spray round.

Differences in the abundance and arrangement of leaves on the plants as well as the chemical properties of leaf cuticular wax could influence the residual efficacy of the insecticide sprayed on the foliage. Variations in the leaf cuticular wax have been shown to affect the transfer and contact of pesticide to the target insect, leading to different residual efficacy of the pesticide (Ford & Salt, 1987; Kirkwood, 1987; Doyle *et al.*, 2009). In a previous study by Chowdhury *et al*. (2001), sixteen leaf types treated with deltamethrin were tested against *Folsomia candida* Willem in leaf bioassays. Plant species with low cuticular wax would increase insecticide active ingredient movement into the plant cuticle and consequently reduce the chance of contact between the insecticide sprayed on the leaf surface and the target insect (Chowdhury *et al.*, 2001). A limitation in our study was that we did not investigate the leaf cuticular wax properties on the plant species that we used, which could have affected the bioassay results. Further studies may be warranted to investigate the effect on the residual efficacy of insecticides applied to different species of landscape plants in Malaysia.

In conclusion, results from this study suggest that the use of insecticide sprayed on foliage as the insecticide barrier treatment may serve as an effective and promising tool against dengue vectors. Barrier treatment using deltamethrin could be used along with other existing methods such as IRS and ULV fogging for integrated vector management of *Aedes* mosquitoes. However, the scale size of the barrier spraying area should be relatively limited, such as house backyards or recreational areas where mosquito populations are pestiferous. This is important to avoid insecticide resistance that occurs due to repeated application of persistent insecticide. Variables such as leaf types, leaf arrangement, leaf wax properties, and environmental factors like rainfall may influence the residual activity and should be considered.

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Conflicts of Interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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