Journal of the American Mosquito Control Association, 39(3):183–191, 2023

PROFILING INSECTICIDE SUSCEPTIBILITY OF *AEDES ALBOPICTUS* FROM HOT SPRINGS IN SELANGOR, MALAYSIA

OTHMAN WAN-NORAFIKAH,[1,2,3,5] NARIMAH ABDUL HAMID HASANI,[1,2] ABU BAKAR NABILA,[1] IBRAHIM NAJIBAH,[1] ABDUL HADI HAZIRAH NURJUANI,[1] MUHAMAD MASLIANA,[1] SHARIFAH ALIAH-DIYANAH,[1] ZAKARIA ALIA-YASMIN,[1] IBRAHIM YASMIN-ZAFIRAH,[1] AZHAR FARAH-FARHANI,[1] ABDUL HADI AZAHARI,[4] MAZKAMAL FAIQAH-NADHIRAH[1] AND MOHD SHAH NURUL-AZIRA[1,2]

ABSTRACT. The present study establishes insecticide susceptibility profiles of *Aedes albopictus* adult populations from 4 hot springs in Selangor, Malaysia, against 7 pyrethroids through an adult mosquito susceptibility bioassay. All *Ae. albopictus* populations were subjected to a 1-h exposure to each pyrethroid following the World Health Organization. The mortalities were recorded at 60 min of exposure to bifenthrin, 30 min for other pyrethroids, and 24 h posttreatment for all pyrethroids. Complete mortalities were observed upon exposures to the pyrethroids under 60 min and at 24 h posttreatment, excluding permethrin 0.25%, alpha-cypermethrin 0.05%, and bifenthrin 0.2%. These findings indicated that permethrin, deltamethrin, lambda-cyhalothrin, cyfluthrin, and etofenprox possess the recommended pyrethroid adulticide active ingredients that could be applied in vector control programs at these hot springs in the future. Nevertheless, the application of pyrethroids should be carefully monitored in rotation with other insecticide classes, including organophosphates and carbamates to avoid the development of insecticide resistance among mosquito vectors towards all insecticides. Although there were no reported cases of *Aedes*-borne pathogens at these hot springs to date, the current study results could still assist the Malaysian health authorities in determining approaches to control *Aedes* populations in these hot springs, if required in the future.

KEY WORDS *Aedes albopictus*, hot springs, insecticide susceptibility, Malaysia, pyrethroids

INTRODUCTION

In recent decades, the spread of mosquito-borne arboviral diseases, namely, dengue, chikungunya, and Zika, have increased dramatically on a global scale. The mosquito-borne arboviral pathogens are transmitted through the bite of infected *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) mosquitoes (Peinado et al. 2022). High human morbidity and mortality rates due to these diseases have triggered public health concerns worldwide.

The control of mosquito-borne arboviral diseases depends on vector management due to the shortcomings of effective treatment or vaccines for the illnesses. Reducing human-mosquito interactions is also the essence of an effective control strategy. Nonetheless, chemical insecticides remain the primary vector control tools (Fansiri et al. 2022) despite the numerous mosquito control approaches, such as source reduction, habitat manipulation, and biological larvicide applications, delineated by the World Health Organization (WHO 2012).

Aedes aegypti and *Ae. albopictus* tend to blood feed on human hosts over other warm-blooded species (Kamgang et al. 2012, Pruszynski et al. 2020). *Aedes aegypti* is predominantly related to human population densities, while *Ae. albopictus* abundance coincides with vegetation (Tsai and Teng 2016, Dalpadado et al. 2022). Consequently, mosquito control operations are conducted in human habitation and public recreational sites typically surrounded by vegetation following the increased risk of mosquito-borne pathogen transmissions in these areas.

Hot springs are natural geothermal water environments that can be found worldwide. Humans visit hot springs to bathe in the hot water for therapeutic and stress-relieving purposes. Therefore, visitor traffic is commonly heavy, especially during weekends and holidays. Furthermore, ornamental plants and dense vegetation within the hot spring environments offer harborage for certain mosquito species, including *Ae. albopictus*.

Aedes albopictus reportedly prevail in outdoor vegetation (Dalpadado et al. 2022), leading to expectations of mosquito-borne infections in hot spring environments. In Malaysia, several hot springs scattered in some states, including Selangor, have become popular attractions among local and foreign tourists. The hot springs are located in suburban and rural areas and primarily surrounded by vegetation. In an unpublished study, *Ae. albopictus* was the most common mosquito species collected through ovitrapping performed in all 4 hot springs selected in the current study.

[1] Faculty of Medicine, Universiti Teknologi MARA (UiTM), Selangor Branch, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia.

[2] HW ReNeU, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia.

[3] Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA (UiTM), Selangor Branch, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia.

[4] Medical Entomology Unit, WHO Collaborating Centre for Vectors, Institute for Medical Research (IMR), Ministry of Health Malaysia, National Institute of Health, Block C, Jalan Setia Murni U13/52, Seksyen U13, Setia Alam, 40170 Shah Alam, Selangor, Malaysia.

[5] To whom correspondence should be addressed.

Table 1. Geographical location of selected hot springs from different localities in Selangor, Malaysia.

