

Evaluating the Adulticidal Effect of Alpha-Cypermethrin Against Malaysian Dengue Vector *Aedes albopictus*

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ABSTRACT

The efficacy of a pyrethroid insecticide; alpha-cypermethrin against dengue vector *Aedes albopictus* adult populations from various types of residential and agricultural areas in West Malaysia was evaluated through the adult mosquito bioassays. Sugar-fed *Ae. albopictus* adult females aged 3-5 days old from each study area were exposed to alpha-cypermethrin 0.05% for an hour. The mortality percentages were calculated at 30 minutes of exposure and 24 h post-exposure. At 30 minutes of exposure, *Ae. albopictus* adult populations from dengue-risk residential areas (34.19 min) and oil palm plantations (56.53 min) exhibited the shortest and longest time to obtain the 50% mortalities of the population (LT_{50}), respectively. At 24 h post-exposure, other than the reference strain, *Ae. albopictus* adult populations from rubber estates (99.67%), dengue-free residential areas (99.67%) and dengue-risk residential areas (98.33%) demonstrated susceptibility to alpha-cypermethrin 0.05%. These results revealed the worthwhile adulticidal effect of alpha-cypermethrin which could be used in the future vector control strategies at rubber estates, dengue-free residential areas and dengue-risk residential areas selected in this study.

Keywords: Pyrethroid, insecticide, adult mosquitoes, mosquito control, Malaysia.

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INTRODUCTION

Dengue, chikungunya, Zika and yellow fever are important arboviral diseases transmitted by both *Aedes aegypti* and *Aedes albopictus* mosquitoes (Zhao et al., 2023). Millions of people from across the globe are affected by these *Aedes*-borne infections especially dengue which sometimes could be life-threatening. Dengue infection alone is endemic in many tropical countries including Malaysia with significantly high number of cases per year (Salim et al., 2021). The spread of dengue is not only confined to urban areas but also prevalent in rural regions (Azami et al., 2020).

The spread of dengue and other *Aedes*-borne diseases are closely associated with urbanization, globalization, climate change and distribution of mosquito vectors (Rocklov & Tozan, 2019; Estevez-Castro et al., 2024). *Aedes aegypti* has a preference for indoor setting while *Ae. albopictus* is more prevalent in outdoor environment (Silalahi, Yasin, Chen, Ahmad, & Neoh, 2024). The wide spread of *Aedes* populations poses significant challenges in mosquito control strategies. Regardless of diverse mosquito control methods recommended by the World Health Organization and the rising trend of insecticide resistance in mosquitoes, the utilization of chemical insecticides targeting both adult mosquitoes and larvae is still mostly preferred across the globe.

Pyrethroids are chemicals used as insecticides and repellents to control mosquito vectors. Type I pyrethroids such as allethrin, bifenthrin, resmethrin and permethrin are less toxic to humans and mammals as compared to type II pyrethroids like cyfluthrin, cyhalothrin, cypermethrin and deltamethrin which contains the α -cyano group in their chemical structure (Hodosan et al., 2023). Pyrethroids are widely utilized for mosquito control via residual spraying or space spraying in several regions of the world (World Health Organization, 2021).

Alpha-cypermethrin is a type II pyrethroid with a higher potency to the voltage-gated sodium channel (VGSC) in nerve membranes of insects than type I pyrethroids (Moyes et al., 2021). Alpha-cypermethrin is one of the frequently selected pyrethroids to control adult mosquito populations particularly during dengue epidemics (Bisset et al., 2013; Nurweni, Kusnanto, Widayani, & Umniyati, 2024). The operational doses of alpha-cypermethrin applied by the health authorities are varied and usually follow the recommendations of the product manufacturers. Information on the susceptibility status of Malaysian mosquito vectors towards alpha-cypermethrin is still inadequate. Hence, this study was carried out to determine the susceptibility level of local *Aedes albopictus* adult populations from various types of localities against alpha-cypermethrin through the conduct of bioassays in order to foresee its potential to be applied in the vector control programme in Malaysia.

MATERIALS AND METHODS

Study localities

Fifteen study localities were selected for this study comprising of residential and agricultural areas within Peninsular Malaysia (Wan-Norafikah et al., 2023a; Wan-Norafikah, Chen, & Sofian-Azirun, 2023b). The residential localities included dengue-risk residential

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areas and dengue-free residential areas. All dengue-risk residential localities possessed the history of regular vector control operations within three recent years which were coherent with the dengue cases reported in the areas. On the contrary, all dengue-free residential localities have no experience of any reported dengue case. Meanwhile, the agricultural localities comprised of oil palm plantations, rubber estates and paddy fields with histories of frequent agricultural pesticide exposures only. Oil palm, rubber and paddy are the main industrial crops grown in Malaysia (Department of Agriculture Malaysia, 2021; Department of Agriculture Peninsular Malaysia, 2022).

