

**EFFECT OF XENOBIOTIC CHALLENGE ON ENZYMATIC ACTIVITY OF  
DETOXIFICATION ENZYMES AT DIFFERENT EXPOSURE PERIODS IN  
*Aedes albopictus* (SKUSE) (DIPTERA: CULICIDAE)**

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**ABSTRACT**

The xenobiotics including insecticides such as malathion and permethrin induce the activities of detoxification enzymes and potentially leading to the development of resistance. In this study, biochemical analysis was used to characterize the time-dependent malathion and permethrin induction profile of Glutathione S-transferase (GST),  $\alpha$ -esterase ( $\alpha$ -est),  $\beta$ -esterase ( $\beta$ -est), Cytochrome P450 (Cyt P450) and Acetylcholinesterase (AChE), enzymes which are known to contribute to metabolic resistance in *Aedes albopictus*. Time-dependent induction of early fourth instar larvae with the sub-lethal concentration (LC<sub>50</sub>) of malathion (0.099 mg/L) and permethrin (0.0023 mg/L) was done at 6, 12 and 24 hours to observe the effect on the enzymatic activity under toxicological challenges. Total protein content of larvae was most elevated when the larvae were exposed to both insecticides for 24 hours. The level of total enzyme activity and specific activity of GST, as well as Cyt P450 were found to be most elevated whereas the level of  $\alpha$ -est and  $\beta$ -est total enzyme and specific activity were decreased at 24 hours of treatment with malathion. A different pattern was observed for permethrin induction whereby the total enzyme and specific activity of all enzymes except Cyt P450 were highly elevated upon 24 hours of acute exposure. The level of total enzyme activity and specific activity of almost all enzymes upon acute induction with malathion and permethrin were statistically significant ( $p < 0.05$ ) when compared between the induced hours and to its susceptible strain. Conclusively, these findings indicate that the continuous and prolonged exposure to sub-lethal concentration of malathion and permethrin influenced the induction of GST,  $\alpha$ -est,  $\beta$ -est, Cyt P450 as well as AChE enzymatic activities.

**Keywords:** *Aedes albopictus*, detoxification enzymes, enzymatic activities, metabolic induction, xenobiotic challenge.

**ABSTRAK**

Insektisid seperti malation dan permetrin ialah xenobiotik yang menggalakkan aktiviti enzim pengnyahtoksikan dan berpotensi untuk menyebabkan perkembangan kerintangan metabolik. Dalam kajian ini, analisis biokimia telah dijalankan untuk mengelaskan profil induksi enzim (GST,  $\alpha$ -est,  $\beta$ -est, Cyt P450 dan AChE) yang menyumbang kepada kerintangan metabolik

*Aedes albopictus* apabila didedahkan secara berkala dengan malation dan permetrin. Proses pendedahan akut secara berkala untuk instar keempat larva *Ae. albopictus* dengan dos subletal ( $LC_{50}$ ) malation (0.099 mg/L) dan permetrin (0.023 mg/L) dijalankan selama 6 jam, 12 jam dan 24 jam untuk memerhatikan tindak balas aktiviti enzim apabila didedahkan dengan cabaran toksik insektisid. Jumlah kandungan protein larva yang paling tinggi dicatatkan apabila larva didedahkan dengan dos subletal ( $LC_{50}$ ) malation dan permetrin selama 24 jam. Jumlah aktiviti enzim dan jumlah aktiviti khusus enzim GST, dan Cyt P450 didapati paling tinggi manakala  $\alpha$ -est dan  $\beta$ -est mencatatkan nilai paling rendah setelah pendedahan akut dengan dos subletal ( $LC_{50}$ ) malation selama 24 jam. Corak aktiviti yang berbeza diperhatikan setelah pendedahan akut larva dengan dos subletal ( $LC_{50}$ ) permetrin selama 24 jam di mana jumlah aktiviti enzim dan jumlah aktiviti khusus enzim bagi kesemua enzim kecuali Cyt P450 berada di tahap yang paling tinggi. Walau bagaimanapun, setelah proses induksi dengan kedua insektisid, jumlah aktiviti enzim dan jumlah aktiviti khusus enzim khusus bagi kebanyakan enzim yang diuji memberikan perbezaan yang signifikan ( $p < 0.05$ ) apabila dibanding dengan masa induksi yang berbeza dan antara nilai kawalannya. Hasil kajian yang diperoleh menunjukkan bahawa pendedahan akut dengan malation dan permetrin yang berterusan dan berpanjangan akan mempengaruhi penghasilan dan aktiviti enzim GST,  $\alpha$ -est,  $\beta$ -est, Cyt P450 dan AChE.