District	Study locality	Geographical location (coordinates and elevation)
Gombak	Selayang hot spring (SEL)	03°15.324′N, 101°38.469′E; 70 m
Hulu Selangor	Hulu Tamu Batang Kali hot spring (HTBK)	03°27.868′N, 101°41.808′E; 66 m
Hulu Selangor	Kuala Kubu Bharu hot spring (KKB)	03°33.895′N, 101°38.767′E; 69 m
Hulu Selangor	Kerling hot spring (KERL)	03°36.580′N, 101°36.555′E; 44 m

Mosquito vectors and humans co-occurrences create a critical necessity for effective mosquito control strategies around hot springs to prevent the pathogen transmissions by mosquitoes, particularly *Aedes* vectors. Selecting the precise insecticides to be employed in vector control operations at each hot spring is based on the susceptibility profiles of targeted mosquito species. Nonetheless, no susceptibility investigations involving mosquitoes from any of the hot springs in Malaysia have been performed. The current study aimed to determine the most suitable insecticide(s) to control the outdoor vector *Ae. albopictus* at selected hot springs in Selangor, Malaysia, according to their susceptibility profiles against the active ingredients (AIs) of different pyrethroids.

MATERIALS AND METHODS

Study localities: Four hot springs popular among tourists were selected as the localities in this study: Selayang (SEL), Hulu Tamu Batang Kali (HTBK), Kuala Kubu Bharu (KKB), and Kerling (KERL). The hot springs are open to visitors daily. The study sites are located in the Gombak and Hulu Selangor districts in Selangor, Malaysia. The hot spring points in the areas are encircled by concrete pools, while their surroundings are skirted by forest vegetation. Table 1 summarizes the geographical descriptions of the sampling sites.

The Ae. albopictus *reference strain:* The present study utilized the *Ae. albopictus* laboratory strain (F80) as a reference strain, which was initially collected from Selangor, Malaysia, and reared in the insectarium of the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia, for almost 20 yr. The *Ae. albopictus* laboratory strain has never been exposed to any insecticide since its colonization in the IMR insectarium.

The Ae. albopictus *field population sampling:* This study employed ovitrapping during *Ae. albopictus* field sampling (Lee 1992, Ministry of Health Malaysia 1997), where 50 ovitraps of 9.1 cm (height) × 6.8 cm (diam) were set in each area. Each ovitrap contained 10% hay infusion water (Wan-Norafikah et al. 2019) and an oviposition paddle. The ovitraps were placed randomly within the hot spring compound, including under the ornamental plants, near vegetation, and under the concrete benches. The ovitraps were deployed for 5 days to allow female adult mosquitoes to lay eggs before being retrieved and returned to the laboratory.

Rearing and identification of Ae. albopictus *adults:* The contents of the ovitraps retrieved in the present study were poured into different containers. Liver powder and small semicooked ox liver pieces were then provided to the hatched *Ae. albopictus* larvae in the containers. All larvae were reared to adulthood, which were identified to species based on the taxonomic and pictorial identification keys of Jeffery et al. 2012). Only *Ae. albopictus* adults (F0) were conserved in the current study, permitted to mate and blood feed to obtain the F1 progeny for use in adult mosquito susceptibility bioassay. The *Ae. albopictus* laboratory strain and hot spring populations were maintained in identical environments within the insectarium at 27 ± 2°C and 75 ± 10% RH.

Active ingredients: In the adult mosquito susceptibility bioassay, impregnated papers of 7 pyrethroids: permethrin 0.25% and 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.05%, cyfluthrin 0.15%, etofenprox 0.5%, alpha-cypermethrin 0.05%, and bifenthrin 0.2% were utilized. The impregnated papers were procured from the WHO Collaborating Centre, Vector Control Research Unit (VCRU), Universiti Sains Malaysia (USM), Penang, Malaysia, which were primarily produced by the VCRU at WHO-recommended diagnostic doses (WHO 1992, 1998, 2016; Table 2).

Adult mosquito susceptibility bioassay: The current study used the adult mosquito susceptibility bioassay outlined by the World Health Organization (WHO 2016). Twenty-five sucrose-fed 3–5-day-old adult female mosquitoes were held in a holding tube for 1 h to confirm their health. The mosquitoes were then transferred into an exposure tube lined with a pyrethroid-impregnated paper of a specific diagnostic dose and left exposed to the pyrethroid AI for 1 h. A silicone oil-impregnated paper produced by VCRU was also utilized in an exposure tube to serve as control. The bioassay in this study was performed in quadruplicate. Mosquito mortality rates were recorded every minute throughout the exposure period. Mosquitoes incapable of stably flying or resting were considered dead. Subsequently, the mosquitoes were transferred into holding paper cups and provided with 10% sucrose-soaked cotton balls. Cumulative mortality results were taken at 24 h postexposure.

Data analysis: The mortality results of each *Ae. albopictus* population during the 1 h of pyrethroid AIs exposure were statistically analyzed to obtain the 50% lethal time (LT_{50}) value. The resistance ratio (RR) was calculated as

Table 2. Diagnostic doses of pyrethroids used against *Aedes albopictus* in this study (WHO 1992, 1998, 2016).