***Aedes albopictus* samples**

An ovitrapping technique was implemented at each study locality to collect *Aedes albopictus* juveniles. Fifty (50) ovitraps (9.1 cm in height x 6.8 cm in diameter) which contained 10% hay infusion water (Wan-Norafikah et al., 2019) and an egg-laying paddle each, were randomly put in each study locality. All ovitraps were placed at the study localities for five days to permit the egg-laying by female adult mosquitoes before being retrieved and transported to the laboratory. All mosquito juveniles collected in the ovitraps were supplied with bovine liver powder and slices, and reared to adulthood. Newly emerged adult mosquitoes were taxonomically identified (Jeffery et al., 2012) and only *Ae. albopictus* adults were maintained while other mosquito species were disposed. *Aedes albopictus* adults were permitted to copulate to produce eggs of their successive generation (F1). Hatched eggs were nurtured to adulthood. Sugar-fed *Ae. albopictus* adult females aged 3-5 days old were subjected to the adult bioassays.

Additionally, the laboratory strain of *Ae. albopictus* (F86) was utilized as a reference strain for this study. This strain was primarily captured from Selangor, Malaysia and has been reared in the insectarium of the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia, for nearly two decades without any insecticide selection. *Aedes albopictus* field populations and laboratory strain were maintained in the same manner in the insectarium at 27 ± 2 °C and $75 \pm 10\%$ relative humidity.

Alpha-cypermethrin

Pyrethroid alpha-cypermethrin impregnated papers (0.05 %) were procured from the WHO Collaborating Centre; Vector Control Research Unit (VCRU) in Universiti Sains Malaysia (USM), Penang, Malaysia.

Bioassay of adult mosquitoes

Bioassays were performed on *Ae. albopictus* laboratory strain and field populations as defined by the World Health Organization (2022). Twenty-five (25) sugar-fed adult females aged 3-5 days old were gathered in a holding cup and left for an hour to ensure their healthiness. A total of four test replicates and two control replicates were prepared for each bioassay. These mosquitoes were then aspirated and gently blown into four exposure tubes containing the alpha-cypermethrin 0.05% impregnated papers and two control tubes lined with silicone oil impregnated papers, respectively. The knockdown rates of mosquitoes were noted at every minute throughout the 1 h exposure time. Mosquitoes were considered knocked down if they failed to fly, stand

or take off stably. After the exposure period, the mosquitoes were aspirated and released back into the holding cups with 10% sugar solution. The total mortalities of mosquitoes were documented after 24 h recovery period by observing the mosquitoes that were unable to fly, stand or take off in a coordinated manner.

Data analysis

All the raw experimental data for each study area were initially analyzed individually and later as groups based on their types of area. The knockdown numbers of *Ae. albopictus* adult mosquitoes per minute were noted during the 1 h alpha-cypermethrin exposure time while the mortality numbers of *Ae. albopictus* adult mosquitoes per minute were recorded at 24 h post-exposure. These knockdown and mortality numbers were transformed into knockdown percentages and mortality percentages, respectively, as follow:

$$\text{Knockdown percentage (\%)} = \frac{\text{Number of knocked down adult mosquitoes}}{\text{Total number of adult mosquitoes evaluated}} \times 100$$

$$\text{Mortality percentage (\%)} = \frac{\text{Number of dead adult mosquitoes}}{\text{Total number of adult mosquitoes evaluated}} \times 100$$

The knockdown data were also statistically analysed to acquire the knockdown time value at 50% (KT_{50}) for each *Ae. albopictus* population in order to calculate the resistance ratio (RR) as follows:

$$\text{Resistance ratio (RR)} = \frac{KT50 \text{ of field population}}{KT50 \text{ of laboratory (reference) strain}}$$

Based on the mortality percentage at 24 h post-exposure, the susceptibility of each *Ae. albopictus* population against alpha-cypermethrin 0.05% was classified following the World Health Organization guidelines (2022): 98-100% denotes susceptibility to alpha-cypermethrin; 90-97% implies possible resistance against alpha-cypermethrin which then to be confirmed by repeated bioassays of adult mosquitoes; and < 90% shows confirmed resistance against alpha-cypermethrin. The correction of mortality data using Abbott's formula (1925) was performed when the mortality of the control replicates was beyond 5% but not exceeding 20%.