**Kata kunci:** *Aedes albopictus*, enzim pengnyahtoksikan, aktiviti enzim, induksi metabolik, tekanan xenobiotik

## INTRODUCTION

*Aedes albopictus* mosquito, also known as Asian tiger mosquito, originates from Southeast Asia and is responsible for the transmission of arboviral as well as filarial infectious diseases of humans and animals (Gratz 2004; Grigoraki et al. 2015). Apart from its ability to transmit a broad spectrum of human pathogens, *Ae. albopictus* also acts as the secondary vector of dengue virus in Malaysia (Caminade et al. 2012; Chua et al. 2005; Rozilawati et al. 2017; WHO 2009). Due to the unavailability of a stable vaccine for the treatment of dengue, the predominant global strategy to reduce the occurrence of Dengue Fever or Dengue Hemorrhagic Fever cases and ultimately control *Aedes* mosquito abundance is focused on the control of immatures as well as adult mosquitoes (El-garj et al. 2015; Farouk et al. 2019; Guzman et al. 2010; Liu et al. 2006; Sabchareon et al. 2012; Wan-Norafikah et al. 2013).

Prevention of transmission is crucial to reduce the burden of dengue, and the only available strategy is by controlling *Aedes* mosquitoes (Vanlerberghe et al. 2009). Control of *Ae. albopictus* relies on the usage of insecticides, source reduction of larval breeding sites as well as the usage of repellents (Grigoraki et al. 2015; Koou et al. 2014; Smith et al. 2016; Wan-Norafikah et al. 2013). Pyrethroids and organophosphates are some of the insecticides used to control the population of adult mosquitoes in disease affected areas due to its effectiveness (Davies et al. 2007; Smith et al. 2016; Wan-Norafikah et al. 2013). Unfortunately, the prolonged intensive as well as extensive usage of insecticides inevitably leads to resistance towards insecticides and this is developing at an alarming rate (Sokhna et al. 2013; Pang & Loh 2016; Paiva et al. 2016; Wan-Norafikah et al. 2013). Among the other factors compromising vector control efforts are the selection pressure of insecticides and the inheritability of resistance among generations of vector populations (Li et al. 2007). Reports of resistance cases involving *Ae. albopictus* are increasing even though the amount of cases is less compared to *Ae. aegypti* (Ishak et al. 2016). There are four types of insecticide resistance mechanisms which have been developed and exhibited by mosquitoes namely metabolic-based resistance, target-

site resistance, physiological resistance and behavioural resistance (Brogdon & McAllister 1998; Hemingway et al. 2004; Kasai et al. 2014).

The biosynthesis of enzymes has been suggested to occur as a result of a direct reaction when exposed to xenobiotics (Poupardin et al. 2008). Evolution of xenobiotic resistance in insects happens primarily by increasing the metabolic capability of detoxification systems and/or reducing the sensitivity of xenobiotic target site (Li et al. 2007). Metabolic based resistance occurs when increased rate of insecticide metabolism lowers the quantity of insecticide reaching the target site (Ranson et al. 2011; Paiva et al. 2016). The most familiar metabolic resistance mechanisms involve four main enzymes namely glutathione s-transferases,  $\alpha$ -est and  $\beta$ -est, Cyt P450 as well as AChE (Amelia-Yap et al. 2018; El-garj et al. 2016ab; Ngoagouni et al. 2016; Paiva et al. 2016). Understanding the underlying insecticide resistance mechanism is crucial for the development of an effective yet sustainable integrated vector control programs (Grigoraki et al. 2015). Therefore, the aims of the present study are to verify the effect of acute time-dependent treatment with LC<sub>50</sub> of permethrin and malathion on the induction of total protein content, total enzyme activity as well as specific enzyme activity in *Ae. albopictus* larvae to determine the mechanisms of toxicological challenge in the larvae.

