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RESEARCH ARTICLE

Bioefficacy of mosquito mat vaporizers and associated metabolic detoxication mechanisms in *Aedes aegypti* (Linnaeus) in Selangor, Malaysia: A statewide assessment

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ABSTRACT

This study aims to examine the efficacy of mosquito mat vaporizers on *Aedes aegypti* and their associated metabolic detoxication mechanisms. For this purpose, *Aedes aegypti* (Linnaeus) was collected from nine districts in Selangor, Malaysia and tested with mosquito vaporizing mat bioassays. The same populations were also subjected to biochemical assays to investigate activities of detoxifying enzymes, namely non-specific esterase (EST), glutathione-S-transferase (GST) and mixed function oxidase (MFO). The efficacy of *Ae. aegypti* on the active ingredients tested in decreasing order were d- allethrin > dimefluthrin > prallethrin with PBO > prallethrin. The results further indicated significant enhancement mean levels of EST, GST and MFO in pyrethroid-resistant populations. The mortality rate of *Ae. aegypti* in response to pyrethroid active ingredients was associated with MFO activity, suggesting it is an important detoxification enzyme for the populations tested. In view of the presence of resistance against household insecticide products, pyrethroid efficacy on *Ae. aegypti* populations needs to be monitored closely to ensure the implementation of an effective vector control program in Malaysia.

Keywords: Resistance; mosquito mat vaporizer; biochemical assays; pyrethroid; *Aedes.*

INTRODUCTION

Dengue and chikungunya are two major public health issues in Malaysia with 130, 101 severe dengue cases and 990 chikungunya cases reported in 2019 (Ministry of Health Malaysia [MOH], 2020). *Aedes aegypti* and *Ae. albopictus* are responsible for the transmissions of dengue and chikungunya viruses in Malaysia. *Aedes aegypti* is the primary dengue vector which lives close to humans in urban surroundings, whereas *Ae. albopictus* serves as the secondary dengue vector which mainly lives outdoor (Vontas *et al.*, 2012).

The mosquito control program in Malaysia has been carried out as an integrated program that involves environmental management and source reduction through public education and enforcement. The control program highlights two new features: cross-sector and inter-agency cooperation; and a decision-making support system based on four fundamental aspects, namely cases, viruses, entomological monitoring and ecological information (Ministry of Health Malaysia [MOH], 2009). Of these, insecticide application is an one of the important control measures to combat mosquito-borne diseases worldwide including Malaysia. In addition to larviciding and adulticiding activities, household insecticide products containing pyrethroid active ingredients have been widely used worldwide. The efficacy of the commonly used household pyrethroid products against *Ae. aegypti*, however, has been understudied. Essentially, this work seeks to examine the susceptibility of *Ae. aegypti* adults to the commercial mosquito mat vaporizers used by the community in Selangor, Malaysia, and attempts to characterize the detoxification mechanisms in pyrethroid-resistant populations.

MATERIALS AND METHODS

Study sites

Aedes aegypti eggs were collected using ovitraps from nine districts: Sabak Bernam, Kuala Selangor, Hulu Selangor,

Figure 1. Ovitrap collection sites in Selangor.

Gombak, Petaling, Hulu Langat, Kuala Langat, Klang and Sepang (Figure 1).

Preparation of ovitrap and sample collection

Ovitraps were used as designed by Lee (1992). The ovitrap consisted of a 300-ml black coloured plastic cup with 9.0 cm in height, diameter base of 6.5 cm with an opening of 7.8 cm. Each ovitrap was fixed with a 2.5 cm × 10.0 cm × 0.3 cm hardboard paddle. The ovitrap was then filled up with 5.5 cm of chlorine-free tap water. For each study site, 40 ovitraps were placed randomly in close proximity with other potential larval habitats which were protected against direct sunlight and rain. After five days, the ovitraps were collected and transported to the laboratory for hatching, rearing and subsequent identification of adult phase.

Colonization of *Aedes aegypti*

Aedes aegypti was identified and colonized according to locations in respective wooden built, and net covered cages (30 cm × 30 cm × 30 cm). The adult mosquitoes were fed with 10% sucrose solution as their food source. Female adults aged 4-5 days were fed with blood meal using a white mouse until full engorgement. An oviposition site consisted of a plastic cup with 200 ml chlorine-free water lined with No 1

Whatman filtered paper and placed into a cage after two days of blood feeding. The eggs were left to hatch with chlorine-free water-filled in plastic containers (25 cm × 30 cm × 5 cm). Larvae were fed with powdered beef liver. Pupae were then placed in a small plastic cup and put into rearing cage to grow as adults. The *Ae. aegypti* Bora-Bora strain obtained from the Universiti Sains Malaysia, same as those in Amelia-Yap *et al*. (2018a, 2019), was used as the susceptible reference population.