Pyrethroid	Diagnostic dose for adult mosquito susceptibility bioassay
Permethrin	0.25%
Permethrin	0.75%[1]
Deltamethrin	0.05%[1]
Lambda-cyhalothrin	0.05%[1]
Cyfluthrin	0.15%
Etofenprox	0.5%
Alpha-cypermethrin	0.05%[1]
Bifenthrin	0.2%[1]

[1] Concentration of impregnated papers produced by VCRU.

$$\text{Resistance ratio (RR)} = \frac{\text{LT}_{50}\ \text{of field population}}{\text{LT}_{50}\ \text{of laboratory (reference) strain}}.$$

The mortality of adult mosquitoes was also noted every minute throughout the 1 h exposure period and 24 h postrecovery. The mortalities at 30-min exposure for all pyrethroid AIs except 60 min for bifenthrin 0.2%, after 24-h postexposure were converted into mortality percentages using the formula

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

The mortality percentages documented in the current study were categorized based on the World Health Organization guidelines (WHO 2016): A 98–100% mortality signified susceptibility to the AI; 90–97% indicated possible resistance, hence requiring further susceptibility bioassays for confirmation. A <90% mortality suggested confirmed resistance in the population. In this study the mosquito mortalities by all pyrethroid AIs were corrected with Abbott's formula (1925) if the mosquito mortality in the control tubes was over 10%. The adult mosquito susceptibility bioassay was discarded and repeated when the corrected mortality in the control tubes exceeded 10%:

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

The raw mortality data generated in this study were also assessed for normality with the Shapiro-Wilk evaluation. The mortality percentages were employed to reveal any significant differences between the *Ae. albopictus* populations via the one-way ANOVA and post hoc analyses, while the Pearson correlation assessment ascertained any significant associations between different pyrethroid AIs. A >0.4 ($r > 0.4$, $P \leq 0.05$) correlation value (r) denoted significant cross-resistance, whereas an r of >0.8 showed strong cross-resistance between 2 pyrethroid AIs. The statistical analyses in this study were performed with IBM SPSS Statistics version 23.0 with a $P = 0.05$ significance.

RESULTS

The susceptibility of *Ae. albopictus* adult mosquitoes of the reference strain and those of the hot spring study localities against several pyrethroid adulticide AIs were evaluated through a 1 h exposure in adult bioassays. Based on the normality test conducted, all raw mortality data produced in this study were normally distributed ($P > 0.05$).

The 50% lethal time (LT_{50}) values of the *Ae. albopictus* adult populations in the current study exposed to 0.25% and 0.75% permethrin ranged from 45.69 min to 85.48 min and 19.78 min to 23.86 min, respectively (Table 3). The resistance ratios for all field populations subjected to both permethrin concentrations were also below 1.00. Meanwhile, all *Ae. albopictus* adult populations exposed to deltamethrin 0.05%, lambda-cyhalothrin 0.05%, cyfluthrin 0.15%, and etofenprox 0.5% generated LT_{50} values within approximately similar ranges (16.90 min to 33.46 min). The resistance ratios of the mosquitoes exposed to the pyrethroid AIs were under 1.50.

Exposing the *Ae. albopictus* adult populations to alpha-cypermethrin 0.05% resulted in a higher LT_{50} range (23.48 min to 38.78 min) with resistance ratios of 1.02 or below. Conversely, the LT_{50} values (70.83 min to 237.83 min) for bifenthrin 0.2% were obtained only from samples collected in SEL, HTBK, and KERL hot springs, as only a few mosquitoes died during the exposure. The mortality value of *Ae. albopictus* of the reference strain and Kuala Kubu Bharu (KKB) hot spring exposed to the same pyrethroid AI did not reach the minimum of 5.00% ($P = 0.05$), thus, rendering their LT_{50} values indeterminable.

Within the 1-h exposure period, the cyfluthrin 0.15% exposure exhibited the fastest effects in causing 50.00% mortalities among the *Ae. albopictus* adult populations. In contrast, permethrin 0.25% and bifenthrin 0.2% recorded the slowest effects in exterminating 50.00% of each *Ae. albopictus* adult population within the same exposure period.

In this study, mortality percentages were calculated at 30 min of exposure to all pyrethroids, excluding bifenthrin 0.2%, which was determined at 60 min (Table 4). The *Ae. albopictus* adult populations subjected to permethrin 0.25% recorded not more than 10.00% mortality after 30 min. Nonetheless, the mortality increased between 76.00% and 96.00% upon exposure to permethrin 0.75%.

Table 3. Susceptibility to pyrethroids of adult *Aedes albopictus* of the reference strain and 4 from hot springs in Selangor, Malaysia.[1]

Types of area	Pyrethroids / Study areas	Permethrin 0.25% LT$_{50}$ (min) 95% C.L.	Permethrin 0.25% Resistance Ratio (RR)	Permethrin 0.75% LT$_{50}$ (min) 95% C.L.	Permethrin 0.75% Resistance Ratio (RR)	Deltamethrin 0.05% LT$_{50}$ (min) 95% C.L.	Deltamethrin 0.05% Resistance Ratio (RR)
Reference	Laboratory	85.48 (76.80–101.39)	—	26.20 (25.54–26.81)	—	26.06 (25.58–26.52)	—
Hot springs	Selayang hot spring (SEL)	45.69 (45.04–46.38)	0.53	21.55 (21.24–21.86)	0.82	21.72 (21.38–22.05)	0.83
	Hulu Tamu Batang Kali hot spring (HTBK)	53.38 (52.13–54.82)	0.62	23.86 (15.44–34.67)	0.91	24.44 (23.90–25.00)	0.94
	Kuala Kubu Bharu hot spring (KKB)	49.49 (48.67–50.37)	0.58	19.78 (19.37–20.17)	0.75	20.46 (19.76–21.11)	0.79
	Kerling hot spring (KERL)	56.00 (54.62–57.67)	0.66	20.72 (20.33–21.10)	0.79	18.15 (17.40–18.82)	0.70

[1] RR = resistance ratio; C.L. = confidence limit (95%); N.D. = not determined due to no mortality; LT$_{50}$ with no lower and upper C.L. = insufficient data.