## MATERIALS AND METHODS

### Mosquitoes and Insecticides Treatment

An established laboratory colony (susceptible strain) of *Ae. albopictus* was supplied by Vector Control Research Unit (VCRU), Universiti Sains Malaysia. Early fourth instar of susceptible laboratory strain of *Ae. albopictus* larvae were subjected to baseline toxicity testing according to diagnostic determination protocol used by WHO (1981, 2005). Malathion (organophosphate) and permethrin (pyrethroid) were selected to be used in this study because both insecticides are the primary choice of insecticides applied in the vector control programs in Malaysia (Hamzah et al. 2019; Rozilawati et al. 2017).

In all experiments, preliminary tests were conducted to determine LC<sub>50</sub> dosages of malathion and permethrin according to WHO standard procedure (WHO 2005). Five concentrations (treatments) of each insecticide were used to determine concentration with a mortality range of 15% to 95%. A minimum of eight replicates (25 larvae each) per treatment were performed with each concentration. The data were analysed using probit analysis (SPSS software version 24) in order to determine the lethal concentration required to kill 50% of the larvae (LC<sub>50</sub>). Then, the fourth instar larvae were exposed to the insecticides according to its LC<sub>50</sub> value at different time durations namely 6, 12 and 24 hours. All treated larvae were collected and used for further analysis.

### Protein Concentration

The protein concentration in the enzyme source for all biochemical assays was determined according to Bradford (1976) using Coomassie Brilliant Blue R-250. Bovine serum albumin was used as a standard to normalize the protein activities (Hamzah & Alias 2016a).

### Enzyme Assays

Early fourth instar larvae were individually homogenized in 200  $\mu$ l of season water on ice. 25  $\mu$ l of homogenate were used for the AChE assay. The rest of the homogenate were centrifuged at 14,000 rpm, 4°C for 30 seconds, and the supernatant was used as a source of enzyme for all biochemical enzyme assays in this research. At total of 100 replicates were used per assay. All

of the assays were performed according to Hemingway (1998) which adheres to WHO procedure.

#### *Acetylcholinesterase (AChE) assay*

For each sample, 25  $\mu\text{l}$  of insect homogenate was mixed with 145  $\mu\text{l}$  of triton phosphate buffer. Then, 10  $\mu\text{l}$  of 0.01 M dithiobis 2-nitrobenzoic acid solution and 25  $\mu\text{l}$  of 0.01 M acetylthiocholine iodide were added to initiate the reaction. Two similar reactions were prepared for each sample. One reaction was allowed to progress, the other was inhibited using 0.05  $\mu\text{l}$  of 0.1 M propoxur. After one hour of incubation, the Optical Density (OD) of both reactions were measured at 405 nm. Specific AChE activity was calculated according to Beer's Law ( $A = \epsilon cl$ ) and expressed as nmol of AChE/min/mg.

#### *Non-specific esterases ( $\alpha$ -est and $\beta$ -est) Assay*

For each replicate, 20  $\mu\text{l}$  of supernatant extracted from the insect homogenate were mixed with 200  $\mu\text{l}$  of the substrate, 30 mM  $\alpha$ -naphthyl acetate. At the same time, another 20  $\mu\text{l}$  of supernatant from the same sample were also incubated with 200  $\mu\text{l}$  of 30 mM  $\beta$ -naphthyl acetate. 50  $\mu\text{l}$  of fast blue stain was added to each reaction after 15 minutes of incubation and the OD value was measured at 570 nm. The activity against each substrate was calculated from standard curves of absorbance for known concentrations of  $\alpha$ -naphthol or  $\beta$ -naphthol. Enzyme activities were expressed as nmol of  $\alpha$ -naphthol or  $\beta$ -naphthol/min/mg protein.

