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Original article

Susceptibility of *Aedes albopictus* Skuse (Diptera: Culicidae) to permethrin in Kuala Lumpur, Malaysia

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Background: Insects control using insecticides is used extensively and intensively in vector control programs in many countries including Malaysia. Because of this, mosquito species have been found to develop various levels of resistance towards these insecticides, leading to failure in vector control activities.

Objectives: We determined permethrin resistance status in laboratory susceptible, permethrin-selected, and field strains of *Aedes albopictus*.

Methods: The susceptibility status of laboratory susceptible strain, permethrin-selected strain, and four field strains of *Aedes albopictus* collected from Kuala Lumpur were determined using three standard laboratory tests, WHO larval bioassay, WHO adult mosquito bioassay, and microassay of mixed function oxidases (MFOs).

Results: The LC_{50} values of permethrin-selected strain and field strains obtained from the WHO larval bioassay were almost two times higher (0.38-0.44 mg/L) than the LC_{50} value of the laboratory strain (0.20 mg/L). In the WHO adult bioassay, the susceptibility of permethrin-exposed of both permethrin-selected strain, and field strains (LT_{50} = 19.39 to 20.65 min) were reduced for 1.31 to 1.72 times after been exposed to the synergist, piperonyl butoxide (PBO) prior to permethrin. Complete mortalities were also recorded in both permethrin-exposed and PBO + permethrin-exposed *Ae. albopictus* of all strains, twenty-four hours post-exposure. For the MFOs enzyme microassay, a significant difference (p <0.05) in the mean absorbance of elevated oxidase activity at 630 nm was observed between all strains of both the non-exposed and PBO-exposed *Ae. albopictus*. Strong and significant positive correlations were also observed between LT_{50} values of permethrin-exposed and PBO + permethrin-exposed with oxidase level in *Ae. albopictus* tested (r = 0.943; p <0.05).

Conclusion: These results indicate the association of oxidase activity with permethrin resistance development in *Ae. albopictus*.

Keywords: *Aedes albopictus*, bioassay, Malaysia, mixed function oxidases, permethrin

Aedes albopictus has been incriminated as the vector of dengue in Malaysia and other Southeast Asian countries [1-4]. Chikungunya virus had also been isolated from field-collected *Ae. albopictus* in several countries including Malaysia, Africa, and Italy [5-7]. Since no effective vaccines and specific treatment against these diseases are available, mosquito control remains the main method for prevention. Insecticides, including pyrethroids play a crucial role in vector control activities. However, intensive and extensive use of these insecticides has led to the development of resistance among mosquito vectors. In pyrethroids resistance, the increase in the rate of metabolic detoxification of the insecticide which often associated with changes in monooxygenase or mixed function oxidases (MFOs) activity and changes in target site sensitivity, are the

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two major mechanisms that cause resistance [8]. The World Health Organization (WHO) standard bioassay technique has been consistently used in detecting resistance in these mosquito vectors. In addition, biochemical assays are also performed in order to determine the resistance mechanisms.

Permethrin is the first potent, photostable pyrethroid discovered in 1973 [9]. It has been widely used because of its effectiveness and low mammalian toxicity [10]. It is often used in combination with synergists such as piperonyl butoxide (PBO) to prolong effectiveness. Permethrin has also been applied in dengue vector control in Malaysia, especially in Kuala Lumpur. In fact, permethrin resistance development had been detected among *Ae. aegypti* mosquitoes obtained from Kuala Lumpur [11]. The main purpose of this study was to determine permethrin resistance in laboratory susceptible, permethrin-selected, and field strains of *Aedes albopictus*. Besides, this study also attempted to determine the presence of knockdown resistance (kdr) alleles using the synergist, piperonyl butoxide (PBO) as well as to determine and evaluate the presence and the level of oxidases in the mosquitoes tested.

Materials and methods

Samples

Three strains of mosquitoes were used for this study, i.e. a laboratory strain, a permethrin-selected strain and four field strains of *Ae. albopictus.* The laboratory strain was used as a reference in this study. It was originally collected in Selangor, Malaysia and has been maintained in the insectarium of the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia for five years. It is free of past insecticide exposure. In contrast, the permethrin-selected strain of *Ae. albopictus*, which was also collected in Selangor in 2008, was constantly selected for permethrin resistance in each generation at a concentration causing 50% mortality (LC_{50}). Only the first generation (F1) of the field-collected *Ae. albopictus* was used in this study in order to maintain the representation of the mosquitoes as field strain samples. The F16 of the laboratory strain and F5 of the permethrin-selected strain were employed for this study. The results for the laboratory strain, permethrin-selected strain and Jalan Fletcher field strain were as reported in previous studies [12].

All strains of *Ae. albopictus* used in this study were supplied ad libitum with 10% sucrose solution. These mosquito colonies were reared simultaneously in separate rooms and handled in the same manner. The temperature and relative humidity (R.H.) of each colony was maintained at 25°C±2°C and 80%, respectively [13].

Study areas

The field strain samples used in this study were collected from four different study sites in Kuala Lumpur: the Institute for Medical Research (IMR) staff quarters in Jalan Fletcher (N03°11.384', E101°41.769'), Titiwangsa Zone; Taman Melati (N03 13.434', E101°43.528'), Setapak Zone; Vista Angkasa (N03°06.778', E101°39.736'), Klang Lama Zone, and Desa Tasik (N03°13.292', E101°43.513'), Cheras Zone. These study areas were selected according to the high numbers of dengue cases reported as provided by the Health Department, Kuala Lumpur City Hall.