The *Ae. albopictus* populations subjected to deltamethrin 0.05%, lambda-cyhalothrin 0.05%, cyfluthrin 0.15%, and etofenprox 0.5% for 30 min documented 36.00% to 99.00% mortality. In contrast, the mortality percentages of all *Ae. albopictus* adult populations exposed to alpha-cypermethrin 0.05% were below 32.00% except for HTBK, at 85.00%, within a similar time frame. Nevertheless, the mortality percentage of the *Ae. albopictus* adult populations exposed to bifenthrin 0.2% were much lower, 2.00% to 27.00%.

The *Ae. albopictus* adult populations exposed to cyfluthrin 0.15% demonstrated the highest mortalities after 30 min of exposure, while the lowest were demonstrated among *Ae. albopictus* adult populations exposed to permethrin 0.25%. On the other hand, under 30.00% mortality of the populations evaluated was documented by bifenthrin 0.2% at 1-h exposure.

The *Ae. albopictus* reference strain adults demonstrated full susceptibility to almost all pyrethroid AIs after 24 h of recovery period, excluding the mosquitoes subjected to permethrin 0.25% exposure (51.00%) and bifenthrin 0.2% (67.00%; Table 5). The *Ae. albopictus* adults from SEL, HTBK, and KKB exhibited possible resistance against permethrin 0.25%, while the *Ae. albopictus* adults from KERL were resistant to permethrin 0.25%. All *Ae. albopictus* field populations revealed full susceptibility to permethrin 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.05%, cyfluthrin 0.15%, and etofenprox 0.5%.

After the 24-h recovery period, only the *Ae. albopictus* adults from SEL demonstrated possible resistance against alpha-cypermethrin 0.05%, while the rest indicated susceptibility. On the other hand, only *Ae. albopictus* adults from SEL were fully susceptible to bifenthrin 0.2%, while *Ae. albopictus* adults from the other hot springs exhibited resistance against bifenthrin 0.2% 24 h postexposure (86.00% to 97.00%).

Based on the one-way ANOVA performed in the present study, significant differences ($P \leq 0.05$) in mortality percentages at 30-min or 60-min and 24-h postexposure were documented in only a few pyrethroid exposures. Significant LT$_{50}$ correlations were revealed between permethrin 0.75% and deltamethrin 0.05% ($r = 0.909$, $P = 0.032$), deltamethrin 0.05% and cyfluthrin 0.15% ($r = 0.885$, $P = 0.046$), and lambda-cyhalothrin 0.05% and cyfluthrin 0.15% ($r = 0.965$, $P = 0.008$), indicating intraclass cross-resistance between the pyrethroids involved.

DISCUSSION

According to WHO (2016), the mortality percentage of adult mosquitoes should be determined at 24-h postexposure to an insecticide AI. In the current study, *Ae. albopictus* populations subjected to adulticide pyrethroid AIs except for permethrin 0.25%, alpha-cypermethrin 0.05%, and bifenthrin 0.2% resulted in 100.00% mortalities at the end of 1-h exposure and 24-h posttreatment. Consequently, the shortest or longest time required by the populations to be affected by the pyrethroid could not be determined in this study. As a result, besides mortality percentages at 24-h postexposure, the current work recorded mortality percentages at 30-min exposure for all AIs except for bifenthrin 0.2%, where the *Ae. albopictus* adult populations did not demonstrate complete mortalities after 1 h.

As for permethrin 0.25% exposure, the mortality percentages were noted at 30-min exposure time so that these data were comparable with mortality percentages for permethrin 0.75% exposure. The mortality percentage of *Ae. albopictus* adult population from HTBK subjected to alpha-cypermethrin 0.05% was also logged at 30-min exposure, as the population exhibited complete mortality before the end of the exposure time.

The current study employed 0.25% and 0.75% permethrin. Initially, the higher concentration of permethrin impregnated papers was utilized as it

Table 3. Extended

Lambda-cyhalothrin 0.05%		Cyfluthrin 0.15%		Etofenprox 0.5%		Alpha-cypermethrin 0.05%		Bifenthrin 0.2%	
LT_{50} (min) 95% C.L.	Resistance Ratio (RR)	LT_{50} (min) 95% C.L.	Resistance Ratio (RR)	LT_{50} (min) 95% C.L.	Resistance Ratio (RR)	LT_{50} (min) 95% C.L.	Resistance Ratio (RR)	LT_{50} (min) 95% C.L.	Resistance Ratio (RR)
29.94 (29.54–30.33)	—	23.08 (22.69–23.48)	—	25.53 (25.04–26.01)	—	37.89 (36.72–39.09)	—	N.D.	N.D.
26.29 (25.86–26.70)	0.88	19.10 (18.78–19.41)	0.83	29.76 (29.37–30.15)	1.17	38.78 (38.27–39.28)	1.02	70.83 (66.76–77.55)	—
32.16 (31.69–32.63)	1.07	25.70 (25.25–26.14)	1.11	27.28 (26.82–27.73)	1.07	23.48 (23.06–23.90)	0.62	176.55 (111.00–1,020.84)	—
26.56 (26.16–26.94)	0.89	18.65 (18.26–19.02)	0.81	22.43 (21.96–22.89)	0.88	35.57 (35.16–35.99)	0.94	N.D.	N.D.
26.59 (26.16–27.00)	0.89	16.90 (16.52–17.26)	0.73	33.46 (32.82–34.11)	1.31	34.67 (34.16–35.17)	0.92	237.83	—

was the only concentration available from VCRU. The permethrin 0.25% impregnated papers were later available following the WHO (2016) recommended diagnostic dose for *Aedes* mosquitoes. Nevertheless, WHO established a 0.4% diagnostic dose for permethrin in 2022, which was revealed after the completion of this study.