Mosquito vaporizing mat bioassay

Four commercial mosquito mat vaporizers, prallethrin 15.0 mg/mat with piperonyl butoxide [PBO] 18.0 mg/mat, dimefluthrin 7.4 mg/mat, prallethrin 15.0 mg/mat and d-allethrin 40.0 mg/mat were used in the present study. The bioassays were performed using the standardized protocol defined by the World Health Organization [WHO] (2009), and the World Health Organization [WHO] (2016) resistance indicator was adopted. Transparent glass chambers (70 cm × 70 cm × 70 cm) that included a sliding window (18 cm × 20 cm) were used for bioassays. Temperatures and relative humidity were maintained at 27 ± 2°C and 80 ± 10% for the duration of bioassays.

Mosquito mat was inserted into its vaporizing device and was heated outside the test chamber. At the intended test intervals, the device was introduced into the centre of the glass chamber and allowed to operate continuously. At suitable intervals, the amount of knocked down specimens were observed for 60 minutes. In total, twenty-five, 2 to 5-d-old sugar-fed *Ae. aegypti* females were released into the chamber and exposed to the mats. The number of knocked-down mosquitoes was calculated and documented per minute, up to 60 minutes. Mosquitoes that were unable to fly or in imbalance posture would be considered as a knockdown. After 60 minutes of exposure time, tested mosquitoes were transferred into a clean plastic container size 9.0 cm in height, diameter base of 6.5 cm with an opening of 7.8 cm using an electric aspirator and held for 24-h post-exposure observation. Containers were covered with a mesh and mosquitoes were provided a 10% sucrose solution via a soaked cotton wool. Mosquitoes were maintained at 27 ± 2°C and relative humidity of 80 ± 10%. Mortality readings were taken 24-h after mosquitoes had been removed from vapor exposure. Following the mortality reading, dead and alive mosquitoes were transferred to individual microfuge tubes and stored at -20°C.

Before subsequent test, the chamber was cleaned with detergent and water. For control experiments, 25 female mosquitoes were released in the cleaned chamber for 60 minutes to avoid any insecticide contamination after cleaning without exposing them to any mats. For each study location and active ingredient, toxicological tests were conducted in three replicates.

Enzyme assays

For each of the three enzyme assays, 24 individual *Ae. aegypti* females from each location were used for a total of 720 individuals assayed. The non-specific esterase (EST) enzyme assay was carried out according to the protocol by Brogdon *et al*. (1988) and Lee (1990). A total of 24 single mosquitoes were homogenized and centrifuged at 4°C in phosphate-buffered solutions for 10 minutes at 15,000 rpm. This assay then obtained four supernatant aliquots (50 µl) derived from single mosquito homogeneity. In a 96-well plate, a 50 µl of indicator (fast blue B salt) was placed on substrate solution (either α-naphthyl acetate or β-naphthyl acetate) and left up to one minute. After an incubation period of 10 minutes, 50 µl 10% acetic acid was added to stop the reaction. An absorbance reader for the optical dense (BIO-TEK ELx800) was used to measure the density of 450 nm.

Glutathione-S-transferase (GST) enzyme assay was performed according to the protocol by Lee & Chong (1995). In the potassium phosphate buffer solution, 24 individual mosquitoes were homogenized. Subsequently, centrifugation was conducted at 14,000 rpm at 4°C for 10 minutes. Four homogeneous aliquots (each 50 µl) from each mosquito were added in a 96-well plate, followed by the addition of 50 µl of 2-mm glutathione and 50 µl 1mM of 1-chloro-2,4-dinitrobenzene. The reaction was incubated within 30 minutes. The data was then recorded at 410 nm of the optical density.

The mixed function oxidases (MFO) enzyme assay was conducted based on the method by Brogdon *et al*. (1997). A total of 24 individual mosquitoes were homogenized in a sodium acetate buffer solution. Four homogeneous aliquots (100 µl) were obtained from all specimens. After 5-minutes incubation, absorption was determined at 630 nm with the addition of 200 µl of 2-mm 3,3',5'-tetramethylbenzidine and 25 µl of 3% hydrogen peroxide.

Data analysis

Bioassay data from at least three mosquito mat vaporizer replicates were collected and analyzed. Time to knockdown (KT_{50}) was calculated by using probit analysis with SPSS software (version 20) (Finney, 1971). Resistance ratios were calculated using the following formula from

$$RR = \frac{KT_{50} \text{ of field strain}}{KT_{50} \text{ of reference strain}}$$

RR values of <5 imply low resistance, 5–10 imply medium resistance, while >10 imply high resistance (Mazzarri & Georghiou, 1995). A one-way variance analysis (ANOVA) was performed using SPSS Version 20 to compare the knockdown and mortality rates in all study sites. Tukey's test used to determine the mean for significant ANOVAs, $P < 0.05$. In order to examine the presence of cross-resistance of the active ingredient tested, Spearman's rank-order correlation analysis between knockdown rates was performed (Bisset *et al*., 1997). To assess mosquito susceptibility, the mortality rate after 24-h post-treatment was recorded (WHO, 2016).

