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

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\textbf{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). \textit{Aedes aegypti} and \textit{Ae. albopictus} are responsible for the transmissions of dengue and chikungunya viruses in Malaysia. \textit{Aedes aegypti} is the primary dengue vector which lives close to humans in urban surroundings, whereas \textit{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 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 \textit{Ae. aegypti}, however, has been understudied. Essentially, this work seeks to examine the susceptibility of \textit{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.

\textbf{MATERIALS AND METHODS}

\textbf{Study sites} \textit{Aedes aegypti} eggs were collected using ovitraps from nine districts: Sabak Bernam, Kuala Selangor, Hulu Selangor,
Preparation of ovitrap and sample collection

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), 
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 
cage 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. (1998) 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 
than 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 
icubation, 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 
(KT50) was calculated by using probit analysis with SPSS 
software (version 20) (Finney, 1971). Resistance ratios were 
calculated using the following formula from

\[ RR = \frac{KT50 \text{ of reference strain}}{KT50 \text{ of field strain}} \]

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 
exclude 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%: 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<sub>50</sub> 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<sub>50</sub> of field population exposed to prallethrin with PBO, dimefluthrin, prallethrin and d-allethrin ranged from 2.56 to 13.06 minutes (the longest KT<sub>50</sub> population: Hulu Selangor); 1.44 to 4.41 minutes (the longest KT<sub>50</sub> population: Kuala Selangor); 2.72 to 23.46 minutes (the longest KT<sub>50</sub> 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: Kuala 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 Langat, 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, Kuala 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 Kuala 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, Kuala 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<sub>50</sub> 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>prallethrin with piperonyl butoxide</th>
<th>dimefluthrin</th>
<th>prallethrin</th>
<th>d-allethrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>KT&lt;sub&gt;50&lt;/sub&gt; (min) (95% CL)</td>
<td>RR</td>
<td>KT&lt;sub&gt;50&lt;/sub&gt; (min) (95% CL)</td>
<td>RR</td>
</tr>
<tr>
<td>Sabak Bernam</td>
<td>4.39 (3.83-4.95)</td>
<td>11.26</td>
<td>3.17 (2.84-3.47)</td>
<td>2.35</td>
</tr>
<tr>
<td>Kuala Selangor</td>
<td>7.37 (6.61-8.13)</td>
<td>18.90</td>
<td>2.01 (1.59-2.41)</td>
<td>1.49</td>
</tr>
<tr>
<td>Hulu Selangor</td>
<td>13.06 (11.71-14.35)</td>
<td>33.49</td>
<td>4.41 (4.09-4.70)</td>
<td>3.27</td>
</tr>
<tr>
<td>Gombak</td>
<td>6.62 (5.81-7.41)</td>
<td>16.97</td>
<td>3.05 (2.06-3.84)</td>
<td>2.26</td>
</tr>
<tr>
<td>Petaling</td>
<td>4.97 (4.43-5.51)</td>
<td>12.74</td>
<td>6.02 (5.42-6.62)</td>
<td>4.46</td>
</tr>
<tr>
<td>Hulu Langat</td>
<td>5.35 (4.03-6.65)</td>
<td>13.72</td>
<td>3.11 (2.88-3.34)</td>
<td>2.30</td>
</tr>
<tr>
<td>Kuala Langat</td>
<td>7.62 (6.79-8.46)</td>
<td>19.54</td>
<td>2.50 (2.28-2.70)</td>
<td>1.85</td>
</tr>
<tr>
<td>Klang</td>
<td>2.56 (2.17-2.95)</td>
<td>6.56</td>
<td>1.44 (1.31-1.57)</td>
<td>1.07</td>
</tr>
<tr>
<td>Sepang</td>
<td>3.19 (2.72-3.64)</td>
<td>8.18</td>
<td>2.67 (2.43-2.91)</td>
<td>1.98</td>
</tr>
</tbody>
</table>

CL – confidence limit. CL does not overlap with the reference strain are significantly different from the reference strain.
Notably, these populations in Malaysia were resistant to pyrethroids. (Figure 2). Table 5 shows a summary of insecticide resistance as determined by WHO (2016). Knockdown rate was determined after 60-min exposure; mortality was calculated 24 h post-exposure.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Knockdown</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.0 mg/mat</td>
<td>7.4 mg/mat</td>
</tr>
<tr>
<td>Reference</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Sabak Bernam</td>
<td>96.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Kuala Selangor</td>
<td>89.33</td>
<td>100.00</td>
</tr>
<tr>
<td>Hulu Selangor</td>
<td>84.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Gombak</td>
<td>90.67</td>
<td>100.00</td>
</tr>
<tr>
<td>Petaling</td>
<td>92.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Hulu Langat</td>
<td>80.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Kuala Langat</td>
<td>84.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Klang</td>
<td>97.33</td>
<td>100.00</td>
</tr>
<tr>
<td>Sepang</td>
<td>98.67</td>
<td>100.00</td>
</tr>
</tbody>
</table>

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 ($\alpha$- and $\beta$-EST), glutathione-S-transferase (GST) and mixed function oxidases (MFO) activities of Aedes aegypti sampled from different localities in Selangor