#### *Glutathione s-transferase (GST) assay*

A sum of 200  $\mu\text{l}$  of 10 mM reduced glutathione (GSH) and 63 mM 1-chloro-2,4-dinitrobenzene (CDNB) mixture were added to 10  $\mu\text{l}$  of supernatant which were extracted from the insect homogenate. Absorbance was determined at 340 nm after 20 minutes of incubation. The OD value (A) was transformed to  $\mu\text{mol}$  of CDNB conjugates using the extinction coefficient ( $\epsilon$ ) of  $9.6 \text{ mM}^{-1}$ . GST activity was calculated according to Beer's Law ( $A = \epsilon cl$ ) and reported as  $\mu\text{mol}$  of CDNB/min/mg protein.

#### *Cytochrome P450 (Cyt P450) titration assay*

80  $\mu\text{l}$  of 0.625 M potassium phosphate buffer (pH 7.2), 200  $\mu\text{l}$  of 3,3',5,5'-tetramethylbenzidine (TMBZ) in methanol solution, and 25  $\mu\text{l}$  of hydrogen peroxide (3%) were added to 2  $\mu\text{l}$  of supernatant derived from the insect homogenate to initiate its activity. The reaction was allowed to oxidize for 2 hours at room temperature before the OD value was read at 650 nm. Cytochrome P450 activity was calculated from standard curve of absorbance for known concentration of cytochrome C (Brogdon & McAllister 1997). Enzyme activity was expressed as nmol equivalent units of cytochrome P450/min/mg protein.

### **Statistical Analysis**

The data obtained in diagnostic bioassays were statistically analysed by using probit analysis computer program SPSS (version 24) to determine the  $LC_{50}$  values to be used as the standard dosages for this study. One-way analysis of variance (ANOVA) was used to compare the total protein content, total and specific enzyme activity levels of each enzyme between different time-dependent insecticide-induced strains including the non-induced susceptible strain as a control, at  $P=0.05$ .

## RESULTS

Results obtained from probit analysis are presented in Table 1. From the larval bioassay, the LC<sub>50</sub> of malathion established was 0.099 mg/L (0.090-0.108) while the LC<sub>50</sub> of permethrin is 0.023 mg/L (0.019-0.027).

Table 1. Sub-lethal (LC<sub>50</sub>) doses of susceptible *Aedes albopictus* larvae against permethrin and malathion.

	Population	LC <sub>50</sub> (95% CI) mg/L	Slope±SD
Permethrin	Susceptible	0.023 (0.019-0.027)	3.692±0.228
Malathion	Susceptible	0.099 (0.090-0.108)	3.482±0.223

Figure 1 indicated the changes in the total protein content ( $\mu\text{g}$ ) of larvae upon acute treatment with either malathion or permethrin. Results represent the mean of at least ten separate experiments. Comparison by using 1-way ANOVA was made between each treated hour respectively and to its control from the same insecticide only. Protein content with same letters show significant difference at  $p=0.05$ . Upon acute treatment with malathion and permethrin, the total protein content of larvae was at its highest peak at 24 hours of the acute treatment ( $p<0.05$ ). Cumulatively, there was a significant difference ( $p<0.05$ ) in the total protein content of larvae upon acute treatment with malathion as well as permethrin when compared between the treated hours and to the control respectively.