- Mortality rate of ≥98-100%: susceptible to insecticide
- Mortality rate of <98%: possible development of resistance to insecticide
- Mortality rate of <90%: resistance to insecticide

The Spearman rank-order correlation analysis was correlated with the mortality rate of mosquito mat vaporizing bioassays tested on 24 samples per test with triplicates of each population. The ratio of enzyme activity was determined by dividing the mean enzyme level of the field strain, and the mean enzyme level of the laboratory reference strain. Using SPSS version 20, a one-way variance analysis (ANOVA) was run to compare mean enzyme activity between study sites. The Tukey test was used to determine the mean for ANOVAs, $P < 0.05$. An independent-sample t-test was performed to show any differences in the mean of enzyme activity.

RESULTS

Mosquito vaporizing mat bioassay

Aedes aegypti populations tested exhibited different trends in susceptibility to pyrethroid active ingredients. Bora-Bora laboratory reference that was tested with mosquito mat vaporizer resulted in 100% mortality in all replicates, with KT_{50} 0.39 minutes to prallethrin with PBO, 1.35 minutes to dimefluthrin, 0.91 minutes to prallethrin and 0.38 minutes to d-allethrin. The KT_{50} of field population exposed to prallethrin with PBO, dimefluthrin, prallethrin and d-allethrin ranged from 2.56 to 13.06 minutes (the longest KT_{50} population: Hulu Selangor); 1.44 to 4.41 minutes (the longest KT_{50} population: Hulu Selangor); 6.58 to 37.07 minutes (the longest KT_{50} population: Hulu Langat) and 2.72 to 23.46 minutes (the longest KT_{50} population: Kuala Langat) (Table 1).

Aedes aegypti populations demonstrated different percentages of knockdown from 80.00 to 98.67% (the lowest knockdown rates: Hulu Langat population), 96.00 to 100% (the lowest knockdown rates: Petaling population), 50.67 to 90.67% (the lowest knockdown rates: Hulu Langat population) and 76.00 to 100.00% (the lowest knockdown rates: Kuala Langat population) for prallethrin with PBO, dimefluthrin, prallethrin and d-allethrin, respectively (Table 2).

Mortality was observed after exposure to prallethrin with PBO, dimefluthrin, prallethrin and d-allethrin, respectively in *Ae. aegypti* populations ranging from 69.33-100%, 73.33-100%, 72-97.33% and 85.33-100%. Populations from Kuala Selangor, Gombak, Petaling and Sepang showed high susceptibility to d-allethrin with 100% mortality at the end 24-hr reading. Meanwhile, the population from Sabak Bernam, Kuala Selangor, Hulu Langat and Kuala Langat showed < 90% mortality, suggesting that they were resistant to prallethrin. Spearman rank analysis showed significant correlations between prallethrin with PBO and dimefluthrin mortality rates (r = 0.828; P = 0.003), prallethrin with PBO prallethrin and d-allethrin (r = 0.839; P = 0.002) as well as dimefluthrin and d-allethrin (r = 0.822; P = 0.004).

Enzyme assays

Non-specific esterases (EST) assay demonstrated enzyme ratios ranging from 1.00 to 2.07 fold for α-esterases activity and from 1.00 to 2.08 fold for β-esterases activity. Activities of α-esterases and β-esterases had significantly increased in all populations except Hulu Selangor and Klang. All populations at nine sites showed higher α-esterase activity compared to β-esterase activity, except for Kuala Selangor, Kuala Langat and Klang populations. The ratios of glutathione-S-transferase (GST) ranged from 1.14 to 1.71 folds were recorded. Seven populations (i.e., Sabak Bernam, Kuala Selangor, Hulu Selangor, Hulu Langat, Kuala Langat, Klang and Sepang) showed a significant increase of glutathione-S-transferase activity. Slightly elevated of mixed function oxidases (MFO) activity was found in all populations (except Petaling) with ratios ranging from 1.19 to 3.76 folds. Furthermore, one way ANOVA showed that the mean for all enzyme activity tested in *Ae. aegypti* was significantly different across all study sites (P < 0.001) (Table 3).

A significant correlation between prallethrin and PBO survivability rate and GST (r = -0.683; P = 0.030) and prallethrin survivability rate and GST (r = -0.642; P = 0.045) were recorded

Table 1. KT_{50} and resistance ratio (RR) of *Aedes aegypti* adults against prallethrin 15.0 mg/mat with piperonyl butoxide 18.0 mg/mat, dimefluthrin 7.4 mg/mat, prallethrin 15.0 mg/mat and d- allethrin 40.0 mg/mat