Collection of field strain Ae. albopictus

The collection of field strain *Ae. albopictus* from all study areas chosen was performed using standardized ovitraps [14]. The ovitrap consists of a 300 ml black plastic container with straight, slightly tapered sides. The opening measures 7.8 cm in diameter, the base diameter is 6.5 cm, and the height is 9.0 cm. An appropriate label was attached on the outer wall of each ovitrap. An oviposition paddle (10 cm × 2.5 cm × 0.3 cm) made from hardboard consisting of two different types of surfaces was placed diagonally into each ovitrap with the rough surface of the oviposition paddle upwards. Every ovitrap was filled with tap water to a level of 5.5 cm. These ovitraps were used in accordance with guidelines of the Ministry of Health [15]. Eighty ovitraps were placed indoor and outdoor randomly in every study area. All ovitraps were placed in proximity to other potential breeding containers with minimum physical and environmental disturbance. The locations for ovitrap placements were either partially or totally shaded to avoid direct sunlight and heavy rain that may cause water spillage. Ovitraps placed at every study site were collected after five days of trapping and transported back to the laboratory. The contents were poured into individual plastic containers and topped up with fresh water. A mixture of liver powder, cereals, yeast, and a small piece of partially-cooked cow liver were added into each container as larval food. The containers were kept covered to avoid other

mosquitoes in the vicinity from depositing into the containers. All hatched larvae were bred and subsequently identified at adult stage. Only adult *Ae. albopictus* were selected and reared as zero filial generation (F0) in the laboratory while others were destroyed. These F0 *Ae. albopictus* were reared in cages and supplied with white mice for blood-feeding to obtain eggs. These eggs were hatched and reared to the larval stage. Some early fourth larvae of F1 were used for the testing, while the rest were further reared until they pupated. Pupae were collected and transferred to emergence cages. These adult F1 *Ae. albopictus* were supplied with 10% sucrose solution. Only sucrose-fed three to five-days-old adult females were tested.

Insecticide

A commercial grade of permethrin (10.9 % a.i. w/v) was used in WHO larval bioassay while permethrin impregnated papers at diagnostic dosage (0.75 %) was used in WHO bioassay for adult mosquitoes.

Selection of permethrin-selected strain

Selection pressure using permethrin was performed constantly at the concentration that caused 50% mortality (LC_{50}) to obtain the permethrin-selected strain. The progress of selection was monitored using the WHO diagnostic test [16].

Preparation of piperonyl butoxide (PBO) impregnated papers

Impregnated papers of piperonyl butoxide (PBO) as a synergist were prepared locally as described by Herath and Davidson [17] with a ratio of 1:5 (permethrin:PBO) [18]. A similar procedure was used to impregnate control papers except that the papers were soaked in absolute ethanol only.

Bioassay of mosquito larvae

Larval bioassay was performed according to the standard WHO susceptibility or resistance test protocol [19]. Twenty-five early fourth instar larvae were introduced into 250 ml test solution in a 300 ml paper cup and exposed continuously for 24 hours. The concentrations were obtained by diluting the commercial grade of permethrin stock solution (10.9% a.i. w/v) with absolute ethanol. For the control, 1 ml of absolute ethanol was added into 249 ml distilled water. Test concentrations of permethrin were selected to cause 10% to 90% mortality. The tests were replicated three times per concentration. The mortality of larvae was assessed after 24 hours. These larvae were considered dead if they sank to the bottom of the paper cups and failed to move or float after being probed with an applicator stick [20, 21].

Bioassay of adult mosquitoes

Adult mosquito bioassay was conducted by following the standard WHO susceptibility or resistance test protocol [19]. Fifteen sucrose-fed three to five-days-old adult female mosquitoes per replicate were used for this bioassay. This test was divided into three different bioassays with similar test procedure but using different types of impregnated papers. The first bioassay consisted of exposure of the mosquitoes to the permethrin impregnated papers with the diagnostic dosage (0.75%) as recommended by WHO. Mosquitoes employed for the second bioassay were only exposed to the impregnated papers of PBO. The third bioassay required the mosquitoes to be exposed to the PBO impregnated papers prior to exposure to 0.75% permethrin impregnated papers. For the control, paper with 1 ml ethanol was used. There were three replicates per bioassay including the control. All mosquitoes were exposed to the diagnostic dosage of permethrin and / or PBO at the respective exposure period. Cumulative mortality counts were recorded every minute within the exposure time. After the exposure period, the mosquitoes were held for a 24-hour recovery period before the mortality was recorded again. Sucrose solution was provided for the mosquitoes. All survivors were then collected and kept in the freezer at -70 C before being used for microassay of mixed function oxidases (MFOs).

Microassay of mixed function oxidases (MFOs)

The biochemical assay for mixed function oxidase (MFOs) activities was conducted [22] and modified [23]. Surviving adult mosquitoes from bioassay tests kept at -70°C were prepared. Every single adult mosquito was homogenized individually in 100 µl sodium acetate buffer in a microcentrifuge tube at 4°C using a pestle. Another 900 µl of buffer was added to a total of 1 ml. Using a micropipette, 100 µl homogenate was transferred into each well of a microtiter plate. Four replicate aliquots of the homogenate from a single adult mosquito were obtained for this assay. Therefore, four wells of

microtiter plate were used per adult mosquito. Two hundred l 3,3'5,5'-tetramethylbenzidine (TMBZ) solution, which is a substrate solution was added into each well of the microtiter plate and left for one minute. Twenty-five l of 3% hydrogen peroxide solution, which is an indicator solution was added into each well of the microtiter plate. Change of color reaction took place immediately. The microtiter plate was incubated for 10 minutes before being read using an immunoassay (ELISA) reader (Dynatech MR 5000) at a wavelength of 630 nm. The presence and level of MFOs was indicated quantitatively by the color intensity, which is directly proportional to the activity of MFOs.

Statistical analysis

If the control mortality in bioassay was between 5% and 20%, the percentage mortalities were corrected by Abbott's formula [24]. Data obtained from all tests were subjected to a probit analysis computer program and 50% lethal time (LT_{50}) was obtained [25]. Resistance ratios for both permethrin-selected and field strains of *Ae. albopictus* tested were calculated using the following formula: resistance ratio (RR) = LT_{50} or LC_{50} of the permethrin-selected or field strain/ LT_{50} or LC_{50} of the laboratory strain. The presence of resistance is indicated when the value of RR >1 while the value of RR ≤ 1 indicated the susceptibility of mosquitoes towards permethrin [26]. For the synergistic effect of PBO, the following formula was used: synergistic ratio (SR) = LT_{50} or LC_{50} of the permethrin / LT_{50} or LC_{50} of the PBO + permethrin. The mean absorbance of oxidase activity was detected using an immunoassay reader.

Results

From the WHO larval bioassay performed, the LC_{50} value of *Ae. albopictus* permethrin-selected strain was about two times higher (0.44 mg/L) than the LC_{50} value of the laboratory susceptible strain (0.20 mg/L) as shown in **Figure 1**. Nevertheless, the LC_{50} values of the field strains from Jalan Fletcher (0.38 mg/L), Taman Melati (0.41 mg/L), Vista Angkasa (0.43 mg/L) and DesaTasik (0.38 mg/L) were slightly lower than the permethrin-selected strain. A narrow range of resistance ratio was observed for these *Ae. albopictus* larvae, which was between 1.90 folds and 2.20 folds, respectively (**Table 1**).