<table>
<thead>
<tr>
<th>Strain</th>
<th>$\alpha$-ESTs</th>
<th>$\beta$-ESTs</th>
<th>GSTs</th>
<th>MFOs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($\alpha$-Na ñmol/min</td>
<td>ER</td>
<td>($\beta$-Na ñmol/min</td>
<td>ER</td>
</tr>
<tr>
<td>Reference</td>
<td>0.14 ± 0.01</td>
<td>–</td>
<td>0.13 ± 0.02</td>
<td>–</td>
</tr>
<tr>
<td>Sabak Bernam</td>
<td>*0.22 ± 0.01</td>
<td>1.57</td>
<td>*0.21 ± 0.01</td>
<td>1.62</td>
</tr>
<tr>
<td>Kuala Selangor</td>
<td>*0.20 ± 0.01</td>
<td>1.43</td>
<td>*0.20 ± 0.01</td>
<td>1.54</td>
</tr>
<tr>
<td>Hulu Selangor</td>
<td>0.14 ± 0.00</td>
<td>1.00</td>
<td>0.13 ± 0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Gombak</td>
<td>*0.29 ± 0.03</td>
<td>2.07</td>
<td>*0.27 ± 0.03</td>
<td>2.08</td>
</tr>
<tr>
<td>Petaling</td>
<td>*0.22 ± 0.01</td>
<td>1.57</td>
<td>*0.18 ± 0.01</td>
<td>1.38</td>
</tr>
<tr>
<td>Hulu Langat</td>
<td>*0.20 ± 0.01</td>
<td>1.43</td>
<td>*0.17 ± 0.00</td>
<td>1.31</td>
</tr>
<tr>
<td>Kuala Langat</td>
<td>*0.17 ± 0.00</td>
<td>1.21</td>
<td>*0.17 ± 0.01</td>
<td>1.31</td>
</tr>
<tr>
<td>Klang</td>
<td>0.16 ± 0.00</td>
<td>1.14</td>
<td>0.16 ± 0.00</td>
<td>1.23</td>
</tr>
<tr>
<td>Sepang</td>
<td>*0.27 ± 0.01</td>
<td>1.93</td>
<td>*0.21 ± 0.01</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Means 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.

**DISCUSSION**

Dengue prevention and control largely depend on insecticide-based strategies. Previous studies showed that Aedes aegypti populations in Malaysia were resistant to pyrethroids. Notably, these Aedes 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 Aedes 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., 1995; Adanan et al., 1999; Liu et al., 2003; El-garj et al., 2015; Chin et al., 2017; Amelia-Yap et al., 2018).

Prolonged use of pyrethroids on Aedes 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 Aedes aegypti showed their recovery...
Table 4. Spearman’s rank order correlation between survivability rates in pyrethroid adult bioassays against nonspecific esterases (α- and β-esterases), glutathione-S-transferase (GST) and mixed function oxidase (MFO) activities in different *Aedes aegypti* populations in Selangor

<table>
<thead>
<tr>
<th>Strain</th>
<th>Active ingredients</th>
<th>r</th>
<th>P</th>
<th>r</th>
<th>P</th>
<th>r</th>
<th>P</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>prallethrin with piperonyl butoxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-esterases</td>
<td>r-esterases</td>
<td>0.365</td>
<td>0.300</td>
<td>0.312</td>
<td>0.380</td>
<td>-0.228</td>
<td>0.527</td>
<td>0.428</td>
<td>0.217</td>
</tr>
<tr>
<td>β-esterases</td>
<td>r-esterases</td>
<td>0.291</td>
<td>0.415</td>
<td>0.372</td>
<td>0.289</td>
<td>-0.415</td>
<td>0.233</td>
<td>0.398</td>
<td>0.254</td>
</tr>
<tr>
<td>GSTs</td>
<td>r-esterases</td>
<td>-0.683</td>
<td>0.030</td>
<td>-0.316</td>
<td>0.374</td>
<td>-0.642</td>
<td>0.045</td>
<td>-0.569</td>
<td>0.086</td>
</tr>
<tr>
<td>MFOs</td>
<td>r-esterases</td>
<td>-0.608</td>
<td>0.062</td>
<td>0.502</td>
<td>0.140</td>
<td>-0.560</td>
<td>0.092</td>
<td>-0.324</td>
<td>0.360</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Insecticide susceptibility</th>
<th>Elevated enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>prallethrin + PBO</td>
<td>dimefluthrin</td>
</tr>
<tr>
<td>Sabak Bernam</td>
<td>R</td>
<td>M</td>
</tr>
<tr>
<td>Kuala Selangor</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Hulu Selangor</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Gombak</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Petaling</td>
<td>S</td>
<td>M</td>
</tr>
<tr>
<td>Hulu Langat</td>
<td>M</td>
<td>R</td>
</tr>
<tr>
<td>Kuala Langat</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Klang</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Sepang</td>
<td>M</td>
<td>S</td>
</tr>
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</table>

* 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. Dimethoat, 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 dimethoat could have faster knockdown activity against Cx. piperi palls 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 might 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-allethrin in specific locations. The use of pyrethroid 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 reports revealed that 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; Kooi 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 gambi 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 vehicles.
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 (Petuhan 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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