Strain	Active ingredients							
	prallethrin with piperonyl butoxide		dimefluthrin		prallethrin		d- allethrin	
	KT_{50} (min) (95% CL)	RR	KT_{50} (min) (95% CL)	RR	KT_{50} (min) (95% CL)	RR	KT_{50} (min) (95% CL)	RR
Reference	0.39 (0.34-0.44)	–	1.35 (0.86-1.88)	–	0.91 (0.80-1.03)	–	0.38 (0.33-0.43)	–
Sabak Bernam	4.39 (3.83-4.95)	11.26	3.17 (2.84-3.47)	2.35	10.77 (9.70-11.81)	11.84	4.22 (3.46-4.98)	11.11
Kuala Selangor	7.37 (6.61-8.13)	18.90	2.01 (1.59-2.41)	1.49	7.61 (6.73-8.50)	8.36	6.63 (5.89-7.37)	17.45
Hulu Selangor	13.06 (11.71-14.35)	33.49	4.41 (4.09-4.70)	3.27	6.58 (5.94-7.22)	7.23	8.93 (8.01-9.83)	23.50
Gombak	6.62 (5.81-7.41)	16.97	3.05 (2.06-3.84)	2.26	27.67 (25.28-30.34)	30.41	8.29 (7.45-9.11)	21.82
Petaling	4.97 (4.43-5.51)	12.74	6.02 (5.42-6.62)	4.46	10.30 (9.59-10.99)	11.32	4.05 (3.52-4.61)	10.66
Hulu Langat	5.35 (4.03-6.65)	13.72	3.11 (2.88-3.34)	2.30	37.07 (33.63-41.32)	40.74	14.20 (12.88-15.50)	37.37
Kuala Langat	7.62 (6.79-8.46)	19.54	2.50 (2.28-2.70)	1.85	31.55 (29.83-33.44)	34.67	23.46 (21.78-25.21)	61.74
Klang	2.56 (2.17-2.95)	6.56	1.44 (1.31-1.57)	1.07	8.44 (7.27-9.57)	9.27	2.72 (1.85-3.63)	7.16
Sepang	3.19 (2.72-3.64)	8.18	2.67 (2.43-2.91)	1.98	8.81 (7.96-9.64)	9.68	3.38 (3.16-3.80)	8.89

CL – confidence limit. CL does not overlap with the reference strain are significantly different from the reference strain.

Table 2. Percentages of knockdown and mortality of *Aedes aegypti* adults against prallethrin 15.0 mg/mat with piperonyl butoxide 18.0 mg/mat, dimefluthrin 7.4 mg/mat, prallethrin 15.0 mg/mat and d- allethrin 40.0 mg/mat

Strain	Knockdown				Mortality			
	prallethrin with piperonyl butoxide	dimefluthrin	prallethrin	d- allethrin	prallethrin with piperonyl butoxide	dimefluthrin	prallethrin	d- allethrin
	15.0 mg/mat with 18.0 mg/mat	7.4mg/mat	15.0 mg/mat	40.0 mg/mat	15.0 mg/mat with 18.0 mg/mat	7.4 mg/mat	15.0 mg/mat	40.0mg/mat
Reference	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Sabak Bernam	96.00	100.00 [a]	81.33	92.00	[R] 88.00	92.00	[R] 72.00	92.00
Kuala Selangor	89.33	100.00 [b]	84.00	89.33	93.33	93.33	[R] 74.67	100.00
Hulu Selangor	84.00	100.00 [c]	89.33	82.67	[R] 80.00 [a]	[R] 73.33 [a]	97.33	92.00
Gombak	90.67	100.00 [d]	58.67	92.00	96.00	92.00	96.00	100.00
Petaling	92.00	96.00 [abcdefgh]	90.67	96.00	100.00	93.33	96.00	100.00
Hulu Langat	80.00	100.00 [g]	50.67	84.00	90.67	[R] 88.00	[R] 76.00	96.00
Kuala Langat	84.00	100.00 [f]	72.00	76.00	[R] 69.33 [a]	[R] 89.33	[R]76.00	94.67
Klang	97.33	100.00 [e]	81.33	85.33	[R] 81.33	[R] 86.67	92.00	[R] 85.33
Sepang	98.67	100.00 [h]	86.67	100.00	97.33	100.00 [a]	96.00	100.00
One-way ANOVA	*P* = 0.421	*P* = 0.020	*P* = 0.097	*P* = 0.448	*P* = 0.010	*P* = 0.020	*P* = 0.067	*P* = 0.580
	F = 1.076	*F* = 3.000	*F* = 1.984	*F* = 1.035	F = 4.465	*F* = 2.214	*F* = 2.994	*F* = 0.852
	df = (9,20)	df = (9,20)	df = (9,20)	df = (9,20)	df = (9,20)	df = (9,20)	df = (9,20)	df = (9,20)

Means followed by a different letter were significantly different, $P < 0.05$, Tukey's test. R = resistant (mortality < 90%) and S = susceptible (mortality > 98%) as determined by WHO (2016). Knockdown rate was determined after 60-min exposure; mortality was calculated 24 h post-exposure.