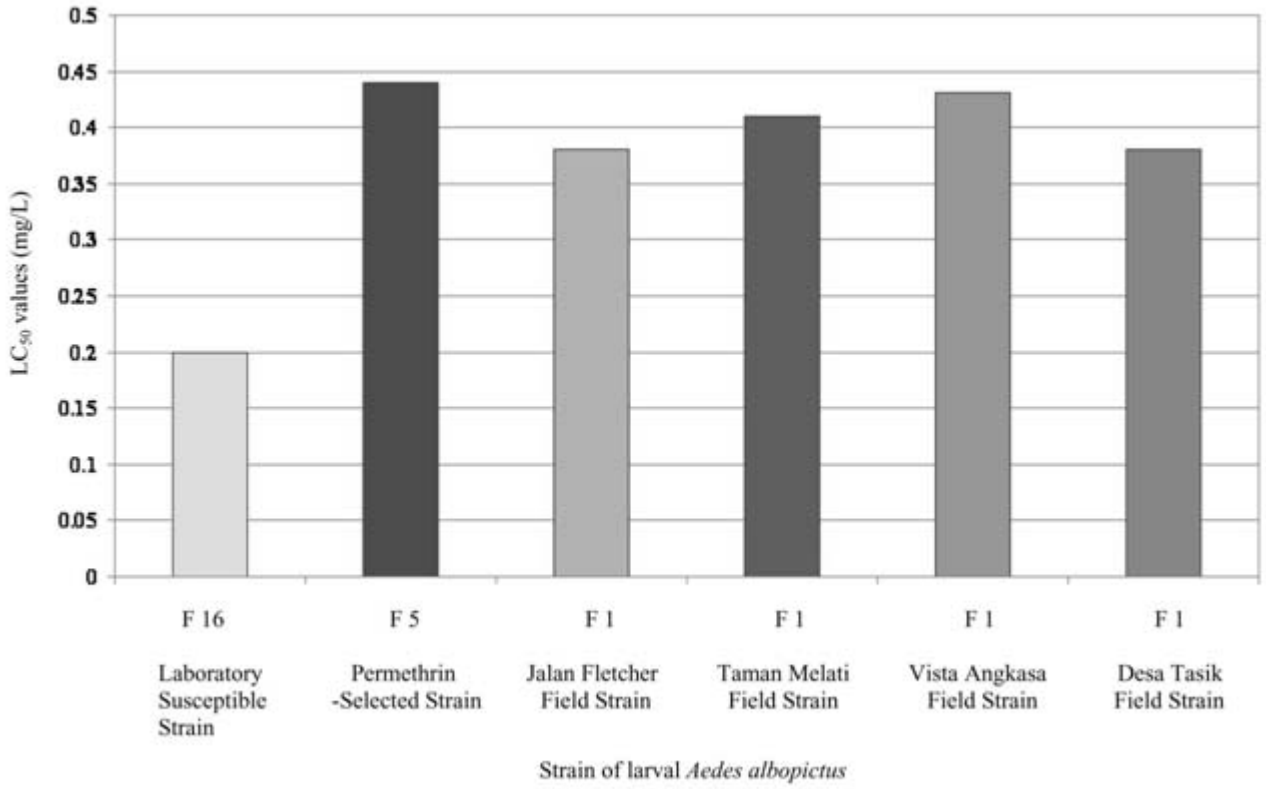

Figure 1. Comparative LC_{50} values for six strains of *Aedes albopictus* larvae exposed to permethrin

Table 1. Susceptibility of strains of *Aedes albopictus* larvae exposed to permethrin

Strain	Generation	LC_{50} (mg/L) 95% C.L.	Regression Line	Resistance Ratio (RR)
Laboratory Susceptible Strain	1 (F 16)	0.20 (0.18-0.21)	Y=5.07x-42.10	-
Permethrin-Selected Strain	1 (F 5)	0.44 (0.42-0.46)	Y=8.99x-81.66	2.20
Field Strain				
Jalan Fletcher	1 (F 1)	0.38 (0.37-0.39)	Y=12.51x-114.77	1.90
Taman Melati	1 (F 1)	0.41 (0.38-0.43)	Y=5.47x-47.59	2.05
Vista Angkasa	1 (F 1)	0.43 (0.41-0.46)	Y=6.09x-53.67	2.15
DesaTasik	1 (F 1)	0.38 (0.37-0.40)	Y=7.78x-69.57	1.90

C.L. = Confidence limit (95%)

For the WHO adult bioassay, the LT_{50} values for all strains of *Ae. albopictus*, which consisted of the permethrin-exposed and PBO + permethrin-exposed mosquitoes are illustrated in **Figure 2** and **Table 2**, respectively. The LT_{50} values of *Ae. albopictus* field strains exposed to permethrin alone ranged from 19.39 to 20.65 minutes, while the LT_{50} values of permethrin-selected strain of the same exposure was 20.02 minutes. The resistance ratios for all strains of permethrin-exposed *Ae. albopictus* tested in this study ranged from 1.22 to 1.30 folds. The permethrin-exposed Vista Angkasa field strain showed the highest resistance ratio (1.30 folds), while the lowest resistance ratio was from the permethrin-exposed Desa Tasik field strain (1.22 folds). Other than that, the highest synergistic ratio was recorded from the PBO + permethrin-exposed permethrin-selected strain (1.72 folds), while the lowest synergistic ratio was shown by the PBO + permethrin-exposed Taman Melati field strain (1.32 folds). Complete mortalities were also demonstrated for all four field-strains of *Ae. albopictus* exposed to both permethrin alone and PBO + permethrin (**Table 3**).

The MFOs enzyme microassay showed that the mean absorbance of oxidase activity at 630 nm for all strains of the non-exposed mosquitoes ranged between 0.34 to 0.50, respectively, which was significantly different (p <0.05) (**Table 4**). Resistance ratios of more than 1.0 fold were found in the permethrin-selected strain and four field-strains. The highest resistance ratio was observed in the Vista Angkasa field strain (1.47 folds), while the Taman Melati field strain showed the lowest resistance ratio (1.07 folds), respectively. Nevertheless, the mean absorbance of elevated oxidase activity of these mosquitoes after exposure to PBO was reduced within the range of 0.41 to 0.45 at 630 nm, respectively.