$$\text{Corrected mortality (\%)} = \frac{\% \text{ mortality of pyrethroid exposure} - \% \text{ mortality of control}}{100 - \% \text{ mortality of control}} \times 100$$

The normality of raw mortality data was also verified by the conduct of Shapiro-Wilk test. Any significant difference between the mortality percentages of *Ae. albopictus* populations from different land use upon exposure to alpha-cypermethrin 0.05% was discovered through the One-way ANOVA and Post Hoc Test analyses. All statistical analyses were performed using IBM SPSS Statistics version 23.0 with significant value set at $P = 0.05$.

RESULTS

The adulticidal effect of alpha-cypermethrin against *Ae. albopictus* adult mosquitoes of the reference strain and field populations from various types of residential and agricultural areas within West Malaysia was evaluated via 1 h exposure of bioassays. All raw data of knockdown and mortalities obtained from this study were normally distributed ($P > 0.05$) and no mortality was observed among *Ae. albopictus* adult populations of the control replicates.

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The 50% knockdown time (KT_{50}) values for all *Ae. albopictus* populations exposed to alpha-cypermethrin 0.05% ranged between 34.19 min and 56.53 min with resistance ratios below 1.50 (Table 1). Interestingly, the fastest adulticidal effect of alpha-cypermethrin 0.05% was observed among *Ae. albopictus* adult populations from dengue-risk residential areas while adult populations of the same species from oil palm plantations took the longest time to reach the 50% mortality in the population upon alpha-cypermethrin 0.05% exposure.

Table 1. The 50% knockdown time (KT_{50}), knockdown percentage at 30 minutes of the exposure time and mortality percentage after 24 hours post-exposure for *Aedes albopictus* from different types of area against alpha-cypermethrin 0.05%.

Types of area	Study areas	KT_{50} (min) 95% C.L.	Resistance Ratio (RR)	Percent knockdown at 30 minutes of the exposure times (%)	Percent mortality after 24 h (%)
Reference	Laboratory	37.89 (36.72-39.09)	-	^R 22.00 ± 7.02	^S 99.00 ± 1.00
Oil palm plantation	Kota Tinggi OP	56.53 ± 20.94 (35.28-98.40)	1.49 ± 0.55	^R 20.00 ± 7.02	^M 90.67 ± 8.84
	Klang OP				
	Temerloh OP				
Paddy field	Kuala Selangor PD	45.36 ± 10.33 (33.70-65.95)	1.20 ± 0.27	^R 26.00 ± 8.54	^M 91.33 ± 8.67
	Kulim PD				
	Kuala Pilah PD				
Rubber estate	Sungai Buloh RB	35.04 ± 2.83 (29.48-38.69)	0.93 ± 0.07	^R 39.33 ± 8.84	^S 99.67 ± 0.33
	Temerloh RB				
	Kota Tinggi RB				
Dengue-free residential area	Shah Alam FF	38.43 ± 0.78 (37.16-39.86)	1.01 ± 0.02	^R 26.67 ± 0.88	^S 99.67 ± 0.33
	Padang Serai FF				
	Temerloh FF				
Dengue-risk residential area	Kota Tinggi DEN	34.19 ± 1.09 (32.46-36.20)	0.90 ± 0.03	^R 32.67 ± 6.23	^S 98.33 ± 0.33
	Shah Alam DEN				
	Cheras DEN				
One way ANOVA				F = 0.961 df = 15 P = 0.485	F = 0.557 df = 15 P = 0.731

RR = resistance ratio; C.L. = Confidence Limit (95%).

Percent knockdown at 30 minutes of the exposure time (%) = Mean of knockdown adult mosquitoes + Standard Error (S.E.).

Percent mortality after 24 h (%) = Mean of mortality adult mosquitoes + Standard Error (S.E.).

S = susceptible, M = possible resistance, R = confirmed resistance as determined by WHO (2022).