Table 3. Mean (±SE) level of non-specific esterases (α-and β-EST), glutathione-S-transferase (GST) and mixed function oxidases (MFO) activities of *Aedes aegypti* sampled from different localities in Selangor

Strain	α-ESTs		β -ESTs		GSTs		MFOs	
	(α-Na çmol/min/ mg protein)	ER	(β-Na çmol/ min/mg protein)	ER	(CDNA-çmol/min /mg protein)	ER	(Absorbance 630nm)	ER
Reference	0.14 ± 0.01	–	0.13 ± 0 .02	–	0.07 ± 0.01	–	0.21 ± 0.04	–
Sabak Bernam	*0.22 ± 0.01 [gkpqr]	1.57	*0.21 ± 0.01 [fk]	1.62	*0.11 ± 0.00 [bekno]	1.57	*0.35 ± 0.02 [dpst]	1.67
Kuala Selangor	*0.20 ± 0.01 [abc]	1.43	*0.20 ± 0.01 [ab]	1.54	*0.09 ± 0.00 [abcd]	1.29	*0.79 ± 0.03 [abcdefgh]	3.76
Hulu Selangor	0.14 ± 0.00 [cfimqs]	1.00	0.13 ± 0.00 [behk]	1.00	*0.09 ± 0.00 [inps]	1.29	*0.37 ± 0.01 [fquwx]	1.76
Gombak	*0.29 ± 0.03 [beklmno]	2.07	*0.27 ± 0.03 [acdfghij]	2.08	0.08 ± 0.00 [hklm]	1.14	*0.50 ± 0.02 [cimpqr]	2.38
Petaling	*0.22 ± 0.01 [nst]	1.57	*0.18 ± 0.01 [i]	1.38	0.08 ± 0.00 [dgjoqst]	1.14	0.25 ± 0.02 [gkrvwy]	1.19
Hulu Langat	*0.20 ± 0.01 [def]	1.43	*0.17 ± 0.00 [c]	1.31	*0.09 ± 0.00 [efg]	1.29	*0.37 ± 0.05 [aijkl]	1.76
Kuala Langat	*0.17 ± 0.00 [hl]	1.21	*0.17 ± 0.01 [g]	1.31	*0.12 ± 0.00 [cflpqr]	1.71	*0.52 ± 0.02 [ejnsuv]	2.48
Klang	0.16 ± 0.00 [jort]	1.14	0.16 ± 0.01 [j]	1.23	*0.10 ± 0.00 [mrt]	1.43	*0.53 ± 0.01 [hlotxy]	2.52
Sepang	*0.27 ± 0.01 [adghijp]	1.93	*0.21 ± 0.01 [de]	1.62	*0.10 ± 0.00 [ahij]	1.43	*0.32 ± 0.01 [bmno]	1.52

SE = standard error; ER = enzyme ratio. Mean followed by a different letter were significantly different, $P < 0.05$, Tukey's test.
*Significant increase in mean differences compared to the laboratory reference strain, $P < 0.05$, t-test.

(Table 4). Besides, an association between α-esterase and β-esterase activity ($r = 0.927$; $P = 0.0001$) was also determined (Figure 2.). Table 5 shows a summary of insecticide resistance and the detoxification mechanism in various populations of *Ae aegypti*. Increased levels of all enzyme activities were found in five populations (i.e., Sabak Bernam, Kuala Selangor, Hulu Langat, Kuala Langat, and Sepang).

DISCUSSION

Dengue prevention and control largely depend on insecticide-based strategies. Previous studies showed that *Ae. aegypti* populations in Malaysia were resistant to pyrethroids. Notably, these *Ae. aegypti* populations were unrelenting in demonstrating the endless evolution of resistance to a wide variety of pyrethroids. The 10-year studies suggested that a specific class of insecticides remains the cornerstone of the mosquito control program. Resistance identification in *Ae. aegypti* populations were found to be consistent with the vast majority of findings from previous studies on mat vaporizer (Chadwick & Lord, 1977; Yap *et al*., 1995; Adanan *et al*., 2005;) and mosquito coil (Jantan *et al*., 1999; Liu *et al*., 2003; El-garj *et al*., 2015; Chin *et al*., 2017; Amelia-Yap *et al*., 2018a).

Prolonged use of pyrethroids on *Ae. aegypti* has resulted in the occurrence of pyrethroid resistance. The use of rapid-acting insecticides for vector control may confer a high selection pressure which could support the survivability of resistant mosquitoes (Chin *et al*., 2017; Amelia-Yap *et al*., 2018a). In this study, most *Ae. aegypti* showed their recovery

Table 4. Spearman's rank order correlation between survivability rates in pyrethroid adult bioassays against nonspecific estrease (α- and β-esterases), glutathione-S-tranferase (GST) and mixed function oxidase (MFO) activities in different *Aedes aegypti* populations in Selangor

Strain	Active ingredients							
	prallethrin with piperonyl butoxide		dimefluthrin		prallethrin		d- allethrin	
	r	P	r	P	r	P	r	P
α-esterases	0.365	0.300	0.312	0.380	-0.228	0.527	0.428	0.217
β-esterases	0.291	0.415	0.372	0.289	-0.415	0.233	0.398	0.254
GSTs	-0.683	0.030	-0.316	0.374	-0.642	0.045	-0.569	0.086
MFOs	-0.608	0.062	0.502	0.140	-0.560	0.092	-0.324	0.360

to all types of mosquito mat vaporizers at 24-h post-exposure. In natural settings, the mosquito mat vaporizer possibly acts as a spatial repellent that inhibits insects rather than kills them (Kawada, 2009; Chin *et al*., 2017). This observation may occur owing to the high frequency of dengue vector control in Malaysia. The ability of wild mosquito populations to become resistant to insecticides containing pyrethroid were documented elsewhere (Chin *et al*., 2017; Amelia-Yap *et al*., 2018b; Sathantriphop *et al*., 2019; Sayono *et al*., 2019).