In contrast, the mean absorbance of oxidase activity at 630 nm for the PBO-exposed permethrin-selected strain (0.51) seemed to be higher than the non-exposed colonies (0.43), while the mean absorbance of oxidase activity at 630 nm for the PBO-exposed laboratory susceptible strains remained the same as the non-exposed colonies (0.34), respectively. Nevertheless, a significant difference (p <0.05) was also observed between all strains of *Ae. albopictus* for both the non-exposed and PBO-exposed.

In addition, a low correlation was presented between LC_{50} values and oxidase level in all strains of *Ae. albopictus* (r = 0.414; p >0.05) (**Figure 3**). Nevertheless, for adult mosquitoes tested, strong significant correlations were observed between LT_{50} values of permethrin-exposed mosquitoes and oxidase level (r = 0.943; p <0.05) (**Figure 4**). Similar results were obtained for the correlation between LT_{50} values of PBO + permethrin-exposed and oxidase level in the same mosquito colonies, respectively (**Figure 5**). These results confirmed the association of oxidase activity with the permethrin resistance development in *Ae. albopictus* tested.

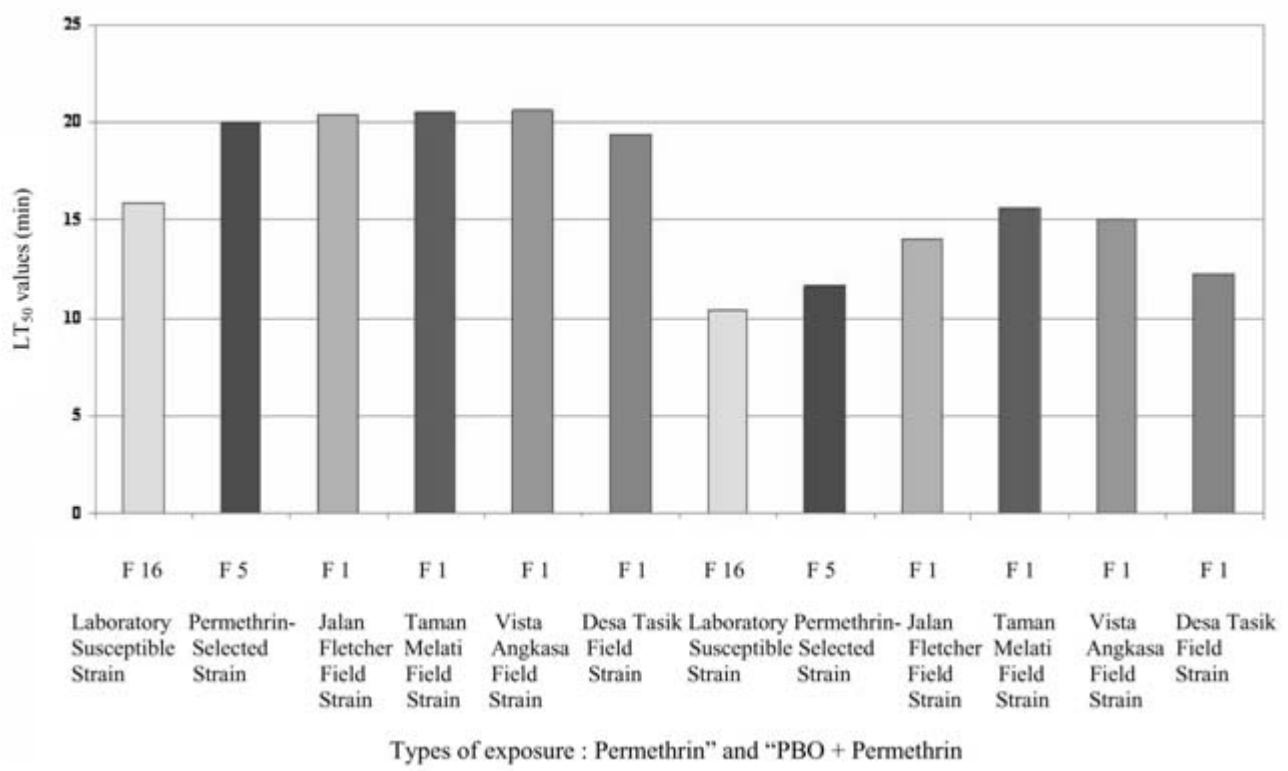

Figure 2. Comparative LT_{50} values for six strains of adult *Aedes albopictus* exposed to permethrin alone and PBO + permethrin

Table 2. Susceptibility of strains of *Aedes albopictus* adult mosquitoes exposed to permethrin alone and PBO +permethrin

Exposure			**Permethrin**			**PBO (Synergist) + Permethrin**	
Strain	**Generation**	**LT_{50} (min) 95% C.L.**	**Regression Line**	**RR**	**LT_{50} (min) 95% C.L.**	**Regression Line**	**SR**
Laboratory Susceptible Strain	1 (F 16)	15.87 (14.98-16.74)	Y=5.47x-56.27	-	10.41 (9.66-11.16)	Y=4.79x-47.80	1.52
Permethrin-Selected Strain	1 (F 5)	20.02 (19.03-20.97)	Y=5.91x-61.77	1.26	11.65 (10.79-12.54)	Y=4.35x-43.13	1.72
Field Strain							
Jalan Fletcher	1 (F 1)	20.39 (19.25-21.49)	Y=4.74x-48.61	1.28	14.05 (13.18-14.90)	Y=5.04x-51.16	1.45
Taman Melati	1 (F 1)	20.53 (19.39-21.65)	Y=5.85x-61.20	1.29	15.67 (14.87-16.47)	Y=3.39x-32.97	1.31
Vista Angkasa	1 (F 1)	20.65 (19.96-21.32)	Y=5.68x-59.29	1.30	15.04 (14.40-15.69)	Y=4.88x-49.56	1.37
DesaTasik	1 (F 1)	19.39 (18.66-20.11)	Y=4.72x-48.29	1.22	12.29 (11.69-12.89)	Y=4.47x-44.56	1.58

PBO = Piperonyl butoxide (Synergist), RR = Resistance Ratio, SR = Synergistic Ratio, C.L. = Confidence Limit (95 %)

Table 3. Mortality of strains of *Aedes albopictus* tested with WHO adult bioassay method

Strain	Generation	Mortality After 24 Hours (% Mortality)			
	Exposure	Non Exposure (Control)	PBO	Permethrin	PBO + Permethrin
Laboratory Susceptible Strain	1 (F 16)	0	0	100	100
Permethrin-Selected Strain	1 (F 5)	0	0	100	100
Field Strain Jalan Fletcher	1 (F 1)	0	0	100	100
Field Strain Taman Melati	1 (F 1)	0	0	100	100
Field Strain Vista Angkasa	1 (F 1)	0	0	100	100
Field Strain DesaTasik	1 (F 1)	0	0	100	100