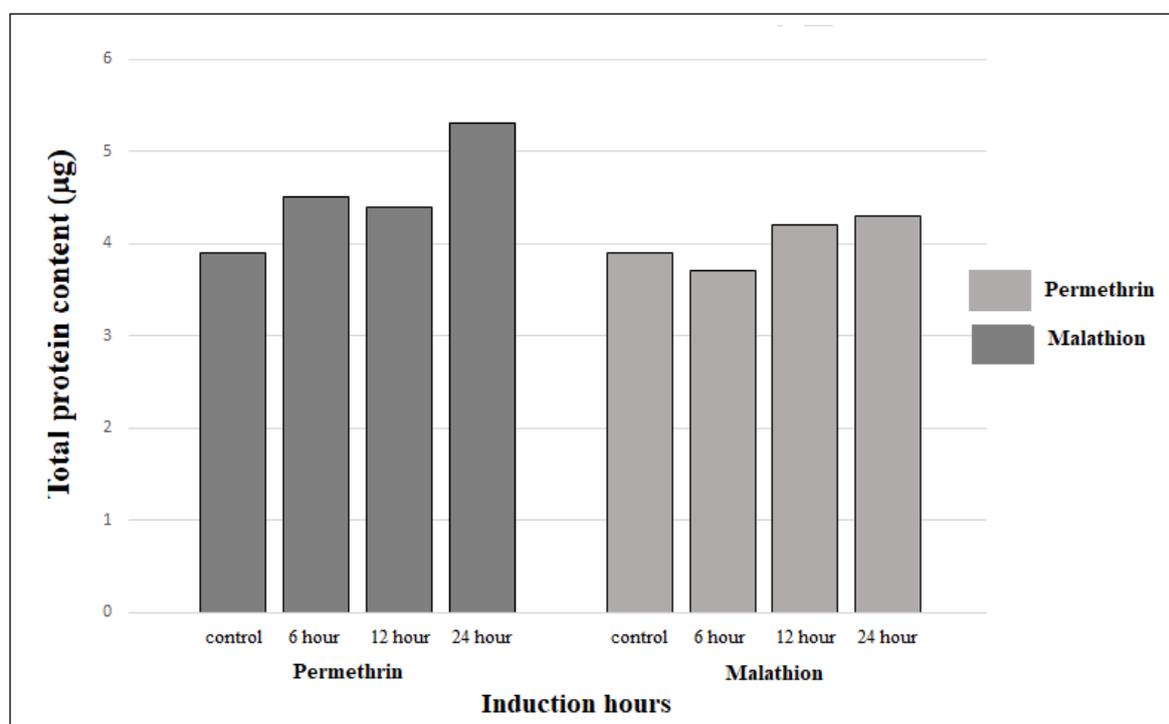


Figure 1. Total protein content ( $\mu\text{g}$ ) upon different time inductions within the same insecticide treatment (malathion or permethrin).

Results represent the averages of at least three separate experiments  $\pm$  standard error. Protein content with same letters for each type of insecticide treatment show significant difference at  $p=0.05$ .  $LC_{50}$  is the dosage resulting in 50% mortality of test insecticides.

Based on the data summarized in Table 2, total GST activity was most elevated at 12 hours and 24 hours of acute treatment with malathion, ( $p<0.05$ ). Comparison by using 1-way ANOVA was made between all induced hours and from the same treatment only,  $p=0.05$ .

Total  $\alpha$ -est activity reduced consistently from 6 hours up to 24 hours ( $p<0.05$ ), while total  $\beta$ -est activity increased after 6 hours of acute malathion treatment ( $p<0.05$ ) and then reduced comparatively until 24 hours of acute malathion treatment ( $p<0.05$ ). Total Cyt P450 activity increased gradually upon acute treatment with malathion and was at the highest peak for 24 hours of exposure ( $p<0.05$ ). A different pattern was observed for total AChE activity whereby the activity increased up to 12 hours and dropped at 24 hours of acute treatment ( $p<0.05$ ).

The total GST, AChE,  $\alpha$ -est and  $\beta$ -est activity was most elevated in larvae which has been acutely treated with permethrin for 24 hours while total Cyt P450 activity was most elevated during the 6th hour of acute treatment. Apart from that, the total enzyme activity of all test enzymes in acute permethrin treated larvae shows a significant difference ( $p<0.05$ ) when compared between different acute treated hours and to its respective control strain.

In comparison, only GST activities for 12 hours and 24 hours (malathion treatment); 6 hours and 12 hours (permethrin treatment) were not significant ( $p>0.05$ ).

Table 2. Mean of total enzyme activity  $\pm$  SE upon time dependent acute treatment with  $LC_{50}$  of malathion and permethrin