In this study, *Ae. aegypti* collected from different study sites displayed varying patterns of resistance to the four active ingredients of the pyrethroid evaluated. The increased resistance degree of *Ae. aegypti* to pyrethroid at the study sites were predictable because the samples were collected from dengue hotspot areas in which dengue control is highly dependent on pyrethroid insecticides. The observation has been documented in various countries, e.g., Malaysia (Ishak *et al*., 2017; Leong *et al*., 2018; Rasli *et al*., 2018), Thailand (Pethuan *et al*., 2007; Katsuda *et al*., 2008; Chuaycharoensuk *et al*., 2011; Chareonviriyaphap *et al*., 2013, Sathantriphop *et al*., 2020), Singapore (Koou *et al*., 2014a, 2014b; Lee *et al*., 2014) and Indonesia (Hamid *et al*., 2017, 2018; Haziqah-Rashid *et al*., 2019; Triana *et al*., 2019). Likewise, a state level study on *Ae. aegypti* showed that these populations were highly resistant to mosquito coils containing pyrethroids with resistance ratios up to 122.87 (Chin *et al*., 2017).

Following a 24-h post-treatment period, gradual recovery was observed in the insecticidal-free setting, suggesting that the knockdown impact could be a transient effect for the species. The variance between the percentage of knockdown and mortality rate might suggest knockdown resistance where the insecticide pressure is removed and recovery is observed. Mosquito mat vaporizer might function as a space repellent to inhibit within insecticide radius, rather than kill (Bibbs & Kaufman, 2017). However, the impact of other insecticide use on certain pests such as houseflies (Bong & Zairi, 2010) and cockroaches (Lee *et al*., 1996) should not be disregarded.

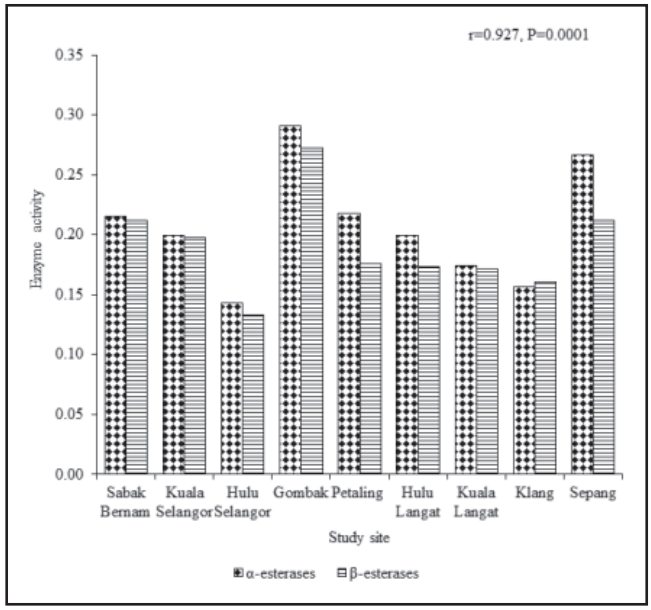

Figure 2. Spearman rank-order correlation between the activity of α-esterases and β-esterases in *Aedes aegypti.*

Table 5. Summary of insecticide susceptibility and prevalence of resistance mechanisms in different *Aedes aegypti* populations in Selangor

Strain	Insecticide susceptibility				Elevated enzyme activity			
	prallethrin + PBO	dimefluthrin	prallethrin	d-allethrin	α-EST	β-EST	GST	MFO
Sabak Bernam	R	M	R	M	+	+	+	+
Kuala Selangor	M	M	R	S	+	+	+	+
Hulu Selangor	R	R	M	M	–	–	+	+
Gombak	M	M	M	S	+	+	–	+
Petaling	S	M	M	S	+	+	–	–
Hulu Langat	M	R	R	M	+	+	+	+
Kuala Langat	R	R	R	M	+	+	+	+
Klang	R	R	M	R	–	–	+	+
Sepang	M	S	M	S	+	+	+	+

* prallethrin 15.0 mg/mat with piperonyl butoxide 18.0 mg/mat, dimefluthrin 7.4 mg/mat, prallethrin 15.0 mg/mat, d-allethrin 40.0 mg/mat, α-EST = α-esterases, β-EST = β-esterases, MFO = mixed function oxidases, GST = glutathione-S-transferase, R = resistant, M = moderate resistant, S = susceptible, + = presence of mechanism, – = absence of mechanism.