Table 4. Mean of oxidase activity (absorbance at 630 nm) in *Aedes albopictus* adult unexposed and exposed to PBO

Exposure		Non Exposure			PBO	
Strain	Generation	Mean±S.E. (Absorbance at 630nm)	Resistance Ratio (RR)	One way ANOVA	Mean±S.E. (Absorbance at 630nm)	One way ANOVA
Laboratory Susceptible Strain	1 (F 16)	0.34 ± 0.01	-	F = 10.29	0.34 ± 0.02	F = 7.15
Permethrin-Selected Strain	1 (F 5)	0.40 ± 0.02	1.18	$p < 0.05$	0.51 ± 0.03	$p < 0.05$
Jalan Fletcher	1 (F 1)	0.46 ± 0.03	1.35		0.44 ± 0.02	
Taman Melati	1 (F 1)	0.48 ± 0.01	1.07		0.45 ± 0.02	
Vista Angkasa	1 (F 1)	0.50 ± 0.02	1.47		0.44 ± 0.01	
DesaTasik	1 (F 1)	0.43 ± 0.01	1.26		0.41 ± 0.02	

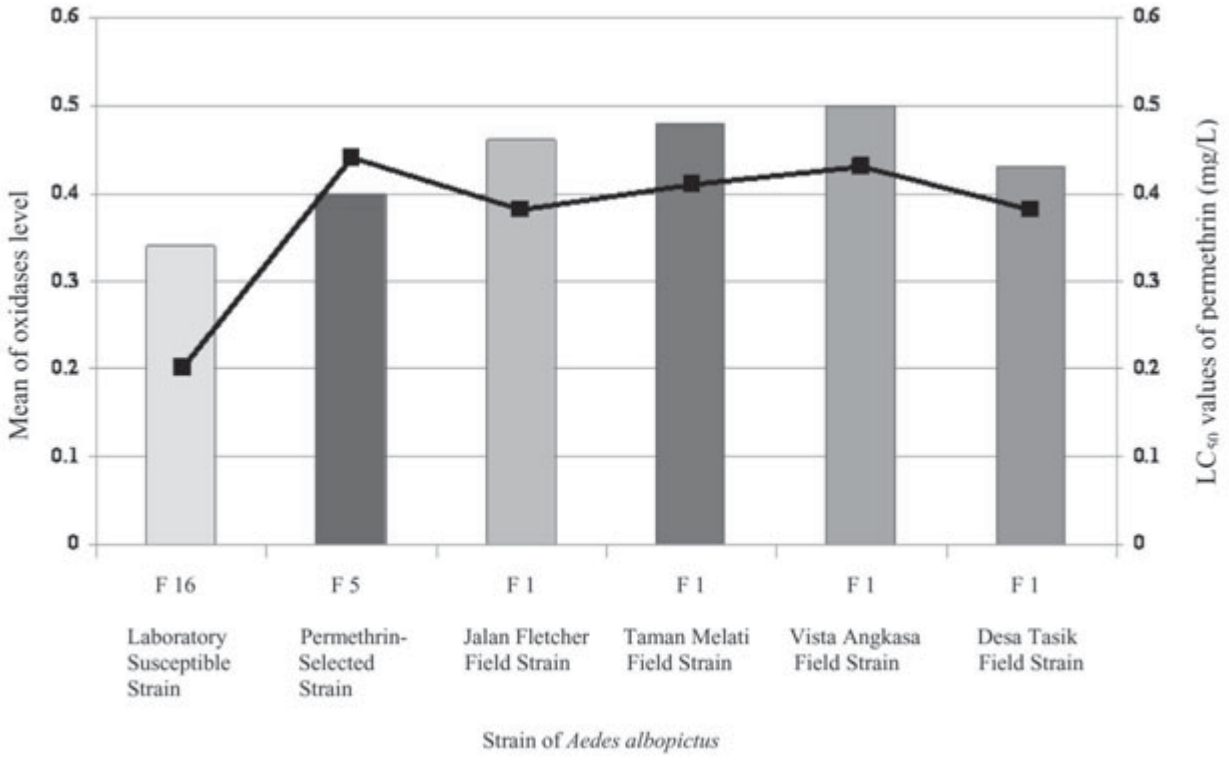

Figure 3. Correlation of LC_{50} values of permethrin and oxidase level for six strains of *Aedes albopictus* (r = 0.414)