Total activity/ insecticide	Treatment duration	GST (nmol/min)	$\alpha$ -est (nmol/min)	$\beta$ -est (nmol/min)	Cyt P450 (nmol/min)	AChE (nmol/min)
Permethrin	Control	76 $\pm$ 1 <sup>a</sup>	83 $\pm$ 6 <sup>a</sup>	132 $\pm$ 8 <sup>a</sup>	18 $\pm$ 0.2 <sup>a</sup>	4.2 $\pm$ 0.03 <sup>a</sup>
	6 hours	109 $\pm$ 2 <sup>b</sup>	108 $\pm$ 1 <sup>b</sup>	169 $\pm$ 8 <sup>b</sup>	29 $\pm$ 0.2 <sup>b</sup>	4.0 $\pm$ 0.09 <sup>b</sup>
	12 hours	111 $\pm$ 2 <sup>b</sup>	102 $\pm$ 3 <sup>c</sup>	151 $\pm$ 2 <sup>c</sup>	26 $\pm$ 0.1 <sup>c</sup>	3.2 $\pm$ 0.02 <sup>c</sup>
	24 hours	141 $\pm$ 1 <sup>c</sup>	144 $\pm$ 8 <sup>d</sup>	215 $\pm$ 2 <sup>d</sup>	21 $\pm$ 0.4 <sup>d</sup>	4.6 $\pm$ 0.05 <sup>d</sup>
Malathion	Control	76 $\pm$ 4 <sup>a</sup>	83 $\pm$ 6 <sup>a</sup>	132 $\pm$ 8 <sup>a</sup>	18 $\pm$ 0.2 <sup>a</sup>	4.2 $\pm$ 0.03 <sup>a</sup>
	6 hours	88 $\pm$ 3 <sup>b</sup>	72 $\pm$ 6 <sup>b</sup>	142 $\pm$ 3 <sup>b</sup>	20 $\pm$ 0.1 <sup>b</sup>	4.6 $\pm$ 0.04 <sup>b</sup>
	12 hours	100 $\pm$ 2 <sup>c</sup>	59 $\pm$ 2 <sup>c</sup>	85 $\pm$ 4 <sup>c</sup>	22 $\pm$ 0.1 <sup>c</sup>	4.9 $\pm$ 0.01 <sup>c</sup>
	24 hours	100 $\pm$ 2 <sup>c</sup>	48 $\pm$ 4 <sup>d</sup>	74 $\pm$ 1 <sup>d</sup>	23 $\pm$ 0.2 <sup>d</sup>	3.6 $\pm$ 0.04 <sup>d</sup>

Comparison was made between the induced hours of each treatment including its control from the same enzyme only. Total activity with same letters shows significant difference at  $p=0.05$ .

Both insecticide treatments on the larvae had significant effect ( $p<0.05$ ) on the enzyme specific activities (Table 3). Highest level of GST and Cyt P450 specific activities were detected in larvae exposed to acute malathion treatment for 24 hours, while the specific  $\alpha$ -est,  $\beta$ -est and AChE specific activities dropped comparatively during 24 hours of acute malathion treatment. Specific activity of  $\alpha$ -est gradually decreased upon acute treatment with malathion and reached the lowest level at 24 hours of exposure. On the other hand, the specific activity of  $\beta$ -est as well as AChE increased upon 6 hours of exposure and then consistently decreased to the lowest level at 24 hours of acute malathion treatment. According to the results obtained

from this study, the specific activity of GST showed no significant difference ( $p>0.05$ ) upon comparison between different acute malathion treatment hours. Apart from that, the specific enzyme activity of all test enzymes of acute malathion treated larvae produced a significant difference ( $p<0.05$ ) when compared between different hours and the control.

Acute permethrin treatment resulted in a different response with the highest peak of specific activity observed on GST,  $\alpha$ -est as well as  $\beta$ -est and the lowest peak for AChE and Cyt P450 specific activity was recorded at 24 hour of acute treatment. Similar response was detected in the specific activity of  $\alpha$ -est as well as  $\beta$ -est whereby the activity increased at 6 hour of exposure, followed by a decrease at 12 hours and ultimately reaching the highest point at 24 hour of acute treatment with permethrin. Specific activity of GST increases gradually at the 6 hour of acute treatment with permethrin with the highest activity observed at the 24 hour. The specific activity of Cyt P450 was at the highest level at 6 hour of acute permethrin treatment and then decreased significantly to the lowest level at 24 hours. Meanwhile, the specific activity of AChE dropped upon acute treatment with permethrin until the 12 hour and increased at the 24 hour of exposure. Collectively, specific enzyme activity of all enzymes in the acute permethrin treated larvae were statistically significant ( $p<0.05$ ) when compared between the acute treated hours and to its control respectively except for the specific GST activity between 6 and 12 hours of acute permethrin treatment ( $p>0.05$ ).