The results in the current study indicated that permethrin, deltamethrin, lambda-cyhalothrin, cyfluthrin, and etofenprox are suitable pyrethroid adulticide AIs and could be employed in vector control activities at the selected hot springs if required. The Ministry of Health Malaysia has also utilized some of the pyrethroids during vector control space treatments (Ong 2016). Nevertheless, the details on the application are currently unavailable for public access.

Although exposure to alpha-cypermethrin 0.05% resulted in various levels of susceptibility at 30 min of exposure, the mortality percentages recorded by the *Ae. albopictus* adult populations increased to over 97.00% at the end of 24-h posttreatment. The application of alpha-cypermethrin-based products could still be considered at selected hot springs if required. Nevertheless, alpha-cypermethrin-based substances necessitate a longer time to reach a high mortality compared to other pyrethroid AIs. Furthermore, the use of alpha-cypermethrin-based products in SEL and KERL should be closely monitored as the *Ae. albopictus* adult populations were no longer fully susceptible to the pyrethroid AI.

Employing the pyrethroids at the hot springs selected in this study should be conducted in rotation with other classes of insecticides, such as organophosphates, that are also utilized in the Malaysian vector control program to prevent or delay insecticide resistance among mosquito vectors. Alpha-cypermethrin has also been employed in indoor residual spraying (IRS) to combat malaria and visceral leishmaniasis in countries including India (Saurabh et al. 2020, Mishra et al. 2021), but information on its application in Malaysian vector control strategies is inadequate.

The *Ae. albopictus* adult populations exposed to bifenthrin 0.2% documented very low mortality percentages by the end of 1 h. Although the figures rose at 24-h posttreatment, the numbers were still less than the results obtained for alpha-cypermethrin 0.05%. If required in the future, bifenthrin could be utilized as an adulticide at the study sites. Nonetheless, careful considerations are necessary as the mosquitoes in this study demonstrated resistance to bifenthrin although only at diagnostic dosage.

Several reports in other countries have demonstrated the effectiveness of bifenthrin as a residual insecticide in barrier spray treatments on foliage and vegetation, thus providing an option for mosquito control (Fulcher et al. 2015, VanDusen et al. 2016). In Malaysia, employing bifenthrin as an adulticidal, larvicidal, and wall residual agent was significantly effective against laboratory-reared *Ae. aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* Say (Lee et al. 1997, Sulaiman et al. 2008). Nevertheless, information on the application of bifenthrin in vector control programs held by the Ministry of Health Malaysia and other local health authorities remains unspecified.

The significant intraclass cross-resistance between permethrin and deltamethrin, deltamethrin and cyfluthrin, and lambda-cyhalothrin and cyfluthrin suggested that similar metabolic enzymes or target-site mutation were involved in the resistance mechanisms against the pyrethroid AIs among *Ae. albopictus* adult populations assessed in this study.

The *Ae. albopictus* laboratory strain from IMR was the best option as a reference strain in the present study, considering that IMR is a research arm of the Ministry of Health Malaysia. Furthermore, access

Table 4. Percent mortality of adult *Aedes albopictus* of the reference strain and 4 from hot springs in Selangor, Malaysia, after exposure to pyrethroids at 30-min and 60-min (bifenthrin 0.2%) exposure time.[1]

	Pyrethroids	Percent mortality at 30-min exposure time							Percent mortality at 60-min exposure time
Type of population	Study area	Permethrin 0.25%	Permethrin 0.75%	Deltamethrin 0.05%	Lambda-cyhalothrin 0.05%	Cyfluthrin 0.15%	Etofenprox 0.5%	Alpha-cypermethrin 0.05%	Bifenthrin 0.2%
Reference	Laboratory	1.00 ± 1.00[a]	76.00 ± 6.73	71.00 ± 15.95	49.00 ± 17.92	90.00 ± 7.57	70.00 ± 4.16	22.00 ± 7.02[a]	2.00 ± 1.15[a]
Hot springs	Selayang hot spring (SEL)	10.00 ± 2.58[ab]	96.00 ± 2.83	91.00 ± 2.52	73.00 ± 4.12	99.00 ± 1.00[b]	52.00 ± 5.89	16.00 ± 1.63[b]	27.00 ± 7.00[ab]
	Hulu Tamu Batang Kali hot spring (HTBK)	8.00 ± 2.83	86.00 ± 8.72	72.00 ± 14.05	36.00 ± 10.58	65.00 ± 10.38[bc]	58.00 ± 12.91	85.00 ± 3.00[abc]	13.00 ± 4.43
	Kuala Kubu Bharu hot spring (KKB)	6.00 ± 1.15	93.00 ± 1.91	90.00 ± 2.58	75.00 ± 3.79	95.00 ± 2.52[c]	74.00 ± 5.03	19.00 ± 1.91[c]	3.00 ± 1.91[b]
	Kerling hot spring (KERL)	1.00 ± 1.00[b]	89.00 ± 1.00	91.00 ± 3.00	67.00 ± 7.90	98.00 ± 1.15[c]	44.00 ± 5.66	32.00 ± 6.73[c]	12.00 ± 7.12
One way ANOVA		F = 4.639 df = 19 $P = 0.012$	F = 2.220 df = 19 $P = 0.116$	F = 1.166 df = 19 $P = 0.365$	F = 2.706 df = 19 $P = 0.070$	F = 5.709 df = 19 $P = 0.005$	F = 2.804 df = 19 $P = 0.064$	F = 37.441 df = 19 $P = 0.000$	F = 4.074 df = 19 $P = 0.020$

[1] Percent mortality at 30-min exposure time (%) = mean of mortality for adult mosquitoes + standard error. Percent mortality with different letter implies significant difference between one another ($P \leq 0.05$) (post hoc Tukey's HSD test): [a] = significantly different with reference strain; [b] = significantly different with SEL strain; [c] = significantly different with HTBK strain; [d] = significantly different with KKB strain.