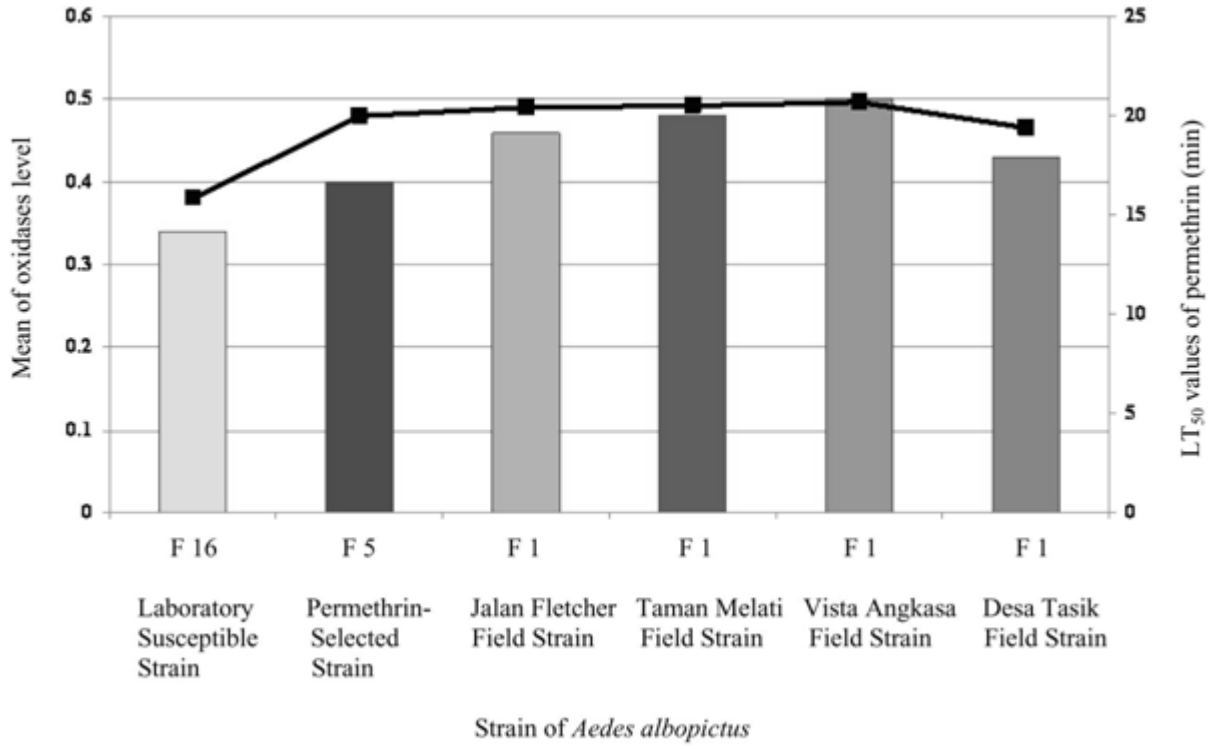

Figure 4. Correlation of LT_{50} values of permethrin and oxidase level for six strains of *Aedes albopictus* (r = 0.943)

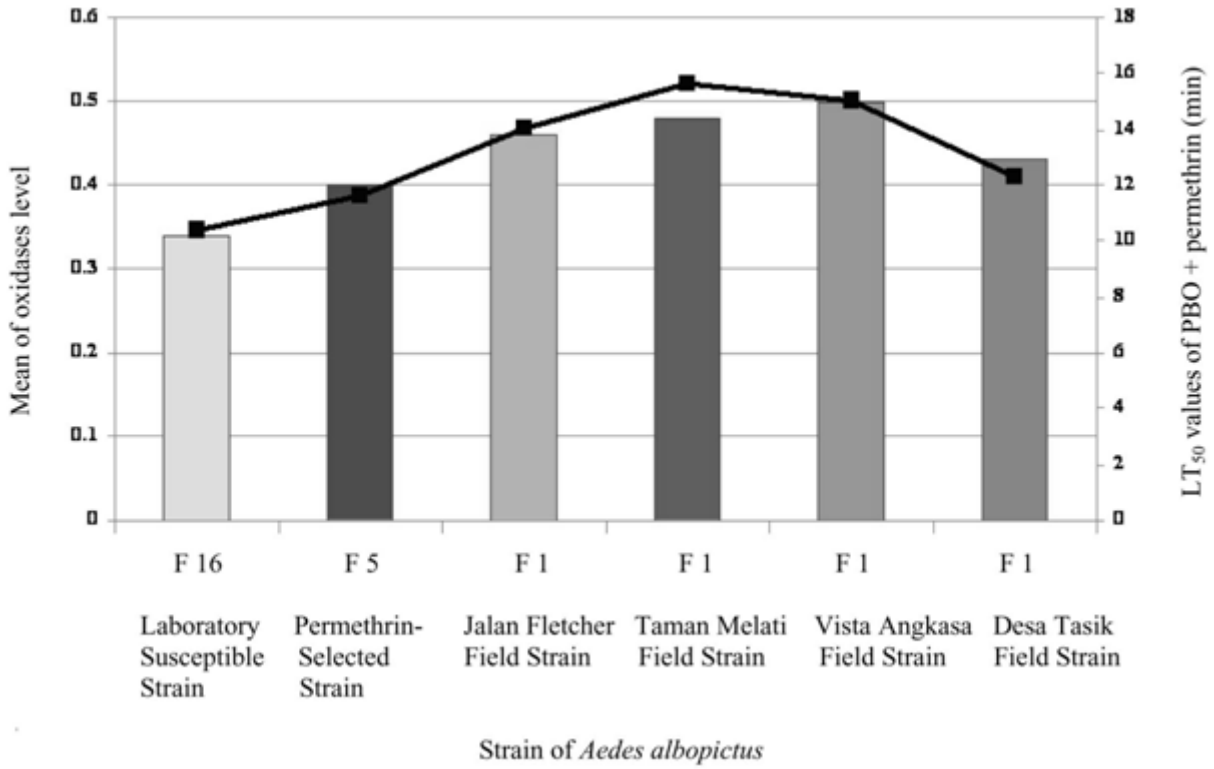

Figure 5. Correlation of LT_{50} values of PBO + permethrin and oxidase level for six strains of *Aedes albopictus* (r = 0.943)

Discussion

Results from the WHO larval bioassay showed that the laboratory susceptible strain remained the most susceptible towards permethrin, while permethrin resistance development among the permethrin-selected strain and the field strains was almost at the same rate. The LC_{50} values obtained in this study for all strains were higher compared to another local study in Penang, Malaysia [27]. It demonstrated that the LC_{50} value of *Ae. albopictus* exposed to permethrin was only 0.0092 ppm (mg/L), which indicated that local *Ae. albopictus* strains are developing permethrin resistance over the years.

Results obtained from the WHO bioassay conducted against adults were in parallel to the larval stage, which showed that the rate of the permethrin resistance development in these mosquitoes was equivalent. Similar findings were also reported by Lee et al. [28] who found that resistance ratio for *Ae. albopictus* throughout ten weeks were between 0.61 and 1.2 folds. However, these colonies showed about 1.31 to 1.72 times reduction in the LT_{50} values after been exposed to PBO prior to permethrin, indicating that the permethrin resistance mechanism in these mosquitoes could still be suppressed with the use of the synergist PBO. These findings not only proved the presence of permethrin resistance within these mosquito populations but also the effectiveness of the synergist PBO as an oxidase-inhibitor. Furthermore, the lower LT_{50} values recorded for PBO + permethrin-exposed mosquitoes compared to the LT_{50} values of the same populations exposed to permethrin alone indicated that the permethrin resistance development occurred in these mosquitoes was due to the oxidase activity. In other words, the knockdown resistance (kdr) alleles, which are associated with the mutation of the sodium channel in mosquitoes, were not yet present in these mosquitoes so far. These findings were further confirmed through the enzyme microassay tests.