A varying degree of fold changes in total enzyme activity as well as specific activity was detected upon time dependent acute malathion and permethrin treatment.

Table 3. Specific enzyme activity against time dependent acute treatment with LC<sub>50</sub> of malathion and permethrin.

Specific activity / Insecticide	Treatment duration	GST $\mu\text{mol}/\text{min}/\text{mg}$	$\alpha$ -est nmol of 1-NA/min/mg	$\beta$ -est nmol of 2-NA/min/mg	Cyt P450 nmol/min/mg	AChE nmol/min/mg
Permethrin	Control	1.947 $\pm$ 0.08 <sup>a</sup>	74.73 $\pm$ 0.62 <sup>a</sup>	65.65 $\pm$ 0.56 <sup>a</sup>	6.32 $\pm$ 0.08 <sup>a</sup>	1.68 $\pm$ 0.02 <sup>a</sup>
	6 hours	2.427 $\pm$ 0.05 <sup>b</sup>	82.58 $\pm$ 0.34 <sup>b</sup>	74.27 $\pm$ 0.42 <sup>b</sup>	9.61 $\pm$ 0.05 <sup>b</sup>	1.46 $\pm$ 0.01 <sup>b</sup>
	12 hours	2.541 $\pm$ 0.12 <sup>b</sup>	80.68 $\pm$ 0.48 <sup>c</sup>	67.71 $\pm$ 0.48 <sup>c</sup>	8.68 $\pm$ 0.04 <sup>c</sup>	1.19 $\pm$ 0.03 <sup>c</sup>
	24 hours	2.635 $\pm$ 0.08 <sup>b</sup>	91.36 $\pm$ 0.70 <sup>d</sup>	80.50 $\pm$ 0.96 <sup>d</sup>	7.23 $\pm$ 0.13 <sup>d</sup>	1.44 $\pm$ 0.01 <sup>d</sup>
Malathion	Control	1.947 $\pm$ 0.08 <sup>a</sup>	74.73 $\pm$ 0.62 <sup>a</sup>	65.65 $\pm$ 0.56 <sup>a</sup>	6.32 $\pm$ 0.08 <sup>a</sup>	1.68 $\pm$ 0.02 <sup>a</sup>
	6 hours	2.397 $\pm$ 0.09 <sup>b</sup>	70.25 $\pm$ 0.62 <sup>b</sup>	75.68 $\pm$ 0.64 <sup>b</sup>	6.97 $\pm$ 0.02 <sup>b</sup>	2.01 $\pm$ 0.02 <sup>b</sup>
	12 hours	2.360 $\pm$ 0.06 <sup>b</sup>	52.09 $\pm$ 0.28 <sup>c</sup>	38.67 $\pm$ 0.22 <sup>c</sup>	7.44 $\pm$ 0.04 <sup>c</sup>	1.92 $\pm$ 0.02 <sup>c</sup>
	24 hours	2.502 $\pm$ 0.05 <sup>b</sup>	46.68 $\pm$ 0.39 <sup>d</sup>	35.47 $\pm$ 0.64 <sup>d</sup>	7.78 $\pm$ 0.06 <sup>d</sup>	1.37 $\pm$ 0.00 <sup>d</sup>

Comparison was made between the induced hours of each treatment including its control from the same enzyme only. The specific activity with same letters shows significant difference at  $p=0.05$ .

## DISCUSSIONS

Elevation of protein content of larvae upon acute time-dependent treatment with two xenobiotics (permethrin and malathion) is suggested to occur due to an increase in metabolism under toxicant stress which is associated with a state of oxidative stress in induced larvae. The exposure to oxidative stress inducers (permethrin and malathion) may contribute to antioxidant defence by repairing the damaged secondary product generated by reactive oxygen species (ROS) (Hamzah & Alias 2016b; Ullah et al 2018; Wang et al. 2016).