During the 1 h exposure period, the knockdown percentages of all *Ae. albopictus* adult populations were calculated at 30 min of exposure. The highest and lowest numbers of knockdown at 30 minutes of alpha-cypermethrin 0.05% exposure were demonstrated among *Ae. albopictus* adult populations from rubber estates (39.33%) and oil palm plantations (20.00%), respectively. However, after 24 h post-treatment, *Ae. albopictus* adults of the reference strain as well as the field populations from rubber estates, dengue-free residential areas and dengue-risk residential areas showed mortality percentages of more than 98.00% which indicate the susceptibility of these populations against alpha-cypermethrin 0.05%. In contrast, *Ae. albopictus* adult populations from oil palm plantations and paddy fields exhibited the mortality percentages of only 90.67% and 91.33%, respectively, after 24 h recovery period. These results signify the possible resistance of these populations against alpha-cypermethrin

0.05% as outlined by the World Health Organization (2022) but this resistance status has actually been confirmed through repeated bioassays.

DISCUSSION

The knockdown percentage and mortality percentage of adult mosquitoes have been recommended by WHO (2022) to be ascertained at the end of 1 h exposure and at 24 h post-treatment of an insecticide, respectively. However, for this study, the knockdown percentages of all *Ae. albopictus* adult populations were noted at 30 min of exposure. The knockdown percentages were not logged at the end of 1 h exposure as some of these *Ae. albopictus* populations had already demonstrated almost 100.00% mortalities at that time which made these data to become statistically incomparable with the data of mortality percentages at 24 h post-treatment.

Following the WHO guideline (2022), the susceptibility of a tested adult mosquito population against an adulticide is classified based on its mortality percentage after 24 hours of recovery period. From the results of mortality percentages at 24 h post-treatment, the application of alpha-cypermethrin as an adulticide of the vector control operations would be effective in the rubber estates, dengue-free residential areas and dengue-risk residential areas selected in this study. On the other hand, the use of alpha-cypermethrin in the future vector control programme at oil palm plantations and paddy fields selected in this study should be carefully considered and monitored as possible resistance against alpha-cypermethrin 0.05% have already been detected among these adult populations of *Ae. albopictus*. The application of pyrethroids like alpha-cypermethrin should be in rotation with insecticides from other classes such as organophosphates and carbamates in order to minimize or delay the development of resistance in *Ae. albopictus* and other mosquito species against these insecticides.

The tentative discriminating concentration of alpha-cypermethrin to be used in the adult bioassay as outlined by WHO (2016) was 0.03 % for *Aedes* mosquitoes and 0.08 % for *Ae. albopictus* (World Health Organization, 2022). The utilization of alpha-cypermethrin 0.05 % in this study was based on the alpha-cypermethrin impregnated papers available to be purchased from VCRU during the conduct of the study. Although the diagnostic dose of alpha-cypermethrin employed in the study was much lower than the operational dose in the field which is usually much higher than the diagnostic dose, the presence of possible resistance against alpha-cypermethrin 0.05% among *Ae. albopictus* adults from oil palm plantations and paddy fields is an early indication of the resistance development against alpha-cypermethrin among these adult populations that should be closely monitored.

The susceptibility of mosquitoes against alpha-cypermethrin as an adulticide has been more frequently studied in other countries than in Malaysia. Resistance against alpha-cypermethrin 99.5% was displayed among six adult populations of *Ae. aegypti* collected in Veracruz state of Mexico (Flores *et al.*, 2013) while *Ae. aegypti* from four cities in Ecuador developed confirmed resistance against established diagnostic dose of alpha-cypermethrin (10 µg / bottle) with mortality levels of less than 80.0% (Ryan *et al.*, 2019). Development of resistance among *Ae. albopictus* against alpha-cypermethrin

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at various concentrations have also been detected in several countries. For example, *Ae. albopictus* populations from Koh Chang and Pong Nom Ron in Thailand were resistant to alpha-cypermethrin 0.035% (Thanispong, Sathantriphop, Malaithong, Bangs, & Chareonviriyaphap, 2015), whereas, several Italian populations of *Ae. albopictus* demonstrated resistance against alpha-cypermethrin 0.05% at various mortality rates (Pichler et al., 2018). Resistance against alpha-cypermethrin 1.4% was also detected among *Ae. albopictus* population from Zhoushan, China (Hou et al., 2020) while *Ae. albopictus* from Lahore, Pakistan was resistant to alpha-cypermethrin 0.03% with mortality percentage of less than 40.0% (Rahman et al., 2021). In southern Benin, West Africa, resistance against alpha-cypermethrin 0.5% was exhibited in both *Aedes* species in which four field populations of *Ae. aegypti* (91.0% - 95.65% mortality) and three field populations of *Ae. albopictus* (83.0% - 95.7% mortality) were either suspected resistant or confirmed resistant to this adulticide (Konkon et al., 2023). Nevertheless, resistance development against alpha-cypermethrin in mosquitoes has been found to be reversible. For instance, although the selection pressure of alpha-cypermethrin for twelve cycles has shorten the larval and pupal duration of *Ae. aegypti* and initiated resistance against this pyrethroid among the mosquito colony at 11.86-fold, the resistance development reduced and recovered as the selection pressure was terminated (Shafiq, Abubakar, Riaz, & Shad, 2023).