For resistance ratios, the values recorded within the permethrin-selected strain and field strains of *Ae. albopictus* tested were lower compared to other studies. Ping et al. [29] reported a resistance ratio of 1.80 folds for a Singapore strain of *Ae. albopictus*, whereas Ponlawat et al. [30] found that *Ae. albopictus* larvae collected from several localities in Thailand demonstrated low levels of permethrin resistance, except Mae Sot and Phatthalung strains, which were resistant to permethrin. Results obtained in this study indicated that permethrin resistance was emerging within the permethrin-selected strain and field strains

of *Ae. albopictus* tested in this study. These findings were in parallel with the studies by Liu et al. [31] who also found that permethrin resistance development in *Ae. albopictus* from Alabama and Florida strains was slow and conventional even though permethrin has been used in the field for a long period.

The ability of mosquitoes to survive twenty-four hours post-exposure to permethrin indicated the presence of resistance in the present population tested. Complete mortalities in all four field strains of *Ae. albopictus*, exposed to both permethrin alone and PBO + permethrin, gave an early indication that these mosquito populations were susceptible to permethrin. However, taking into consideration that the resistance ratio of these adult mosquitoes was in the range of 1.22 to 1.30 folds after the exposure to permethrin alone, these results then clearly showed that permethrin resistance is emerging in these mosquitoes. The early stage of permethrin resistance existing in these mosquito populations could also be due to oxidase activity, as indicated by 100% mortality after the exposure of PBO + permethrin in the same populations.

Similar studies were also conducted in other parts of the world. *Aedes albopictus* adults obtained from the Lower Rio Grande Valley of Texas and Mexico were found susceptible to permethrin [32]. Romi et al. [33] reported that *Ae. albopictus* collected from northern and central Italy were susceptible to permethrin, while Sharma et al. [34] found that similar mosquito species obtained from two airports in Southern India were also susceptible to permethrin. Moreover, almost all strains of *Ae. albopictus* collected from several parts of Thailand were susceptible to permethrin as well [35].

In comparison between the results of both WHO larval bioassay and adult mosquitoes bioassay conducted in this study, it is proven that the larval stage displayed higher expression of the resistance gene(s) compared to the adult stage.

A similar scenario was observed in the MFOs enzyme microassay conducted on *Ae. albopictus* adult mosquitoes. The pre-exposure to PBO reduced the level of MFOs activities in all field strains tested, which suggested that potential permethrin resistance due to the oxidase activity in these mosquitoes was possible.

On the other hand, the mean absorbance of elevated oxidase activity at 630 nm for the permethrin selected strain and laboratory susceptible strain were not obviously reduced and remained the same, even after pre-exposure to PBO. These results suggested that the permethrin resistance within these mosquitoes were not fully reduced by oxidase-inhibiting synergists as these mosquito colonies had not yet been selected for a complete homozygosity of either susceptible or resistant genes [36].

Furthermore, since there was only a small reduction of oxidase levels in all field strains and even no reduction of oxidase level in both laboratory strain and permethrin-selected strain tested, the involvement of other enzyme activities is also suggested in permethrin resistance development detected in these mosquitoes. Previous studies elsewhere have reported that other enzyme activities such as elevated non-specific esterases, reduced sensitivity of sodium ion channels along nerve axons [37-42] and increased levels of glutathione-S-transferases (GSTs) [42] are also associated with the occurrence of pyrethroid resistance among insects including mosquitoes. Nevertheless, in the case of this study, the involvement of the knockdown resistance (kdr) caused by the mutation in sodium ion channels could not be confirmed. Correlations between the LC_{50} and LT_{50} values with oxidase level in all strains of *Ae. albopictus* confirmed the association of oxidase activity with permethrin resistance.

In summary, this study showed that incipient permethrin resistance had started to emerge in local populations of *Ae. albopictus*. However, this resistance is manageable using the combination of a synergist and pyrethroids. A standard vector control program used in all study areas selected, and easy accessibility of these study areas, could be the causes of similarity in permethrin resistance levels of these *Ae. albopictus* populations.

Moreover, although the levels of permethrin resistance in *Ae. albopictus* tested were much lower compared to the permethrin resistance levels in the local *Ae. aegypti* as recently reported by Wan-Norafikah et al. [11], it is crucial to constantly monitor the permethrin resistance development in *Ae. albopictus* since resistance may develop rapidly. In fact, other local studies, for example, that of Hidayati et al. [43] had also found that permethrin resistance in *Ae. albopictus* was developing at a higher rate compared to malathion and temephos. The vector control activities as well as the use of synergists to prolong the usefulness of insecticides will be no longer effective once the kdr mutations have occurred among

these mosquito populations. Hence, a rotational use of insecticides with different modes of action in vector control activities should be implemented to delay the development of insecticide resistance in mosquitoes. In addition, more studies on resistance detection in *Ae. albopictus* should be conducted worldwide so that more information on the permethrin resistance status in this mosquito species could be shared.