Upon acute malathion treatment for 24 hours, a reduction in the fold change in the total and specific enzyme activity were detected in  $\alpha$ -est and  $\beta$ -est. The result obtained in this research appear is contrary to another study by which elevated enzymatic activities was strongly associated to organophosphate resistance (Latif et al. 2010). Cytochrome P450 and Est are the enzymes involved in phase I of detoxification process whereby it is involved in the metabolism of insecticides by oxidation and hydrolysis respectively (Melo-Santos et al. 2010). The results obtained here suggest the likely involvement of Cyt P450 instead of Est in phase I of detoxification of malathion in this strain. In Malaysia, insensitive AChE was discovered in some field collected *Ae. albopictus* populations, indicating the emergence of organophosphate resistance (Chen et al. 2013). On the other hand, Mazzarri & Georghiou (1995) reported that there was no involvement of AChE enzymatic activity and ruled out its role in conferring organophosphate resistance in *Ae. aegypti*.

Elevation in the total and specific activity of Est upon acute permethrin treatment have been observed in this study. Several reports have been filed which revealed the involvement of Est enzymes in organophosphate as well as pyrethroid resistance (Amelia-Yap et al. 2018; Hemingway et al. 2004; Yaicharoen et al. 2005). In some cases, the role of Est in hydrolysing pyrethroids which leads to detoxification activities has been suggested to confer resistance in different species of mosquitoes (Emtithal & Thanaa 2012; Hemingway et al. 2004; Saelim et al. 2005; Somwang et al. 2011; Vulule et al. 1999).

The increment of total and specific enzymatic activity of GST upon acute permethrin treatment concur with a few previous reports regarding the role of GST enzyme activity in insecticide resistance particularly DDT as well as pyrethroids resistance (Dou et al. 2009; Enayati et al. 2005; Hamzah et al. 2019; Vontas et al. 2001). Apart from that, an increase in the production of GST has been reported to be one of the mechanism responsible in conferring resistance in insects including *Ae. albopictus* mosquitoes and are usually found to be elevated in pyrethroid and organophosphate -resistant strains (Hamzah & Alias 2016a; Lumjuan et al. 2005; Lumjuan et al. 2011; Panini et al. 2016; Ranson et al. 2001; Wei et al. 2001; Yang et al. 2009; Vulule et al. 1999).

Difference in the enzymatic activities of Cyt P450 have been detected upon acute permethrin treatment and can be related to several studies which have been conducted in Malaysia stating that detoxification activities involving changes in Cyt P450 monooxygenase activities are responsible for pyrethroid resistance in *Ae. albopictus* mosquitoes instead of knockdown resistance (kdr) mechanism (Avicor et al. 2014; Wan-Norafikah et al. 2013; Wan-Norafikah et al. 2008). Other than that, previous studies revealed the correlation between the elevation of Cyt P450 enzyme activities and the level of insecticide resistance to organophosphate and permethrin (pyrethroid) (Avicor et al. 2014; Etang et al. 2007; Wan-Norafikah et al. 2013; Yaicharoen et al. 2005).

Our results proof that there was more than one detoxification enzyme involved upon acute time-dependent malathion and permethrin treatment. Result obtained from this study is in agreement with earlier results by which the enhanced detoxification activity levels of metabolic enzymes such as GST, Est and AChE in the same mosquito population leads to the emergence of tolerance and ultimately insecticide resistance to all four classes of insecticides (Amelia-Yap et al. 2018; Montella et al. 2007; Smith et al. 2016). Elevated levels of Est and Cyt P450 activities were revealed in pyrethroid-resistant mosquitoes which highlight the role of more than one enzyme in conferring resistance (Liu et al. 2006). Other than that, different

resistance mechanisms can take place concurrently in resistant populations which ultimately results in resistance to one or more insecticides (Nkya et al. 2013).

### CONCLUSION

Acute time-dependent xenobiotic (permethrin and malathion) induction on detoxification enzymes (GST,  $\alpha$ -est,  $\beta$ -est, AChE and Cyt P450) of *Ae. albopictus* larvae altered its total and specific activity levels which indicates the possibility of enhancing the tolerance of *Ae. albopictus* to insecticides in the field. Apart from that, the results obtained from this research indicates that not only one enzyme is solely involved in the detoxification activities of malathion and permethrin upon acute treatment with the respective insecticides, but this could be due to the involvement of multiple metabolic enzymes.

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