The results showed that pyrethroid-resistance was found in all of the targeted populations, suggesting that pyrethroid has been applied in the study areas for a lengthy period. These area have been affected by dengue outbreak in recent years as highlighted by Leong *et al.* (2018, 2019). The finding implied the existence of a regional extension of the population where resistant mosquitoes might move away from previous dengue hotspots. Dimefluthrin, as observed in this study, might stimulate some knockdown activity, thus has higher effectiveness compared to prallethrin with PBO, prallethrin and d-allethrin. It has been experimentally demonstrated that dimefluthrin could have faster knockdown activity against *Cx. pipiens pallens* and *Cx. quinquefasciatus* compared to d-allethrin (Mori, 2017).

In this study, the mosquito mat vaporizer showed effectiveness with high knockdown across all populations tested except Petaling. A lack of efficacy shown in Petaling population is expected as insecticide resistance of *Ae. aegypti* was reported in this area (Besar *et al*., 2019). There were comparative differences between knockdown and mortality rate in this study where d-allethrin recorded the highest mortality rate which was comparable to the finding by Kudom (2020). In the present study, mat vaporizer containing d-allethrin was found to be high insecticidal activity for *Ae. aegypti*.

The percentage on knockdown rate of *Ae. aegypti* to prallethrin with PBO was higher compared to prallethrin, suggesting PBO could provide enhanced protection at this concentration. Previous studies in different parts of the world showed the efficacy of PBO in the management of insecticide resistant vectors (Bingham *et al*., 2011; Fagbohun *et al*., 2020; Kasai *et al*., 2014). However, after 60 minutes of exposure, the mortality rate of prallethrin with PBO decreased in *Ae. aegypti*, nearly similar with mortality rate of prallethrin. When this point reached, such resistant mosquitoes were predicted to be survived. Likewise, similar results were also reported in Rasli *et al*. (2021). Therefore, those populations characterised with high or moderate MFO enzyme activities with less or no impact of PBO along with insecticide pyrethroids, should be further investigated, especially on the significance of *kdr* gene in the development of insecticide resistance.

There were significant associations between knockdown rates of the active ingredients, suggesting cross-resistance in pyrethroid. The cross-resistance arises as those mechanisms overlap due to insecticide strain (Kawada, 2009). Presumably, single pyrethroid insecticide tolerance was suspected of causing cross-resistance to other insecticides in specific class (Du *et al*., 2016). Significant correlations between prallethrin with PBO and dimefluthrin, prallethrin with PBO prallethrin and d-allethrin as well as dimefluthrin and d-allethrin were found from the present study. Detection of cross-resistance in pyrethroid in *Ae. aegypti* was not only confined to Malaysia (Chin *et al*., 2017), as it was also reported in many countries, i.e., Colombia (Ocampo *et al*., 2011; Aponte *et al*., 2018), Mexico (Flores *et al*., 2013), Thailand (Yaicharoen *et al*., 2005) and Indonesia (Amelia-Yap *et al*., 2018a). The relentless incidence of cross-resistance in mosquito mat vaporizer tested might help the local authorities to review the effectiveness of mat vaporizer in the control of mosquitoes.

The recent formulations of the new pyrethroid group of insecticides are d-allethrin, prallethrin, dimefluthrin and metofluthrin (Mori, 2017). Other available household insecticide products apart from mosquito mat vaporizers such as liquid vaporizers, aerosols and coils were widely commercialized, and they were easily accessible in Malaysian markets. The current study showed that most *Ae. aegypti* populations were resistant to mosquito mat vaporizers. It may be due to the endophilic nature of *Ae. aegypti*, which makes it prone to be subjected to, or in touch with, the chemical produced by these materials and build resistance by selection pressure (Carvalho & Moreira, 2017).

This study showed the need to alternate various chemicals such as metofluthrin, transfluthrin or d-transallethrin in specific locations. The use of pyrethroids in *Ae. aegypti* pyrethroid-resistant areas should be monitored by follow up studies and management practices should be amended. The results presented may lead to the evaluation of the susceptibility data to be referred by local authorities in determining effective vector control program. There is a possibility that such chemicals may not yield optimal mortality responses for all strains for end-user as the bioassays were carried out under experimental conditions. Hence it is recommended that a semi-field trial at the natural end-user setting to be conducted in future.

Meanwhile, the enzyme assays revealed that only some detoxifying enzymes (i.e., ESTs, GSTs and MFOs) were expressed in pyrethroid-resistant *Ae. aegypti*. Earlier researches reported the involvement of these enzymes in the contribution of pyrethroid resistance in wild *Ae. aegypti* (Leong *et al*., 2019; Pinto *et al*., 2019; Wan-Norafikah *et al*., 2010). The variation may indicate that there were multiple resistance mechanisms in *Ae. aegypti*.