In addition, cross resistance has been reported in *Aedes* populations that were resistant to alpha-cypermethrin at diagnostic doses. Several studies have shown that these populations also developed resistance against other insecticides simultaneously. For example, four populations of *Ae. aegypti* from French Guiana that were resistant to alpha-cypermethrin 0.05% also exhibited resistance against fenitrothion 0.5%, malathion 0.8% and 5%, bendiocarb 0.1%, and/ or propoxur 0.1% (Guidez et al., 2020). Similarly, an *Ae. albopictus* population from Zhoushan, China, which was resistant to alpha-cypermethrin 1.4% also showed resistance against malathion 0.5% (91.21% mortality), permethrin 3% (94.44% mortality) and deltamethrin 0.1% (89.13% mortality) (Hou et al., 2020). Meanwhile, five *Ae. albopictus* populations from Xi'an, China, which were possibly resistant to alpha-cypermethrin 0.03%, also appeared to be resistant to malathion 0.5%, chlorpyrifos 2%, propoxur 0.05%, beta-cypermethrin 0.1%, and/ or deltamethrin 0.03% (Lei et al., 2024). In Malaysia, an *Ae. albopictus* population from the Selayang hot spring, which showed resistance against alpha-cypermethrin 0.05% (97.00% mortality), also exhibited resistance against DDT 4% (91.00% mortality), dieldrin 4% (93.00% mortality), fenitrothion 1% (74.00% mortality), propoxur 0.1% (92.00% mortality) and bendiocarb 0.1% (58.00% mortality) (Wan-Norafikah et al., 2023c; Wan-Norafikah et al., 2024). However, further statistical analyses are required to determine whether the observed mortality rates following alpha-cypermethrin exposure are significantly correlated with the mortality rates recorded for other insecticide exposures in the same *Aedes* populations.

Documentations on the use of alpha-cypermethrin in the vector control strategies worldwide are still limited. Besides permethrin and deltamethrin, alpha-cypermethrin is another pyrethroid that is commonly used in Italy for adulticide treatments to control invasive colonization of *Ae. albopictus* (Pichler et al., 2018). In Ecuador, alpha-cypermethrin has been used in combination with another pyrethroid deltamethrin and organophosphate malathion to combat

Aedes-borne diseases in certain cities (Ryan et al., 2019). Alpha-cypermethrin has also been applied regularly and extensively in the space spraying operations and epidemic prevention and response programme in Thailand, together with other pyrethroids (Sathantriphop et al., 2020). In Pakistan, alpha-cypermethrin has been utilized as a residual spray on potential mosquito resting sites and all premises within 50 m radius from the index house which has reported dengue case (Rahman et al., 2021). As to date, alpha-cypermethrin has never been utilized by the Ministry of Health Malaysia in the vector control programme. However, the alpha-cypermethrin-based products such as concentrated suspension are still available in the local market to be purchased by the consumers and agricultural personnel. Several products of alpha-cypermethrin have been registered under the Pesticides Board of Malaysia for the use either in public health or agricultural sector. According to Konkon et al., 2023, the indoor biting and resting behaviours of *Ae. aegypti* could increase its exposure to domestic insecticide-based products like aerosols, insecticide sprays, and insecticide-impregnated mosquito bed nets especially in urban areas which later lead to the occurrence of insecticide resistance. In contrast, rapid development of insecticide resistance among *Ae. albopictus* could be due to household or organic pollutants present in the water-holding containers in which this species is commonly found in peri-urban settings and agricultural sites.

CONCLUSION

In summary, the use of alpha-cypermethrin as an effective adulticide for the forthcoming vector control strategies is promising in the rubber estates, dengue-free residential areas and dengue-risk residential areas selected in the present study. More research on the susceptibility of *Aedes* mosquitoes against alpha-cypermethrin covering numerous localities in Malaysia should be carried out to obtain further information on the efficacy of alpha-cypermethrin against *Aedes* adult populations before it could be employed in the local vector control programme.

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