Table 5. Percent mortality of *Aedes albopictus* adults of the reference strain and 4 from hot springs in Selangor, Malaysia, caused by pyrethroids at 24-h posttreatment.[1]

Type of population	Pyrethroid	Percent mortality after 24 h							
	Study area	Permethrin 0.25%	Permethrin 0.75%	Deltamethrin 0.05%	Lambda-Cyhalothrin 0.05%	Cyfluthrin 0.15%	Etofenprox 0.5%	Alpha-Cypermethrin 0.05%	Bifenthrin 0.2%
Reference	Laboratory	^{R}51.00 ± 1.91	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}99.00 ± 1.00	^{R}67.00 ± 7.55
Hot springs	Selayang hot spring (SEL)	^{M}96.00 ± 2.31^{a}	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{M}97.00 ± 1.91	^{S}100.00 ± 0.00^{a}
	Hulu Tamu Batang Kali hot spring (HTBK)	^{M}90.00 ± 1.15^{a}	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{R}90.00 ± 3.46^{a}
	Kuala Kubu Bharu hot spring (KKB)	^{M}91.00 ± 1.91^{a}	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{R}86.00 ± 2.58^{a}
	Kerling hot spring (KERL)	^{R}83.00 ± 5.74^{a}	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}100.00 ± 0.00	^{S}99.00 ± 0.49	^{R}97.00 ± 3.00^{a}
One-way ANOVA		F = 34.649 df = 19 P = 0.000	F = 0.000 df = 19 P = 0.000	F = 0.000 df = 19 P = 0.000	F = 0.000 df = 19 P = 0.000	F = 0.000 df = 19 P = 0.000	F = 0.000 df = 19 P = 0.000	F = 1.324 df = 19 P = 0.306	F = 9.951 df = 19 P = 0.000

[1] Percent mortality after 24 h (%) = mean of mortality for adult mosquitoes + standard error. S = susceptible, M = possible resistance, R = confirmed resistance, as determined by WHO (2016). Percent mortality with different letter implied significant difference between one another ($P \leq 0.05$) (post hoc Tukey's HSD test): [a] = significantly different with reference strain; [b] = significantly different with SEL strain; [c] = significantly different with HTBK strain; [d] = significantly different with KKB strain.

to susceptible *Ae. albopictus* strains from any well-established laboratories were not available.

Although the *Ae. albopictus* laboratory strain required longer than 60-min exposure time to 24-h postexposure to be affected by the insecticide AIs, the *Ae. albopictus* laboratory strain employed in the present study was completely susceptible to numerous other commonly available AIs (Elia-Amira et al. 2019, Lau et al. 2021, Wan-Norafikah et al. unpublished data). Nevertheless, continuous monitoring of its susceptibility against insecticide AIs and precautionary actions have been performed by IMR to maintain or enhance the sensitivity of the strain against the insecticide AIs and prevent further insecticide resistance development in the future.

Generally, the hot springs chosen in this study are equipped with similar essential facilities, such as concrete benches around the hot spring spots, resting gazebos, and washrooms. In terms of location, the KERL site is the only study area located marginally remote from any human dwelling. The other hot springs are situated in proximity to human housing areas, which are more prone to insecticide exposures during vector control operations. Nevertheless, no significant differences in susceptibility levels between the *Ae. albopictus* adult population from KERL and *Ae. albopictus* adult populations from other hot springs and laboratory strains against the same pyrethroid AIs were observed regardless of its unique location.

Close monitoring of the abundance and susceptibility levels of the mosquito vectors from the hot springs against insecticides is necessary because of the dense vegetation around the localities. The lush foliage, especially at KERL, could provide conducive breeding and resting habitats for numerous mosquito species, including *Ae. albopictus*. Consequently, possibilities of mosquito-borne infections spreading are increased in the areas assessed in this study.

The susceptibility status of *Ae. albopictus* adult populations in urban and rural neighborhoods and agricultural plantations against similar adulticide pyrethroid AIs has been reported by researchers globally. For instance, adult *Ae. albopictus* mosquitoes from the USA, Laos, and Papua New Guinea were susceptible to deltamethrin 0.05%, while its counterpart in Fanar, Lebanon, recorded susceptibility to permethrin 0.25% and lambda-cyhalothrin 0.03% (Marcombe et al. 2014, Tangena et al. 2018, Demok et al. 2019, Haddad et al. 2022). In another report, the *Ae. albopictus* adult populations from Andaman and Nicobar Islands in India demonstrated susceptibility to deltamethrin 0.05%, but resistance to permethrin 0.75%, lambda-cyhalothrin 0.05%, and cyfluthrin 0.15% (Sivan et al. 2015).

Two *Ae. albopictus* adult populations from Bangui, Africa, and several adult populations of the same species from Yaounde and Douala in Cameroon were reportedly resistant to deltamethrin 0.03%, 0.05%, alpha-cypermethrin 0.05%, and permethrin 0.75% (Ngoagouni et al. 2016, Kamgang et al. 2017, Yougang et al. 2022). Similarly, resistance against permethrin 0.75% was also recorded among 2 Italian *Ae. albopictus* populations (Pichler et al. 2018).