Various studies have shown that the increased EST activity generally resulted from pyrethroid-resistant *Ae. aegypti* (Lin *et al*., 2013; Koou *et al*., 2014b; Rasli *et al*., 2018) and *Cx. quinquefasciatus* Say (Diptera: Culicidae) (Sarkar *et al*., 2009; Singh & Prakash, 2009; Low *et al*., 2013b; Ramkumar & Shivakumar, 2015). Their results were inconsistent with the present study, which did not reveal any correlation related to the survivability rate of all insecticides analyzed against α-EST activity. However, some pyrethroid-susceptible populations of the field strain demonstrated higher enzyme levels compared to the reference strain. Dichlorodiphenyltrichloroethane (DDT) resistance may have contributed to this detoxification activity (Maestre-Serrano *et al*., 2014), but this hypothesis is yet to be verified.

Additionally, this study revealed a significant association between the survivability rate of pyrethroids in mosquito vaporizing mat bioassays and GST in *Ae. aegypti*. There were concerns that this enzyme might not be prevalent due to pyrethroid resistance. Reasonably, the GST enzyme documented the lowest levels in contrast to other groups of enzymes, including those found in the resistant populations. The mean enzyme activities of GST was inversely correlated to the 24-h percentage mortality of *Ae. aegypti* to prallethrin with PBO and prallethrin, indicating lower mortality rate with increasing activities of GST in this study. Hemingway & Ranson (2000) and Ishak *et al*. (2017) reported that higher rates of GST activity were typically correlated with the exposure to multiple insecticide classes within a large kind of arthropods, primarily due to DDT resistance.

Previous studies also attempted to identify the mechanism of GSTs in DDT resistance in *Anopheles gambie* and *An. funestus* (Matiya *et al*., 2019), *An. maculatus* (Rohani *et al*., 2019), *Ae. aegypti* (Aponte *et al*., 2018) and *Cx. quinquefasciatus* (Lee & Chong, 1995; Corbel *et al*., 2007; Sarkar *et al.*, 2009; Low *et al.*, 2013a, 2013b). To date, associations between insecticide resistance and GST enzyme activity have not been fully identified in a variety of mosquito species worldwide (Amelia-Yap *et al*., 2019). The detectable GST activity might be owing to the use of pyrethroids in mosquito

control activities as both DDT and pyrethroids intended to target the voltage-gated sodium channel of arthropods (Amelia-Yap *et al*., 2018b, 2019; Hemingway & Ranson, 2000; Koou *et al.*, 2014b). Thus, it assumed that pyrethroid-resistant identified in this study was related to the metabolic detoxification or/and target-site insensitivity. Nevertheless, as mentioned above, the role of GSTs has been restricted in most of the populations with low enzyme activities. Therefore, the used of DDT diagnostic doses of WHO adult bioassay would be recommended in order to explain the significant increased in the production of GST in the studied populations.

Meanwhile, the enzyme assay indicated an increasing level of MFO in *Ae. aegypti* populations, suggesting MFOs as primary enzymes that stimulated the pyrethroid resistance. Increased levels of MFOs in *Ae. aegypti* related to pyrethroids resistance were reported elsewhere (Maestre-Serrano *et al*., 2014; Rasli *et al*., 2018; Triana *et al*., 2019; Amelia-Yap *et al*., 2019). Nonetheless, the increased MFO activity in most mosquito populations might reduce the efficacy of insecticides. The MFOs enzymes are most commonly correlated with cross-resistance between pyrethroids and organophosphates (Pethuan *et al*., 2007) and DDT (Ngoagouni *et al*., 2016). Notably, this emphasized the value to evaluate the organophosphate and DDT resistance status in these populations in the years ahead.

A higher RR of the active ingredients tested did not show a consistent activity profile in all enzyme groups, indicating complexity between pyrethroids and enzymes. Thus, metabolic detoxification could not fully explain pyrethroid's elevated resistance status. Several point mutations have been recognized such as F1534C, V1016 G and S989P, homozygous mutations V1016G / S989P (double allele) and F1534C / V1016G / S989P (triple allele) in various dengue vector populations (Leong *et al*., 2019). Inevitable factors for higher pyrethroid-resistant in wild *Ae. aegypti*, e.g. behavioural inhibition, cuticle tolerance or target-site insensitivity, were also anticipated (Amelia-Yap *et al*., 2018b). Further research on synergists would give valuable information on mechanisms of metabolic-mediated resistance.

CONCLUSION

In conclusion, majority of *Ae. aegypti* populations in this study have developed resistance to mosquito mat vaporizer containing pyrethroids. This result revealed that pyrethroid resistance has thrived in this country due to the high dependence on vector control. This study also provided reference data and underlined the need for detailed studies on metabolic resistance in *Ae. aegypti*. In future, resistance might gradually build-up on the susceptible populations if the same control approach was used. Thus, this result urgently suggests reconstructing the national vector control programme in order to monitor the efficacy of pyrethroid against *Ae. aegypti*.

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Conflict of interest

The authors declare there is no conflict of interest.

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