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References

1. Russell PK, Gould DJ, Yuill TM, Nisalak A, Winter PE. Recovery of dengue-4 viruses from mosquito vectors and patients during an epidemic of dengue haemorrhagic fever. Am J Trop Med Hyg. 1969; 18: 580-3.
2. Chan YC, Ho BC, Chan KL. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore city. 5. Observations in relation to dengue haemorrhagic fever. Bull World Health Org. 1971; 44:651-8.
3. Jumali, Sunarto, Gubler DJ, Nalim S, Eram S, Saroso JS. Epidemic dengue haemorrhagic fever in rural Indonesia III. Entomological studies. Am J T Med Hyg. 1979; 28:717-24.
4. Sulaiman S, Omar B, Jefferey J, Busparani V. Evaluation of pyrethroids lambda-cyhalothrin, deltamethrin, and permethrin against *Aedes albopictus* in the laboratory. J Am Mosq Control Assoc. 1991; 7:322-3.
5. Sam IC, AbuBakar S. Chikungunya virus infection. Medical J Malaysia. 2006; 61:264-9.
6. Noridah O, Paranthaman V, Nayar SK, Masliza M, Ranjit K, Norizah I, et al. Outbreak of chikungunya due to virus of Central / East African genotype in Malaysia. Med J Malaysia. 2007; 62:323-8.
7. Leroy EM, Nkoghe D, Ollomo B, Nze-Nkogue C, Becquart P, Grard G, et al. Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007. Emerging Infect Dis. 2009; 15: 591-3.
8. Brengues C, Hawkes NJ, Chandre F, McCarroll L, Duchon S, Guillet P, et al. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. Med Vet Entomol. 2003; 17:87-94.
9. Soderlund DM, Knipple DC. The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochem Mol Biol. 2003; 33:563-77.
10. Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. Permethrin induces overexpression of multiple genes in *Aedes aegypti*. J Med Entomol. 2009; 46:580-7.
11. Wan-Norafikah O, Nazni WA, Lee HL, Zainol-Ariffin P, Sofian-Azirun M. Permethrin resistance in *Aedes aegypti* (Linnaeus) collected from Kuala Lumpur, Malaysia. J Asia Pac Entomol. 2010; 13:175-82.
12. Wan-Norafikah O, Nazni WA, Lee HL, Chen CD, Wan-Norjuliana WM, Azahari AH, et al. Detection of permethrin resistance in *Aedes albopictus* Skuse collected from Titiwangsa Zone, Kuala Lumpur, Malaysia. Proc ASEAN Congr Trop Med Parasitol; 2008 May 22-23; Bangkok, Thailand. 2008; 3: p. 69-77.
13. Division of Medical Entomology, IMR, Kuala Lumpur. Simple and Pictorial Key to Common Genera of Mosquito Adults. In: Abdullah AG, editor. Medical Entomology III. IMR:Kuala Lumpur; 2000. p. 6-13.
14. Lee HL. *Aedes* ovitrap and larval survey in several suburban communities in Selangor, Malaysia. Mosq Borne Dis Bull. 1992; 9:9-15.
15. Ministry of Health Malaysia. Guidelines on the use of ovitrap for *Aedes* surveillance. (Unpublished data). 1997.
16. World Health Organization. Test procedure for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. WHO/CDS/CPC/MAL/98.12. Geneva: World Health Organization; 1998.
17. Herath PRJ, Davidson G. Multiple resistance in *Anopheles albimanus*. Mosq News. 1981; 41:535-9.
18. Kumar S, Thomas A, Pillai MK. Involvement of mono-oxygenases as a major mechanism of deltamethrin-resistance in larvae of three species of mosquitoes. Indian J Exp Biol. 1991; 29:379-84.
19. World Health Organization. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. WHO/VBC/81.805. Geneva: World Health Organization; 1981.
20. Kasai S, Shono T, Komagata O, Tsuda Y, Kobayashi M, Motoki M, et al. Insecticide resistance in potential

vector mosquitoes for West Nile Virus in Japan. J Med Entomol. 2007; 44:822-9.
21. Hardstone MC, Leichter CA, Scott JG. Multiplicative interaction between the two major mechanisms of permethrin resistance, kdr and cytochrome P450-monooxygenase detoxification, in mosquitoes. J Evol Biol. 2009; 22:416-23.
22. Brogdon WG, McAllister JC, Vulule J. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. J Am Mosq Control Assoc. 1997; 13:233-7.
23. Nazni WA, Kamaludin MY, Lee HL, T Rogayah TAR, Sa'diyah I. Oxidase activity in relation to insecticide resistance in vectors of public health importance. Trop Biomed. 2000; 17:69-79.
24. Abbott WS. A method for computing the effectiveness of an insecticide. J Econ Entomol. 1925; 18:265-7.
25. Raymond R. Log-probit analysis basic programme of microcomputer. Cah Orstom Entomol Med Parasitol. 1985; 23:117-21.
26. Cochran DG. Chapter 8: Insecticide Resistance. In: Rust MK, Owens JM, Reierson DA, editors. Understanding and Controlling the German Cockroach, Oxford University Press Inc.:New York; 1995. p. 171-6.
27. Gill SS. Larvicidal activity of synthetic pyrethroids against *Aedes albopictus* (Skuse). Southeast Asian J Trop Med Public Health. 1977; 8:510-4.
28. Lee HL, Nor Asikin, Nazni WA, Sallehuddin S. Temporal variations of insecticide susceptibility status of field-collected *Aedes albopictus* (Skuse) in Malaysia. Trop Biomed. 1998; 15:43-50.
29. Ping LT, Yatiman R, Gek LP. Susceptibility of adult field strains of *Aedes aegypti* and *Aedes albopictus* in Singapore to pirimiphos-methyl and permethrin. J Am Mosq Control Assoc. 2001; 17:144-6.
30. Ponlawat A, Scott JG, Harrington LC. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. J Med Entomol. 2005; 42:821-5.
31. Liu H, Cupp EW, Micher KM, Guo A, Liu N. Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefasciatus*. J Med Entomol. 2004; 41:408-13.
32. Sames IV WJ, Bueno RJr, Hayes J, Olson JK. Insecticide susceptibility of *Aedes aegypti* in the lower Rio Grande Valley of Texas and Mexico. J Am Mosq Control Assoc. 1996; 12:487-90.
33. Romi R, Toma L, Severini F, Di Luca M. Susceptibility of Italian populations of *Aedes albopictus* to temephos and to other insecticides. J Am Mosq Control Assoc. 2003; 19:419-23.
34. Sharma SN, Saxena VK, Lal S. Study on susceptibility status in aquatic and adult stages of *Aedes aegypti* and *Aedes albopictus* against insecticides at international airports of south India. J Commun Dis. 2004; 36:177-81.
35. Jirakanjanakit N, Rongnoparut P, Saengtharatip S, Chareonviriyaphap T, Duchon S, Bellec C, Yoksan S. Insecticide susceptible / resistance status in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Thailand during 2003 – 2005. J Econ Entomol. 2007; 100:545-50.
36. Malcolm CA. Current status of pyrethroid resistance in anophelines. Parasitol Today. 1988; 4:S13-15.
37. Oppenoorth FJ. Biochemical and genetic in insecticide resistance. In: Kerkut GA, Gilbert LI, editors. Comprehensive Insect Physiology Biochemistry and Pharmacology. Pergamon Press; 1985; 12: p. 731-773.
38. Georghiou GP. The Magnitude of Resistance Problem. In: Glass EH, editor. Pesticide Resistance: Strategies and Tactics for Management. National Academy Press : Washington DC; 1986; p. 14-43.
39. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Eastbrook RW, Gunsalus IC, Nebert DW. P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics. 1996; 6:1-42.
40. Roberts DR, Andre RG. Insecticide resistant issues in vectors. Am J Trop Med Hyg. 1994; 50(Supplement): 21-34.
41. Feyereisen R. Insect P450 Enzymes. Annu Rev Entomol. 1999; 44:507-33.
42. Chareonviriyaphap T, Rongnoparut P, Chantarumporn P, Bangs MJ. Biochemical detection of pyrethroid resistance mechanism in *Anopheles minimus* in Thailand. J Vector Ecol. 2003; 28:108-16.
43. Hidayati H, Sofian-Azirun M, Nazni WA, Lee HL. Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos. Trop Biomed. 2005; 22: 45-52.