A study revealed that adulticide pyrethroid AIs required between 1 to 24 h to demonstrate their full effects on 14 *Ae. albopictus* adult populations collected from different districts in Sabah, Malaysia. Although the populations recorded under 90.00% mortalities at 60-min exposure to permethrin 0.75% and to etofenprox 0.50%, it improved to 100.00% mortality at 24-h posttreatment (Elia-Amira et al. 2019). Meanwhile, a majority of the 13 *Ae. albopictus* adult populations obtained from different districts in Sarawak were resistant to etofenprox 0.50%, deltamethrin 0.05%, lambda-cyhalothrin 0.05%, and permethrin 0.25% (Lau et al. 2021). Conversely, the populations were fully susceptible to cyfluthrin 0.15% (Lau et al. 2021). In another local study, 11 *Ae. albopictus* adult populations collected from dengue hotspots in Kuala Lumpur and Selangor documented resistance to deltamethrin 0.03% and permethrin 0.25% (Rasli et al. 2021).

Although no mosquito-borne disease case has been reported from the hot springs selected in this study, the susceptibility levels of *Ae. albopictus* populations should still be considered and utilized to delineate forthcoming plans for tackling *Aedes*-borne diseases. This is the first study to record the susceptibility status of mosquito vectors captured in the hot springs. Consequently, more studies involving mosquito vectors from commonly visited hot springs are needed.

ACKNOWLEDGMENTS

The authors thank the Medical Entomology Unit, Institute for Medical Research, Ministry of Health Malaysia, for supplying the reference strain of mosquitoes. The technical assistance provided by the staff in the Laboratory Animal Care Unit, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, throughout this study is also appreciated. This present study was funded under the Dana UiTM Cawangan Selangor Research Grant (DUCS 2.0) [600-UiTMSEL (PI. 5/4) (021/2020)] by the UiTM Selangor Branch.

REFERENCES CITED

Abbott WS. 1925. A method for computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267.

Dalpadado R, Amarasinghe D, Gunathilaka N, Ariyarathna N. 2022. Bionomic aspects of dengue vectors *Aedes aegypti* and *Aedes albopictus* at domestic settings in urban, suburban and rural areas in Gampaha District, Western Province of Sri Lanka. *Parasit Vectors* 15:148.

Demok S, Endersby-Harshman N, Vinit R, Timinao L, Robinson LJ, Susapu M, Makita L, Laman M, Hoffman A, Karl S. 2019. Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Papua New Guinea. *Parasit Vectors* 12:333.

Elia-Amira NMR, Chen CD, Low VL, Lau KW, Haziqah-Rashid A, Amelia-Yap ZH, Lee HL, Sofian-Azirun M. 2019. Adulticide resistance status of *Aedes albopictus* (Diptera: Culicidae) in Sabah, Malaysia: a statewide assessment. *J Med Entomol* 56:1715–1725.

Fansiri T, Pongsiri A, Khongtak P, Nitatsukprasert C, Chittham W, Jaichapor B, Pathawong N, Kijchalao U, Tiangtrong S, Singkhaimuk P, Ponlawat A. 2022. The impact of insect growth regulators on adult emergence inhibition and the fitness of *Aedes aegypti* field populations in Thailand. *Acta Trop* 236:106695.

Fulcher A, Farooq M, Smith ML, Li C-X, Scott JM, Thomson E, Kaufman PE, Xue R-D. 2015. Evaluation of a new spraying machine for barrier treatment and penetration of bifenthrin on vegetation against mosquitoes. *J Am Mosq Control Assoc* 31:85–92.

Haddad N, Omran H, Amraoui F, Zakhia R, Mousson L, Failloux AB. 2022. The tiger mosquito in Lebanon two decades after its introduction: a growing health concern. *PLoS Negl Trop Dis* 16:e0010206.

Jeffery J, Rohela M, Muslimin M, Abdul Aziz SMN, Jamaiah I, Kumar S, Tan TC, Lim YAL, Nissapatorn V, Abdul-Aziz NM. 2012. *Illustrated keys: some mosquitoes of Peninsula Malaysia*. Kuala Lumpur, Malaysia: University of Malaya Press.

Kamgang B, Nchoutpouen E, Simard F, Paupy C. 2012. Notes on the blood-feeding behavior of *Aedes albopictus* (Diptera: Culicidae) in Cameroon. *Parasit Vectors* 5:57.

Kamgang B, Yougang AP, Tchoupo M, Riveron JM, Wondji C. 2017. Temporal distribution and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in Yaounde, the capital city of Cameroon. *Parasit Vectors* 10:469.

Lau KW, Chen CD, Low VL, Lee HL, Azidah AA, Sofian-Azirun M. 2021. Adulticide resistance status of *Aedes albopictus* (Diptera: Culicidae) in Sarawak state, Malaysia. *J Med Entomol* 58:2292–2298.

Lee HL. 1992. *Aedes* ovitrap and larval survey in several suburban communities in Selangor, Malaysia. *Mosq Borne Dis Bull* 9:9–15.

Lee HL, Khadri MS, Chiang YF. 1997. Preliminary field evaluation of the combined adulticidal, larvicidal, and wall residual activity of ULV-applied bifenthrin against mosquitoes. *J Vector Ecol* 22:146–149.

Marcombe S, Farajollahi A, Healy SP, Clark GG, Fonseca DM. 2014. Insecticide resistance status of United States populations of *Aedes albopictus* and mechanisms involved. *PLoS ONE* 9:e101992.

Ministry of Health Malaysia. 1997. *Guidelines on the use of ovitrap for Aedes surveillance*. Malaysia: Ministry of Health Malaysia.

Mishra AK, Nisar S, Rajvanshi H, Bharti PK, Saha KB, Shukla MM, Sharma RK, Jayswar H, Das A, Kaur H, Wattal SL, Lal AA. 2021. Improvement of indoor residual spraying and long-lasting insecticidal net services through structured monitoring and supervision as part of the Malaria Elimination Demonstration Project in Mandla, Madhya Pradesh. *Malaria J* 20:101.

Ngoagouni C, Kamgang B, Brengues C, Yahouedo G, Paupy C, Nakoune E, Kazanji M, Chandre F. 2016. Susceptibility profile and metabolic mechanisms involved in *Aedes aegypti* and *Aedes albopictus* resistant to DDT and deltamethrin in the Central African Republic. *Parasit Vectors* 9:599.

Ong SQ. 2016. Dengue vector control in Malaysia: a review for current and alternative strategies. *Sains Malaysiana* 45:777–785.

Peinado SA, Aliota MT, Blitvich BJ, Bartholomay LC. 2022. Biology and transmission dynamics of *Aedes* flavivirus. *J Med Entomol* 59:659–666.

Pichler V, Bellini R, Veronesi R, Arnoldi D, Rizzoli A, Lia RP, Otranto D, Montarsi F, Carlin S, Ballardini M, Antognini E, Salvemini M, Brianti E, Gaglio G, Manica M, Cobre P, Serini P, Velo E, Vontas J, Kioulos I, Pinto J, Torre AD, Caputo B. 2018. First evidence of resistance to pyrethroid insecticide in Italian *Aedes albopictus* populations 26 years after invasion. *Pest Manag Sci* 74:1319–1327.

Pruszynski CA, Stenn T, Acevedo C, Leal AL, Burkett-Cadena ND. 2020. Human blood feeding by *Aedes aegypti* (Diptera: Culicidae) in the Florida Keys and a review of the literature. *J Med Entomol* 57:1640–1647.

Rasli R, Cheong YL, Ibrahim MKC, Fikri SFF, Norzali RN, Nazarudin NA, Hamdan NF, Muhamed KA, Hafisool AA, Azmi RA, Ismail HA, Ali R, Hamid NA, Taib MZ, Omar T, Ahmad NW, Lee HL. 2021. Insecticide resistance in dengue vectors from hotspots in Selangor, Malaysia. *PLoS Negl Trop Dis* 15:e0009205.

Saurabh S, Yadav RK, Sharma MP. 2020. Factors associated with variation in insecticide quantity being used for indoor residual spraying (IRS) for visceral leishmaniasis (kala-azar) elimination in Bihar, India. *J Vector Borne Dis* 57:240–248.

Sivan S, Shriram AN, Sunish IP, Vidhya PT. 2015. Studies on insecticide susceptibility of *Aedes aegypti* (Linn) and *Aedes albopictus* (Skuse) vectors of dengue and chikungunya in Andaman and Nicobar Islands, India. *Parasitol Res* 114:4693–4702.

Sulaiman S, Abang Kamarudin DSF, Othman H. 2008. Evaluation of bifenthrin and *Acorus calamus* Linn. extract against *Aedes aegypti* L. and *Aedes albopictus* (Skuse). *Iran J Arthropod-Borne Dis* 2:7–11.

Tangena JAA, Marcombe S, Thammavong P, Chonephetsarath S, Somphong B, Sayteng K, Grandadam M, Sutherland IW, Lindsay SW, Brey PT. 2018. Bionomics and insecticide resistance of the arboviral vector *Aedes albopictus* in northern Lao PDR. *PLoS ONE* 13:e0206387.

Tsai P-J, Teng H-J. 2016. Role of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in local dengue epidemics in Taiwan. *BMC Infect Dis* 16:662.

VanDusen AE, Richards SL, Balanay JAG. 2016. Evaluation of bifenthrin barrier spray on foliage in a suburban eastern North Carolina neighbourhood. *Pest Manag Sci* 72:1004–1012.

Wan-Norafikah O, Chen CD, Mohd-Amir MH, Azahari AH, Zainal-Abidin AH, Nazni WA, Mariam M, Mohd-Shahizan J, Sofian-Azirun M. 2019. Surveillance of *Aedes* vectors in selected agricultural, fogging-free and dengue-prone areas in Peninsular Malaysia. *Southeast Asian J Trop Med Public Health* 50:469–485.

WHO [World Health Organization]. 1992. *World Health Organization Technical Report Series No. 818: Vector resistance to pesticides: Fifteenth report of the WHO Expert Committee on vector biology and control*. Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 1998. *Test procedure for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces* (WHO/CDS/CPC/MAL/98.12). Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 2012. *Handbook for integrated vector management* (WHO/HTM/NTD/VEM/2012.3). Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 2016. *Monitoring and managing insecticide resistance in Aedes mosquito populations. Interim guidance for entomologists* (WHO/ZIKV/VC/16.1). Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 2022. *Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO tube tests*. Geneva, Switzerland: World Health Organization.

Yougang AP, Keumeni CR, Wilson-Bahun TA, Tedjou AN, Njiokou F, Wondji C, Kamgang B. 2022. Spatial distribution and insecticide resistance profile of *Aedes aegypti* and *Aedes albopictus* in Douala, the most important city of Cameroon, *PLoS ONE* 17:e0278779.