SPATIAL DISTRIBUTION, ENZYMATIC ACTIVITY, AND INSECTICIDE RESISTANCE STATUS OF *Aedes aegypti* AND *Aedes albopictus* FROM DENGUE HOTSPOT AREAS IN KUALA LUMPUR AND SELANGOR, MALAYSIA

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ABSTRACT

The effectiveness of insecticide-based dengue control interventions is very much influenced by the insecticide resistance status of the mosquito at the targeted areas. This study aims to determine the insecticide resistance status and the enzymatic activity. WHO adult bioassays conducted on *Ae. aegypti* and *Ae. albopictus* from 12 dengue hotspots outbreak areas in Kuala Lumpur and Selangor towards insecticides currently and historically used for mosquito control in Malaysia which include two pyrethroids, one organoclorine, one organophosphate and one carbamate. Biochemical enzyme assays were conducted and the activity of enzymes α-Esterase, MFO, GST and AChE were examined. Kruskal-Wallis H, Mann-Whitney U and ANOVA test were used to determine the significant difference of the mortality between insecticides and localities, the enzymes activity between the field and the lab strains, and the enzymes activity within all field strains. *Ae. aegypti* from all sites have developed resistance towards all tested insecticides based on WHO adult bioassays; permethrin, DDT, malathion and propoxur. The result of biochemical enzyme assays demonstrated that the activity of enzymes was altered. α-esterase and MFO were altered in both species from all areas. GST was altered in both species as well except in *Ae. albopictus* from sites Bandar Rinching and Taman Gombak Setia. AChE was found significantly demoted in *Ae. aegypti* from Sri Nilam Shah Alam and *Ae. albopictus* from Flat Sri Labuan Cheras only. The resistance detected might be the result of activity by either single or several enzymes combined. The development of resistance is mainly via metabolic mechanism.

Keywords: Insecticide resistance, adult bioassay, biochemical assay, mapping of insecticide resistance.

ABSTRAK

Keberkesanan kawalan denggi berasaskan racun serangga adalah sangat dipengaruhi oleh status kerintangan nyamuk di kawasan sasaran. Kajian ini bertujuan mengenalpasti status kerintangan nyamuk dan aktiviti enzim. Kaedah bioasai WHO nyamuk dewasa dijalankan ke

atas *Ae. aegypti* dan *Ae. albopictus* dari 12 kawasan titik panas wabak denggi di Kuala Lumpur dan Selangor terhadap racun serangga yang telah dan sedang digunakan untuk kawalan nyamuk di Malaysia. Racun serangga yang digunakan adalah dua pyrethroids, satu organoklorin, satu organofosfat dan satu karbamat. Asai enzim biokimia dijalankan dan aktiviti enzim α- Esterase, MFO, GST dan AChE diperiksa. Ujian Kruskal-Wallis H, Mann-Whitney U dan ANOVA digunakan untuk mengenalpasti perbezaan yang signifikan bagi racun serangga dan lokaliti yang berbeza terhadap kematian nyamuk, perbezaan aktiviti enzim di antara nyamuk lapangan dan nyamuk makmal, serta perbezaan aktiviti enzim di antara semua populasi nyamuk lapangan. *Ae. aegypti* lapangan dari semua lokaliti didapati rintang terhadap semua racun serangga yang diuji; permethrin, DDT, malathion dan propoxur. Keputusan asai enzim biokimia menunjukkan bahawa aktiviti enzim-enzim telah berubah. Enzim α-esterase dan MFO telah berubah di dalam kedua-dua spesies nyamuk di semua lokaliti. Perubahan enzim GST juga telah berlaku ke atas kedua-dua spesies nyamuk di semua lokaliti kecuali bagi *Ae, albopictus* Bandar Rinching dan Taman Gombak Setia. Enzim AChE telah dikenalpasti menurun di dalam nyamuk *Ae. aegypti* Sri Nilam Shah Alam dan *Ae. albopictus* Bandar Tun Razak Cheras. Kerintangan yang dikenalpasti mungkin kesan daripada satu atau kombinasi beberapa aktiviti enzim. Pembangunan kerintangan bermula melalui mekanisme metabolik.

Kata kunci: Kerintangan insektisid, bioasai dewasa, asai kimia, pemetaan insektisid.

INTRODUCTION

Dengue has become one of the fastest growing mosquito-borne diseases in the world since 1950s (Alexander et al. 2011) and has caused significant public health burden globally (Garg et al. 2008; Jahan et al. 2016; Murray et al. 2012). In Malaysia, the first known published account of dengue outbreak was reported in 1902 by Skae. It was not until 1962 that the first severe dengue, Dengue Haemorrhagic Fever (DHF) was observed in Malaysia in the city of Georgetown, Penang. From the 1960s, dengue cases began to spread into the urban areas of Penang and Kuala Lumpur (George & Lam 1997). By the early 1970s, DHF had spread to the entire country and has since caused a significant health burden to the population. The dengue situation in Malaysia since then has worsened with an increasing number of reported cases and death during the last decade (Mia et al. 2013). In 2019, there were 130101 dengue cases with 182death reported by the Ministry of Health Malaysia (2020).

Dengue is caused by four serotypes of dengue viruses namely DEN-1, DEN-2, DEN-3 and DEN-4 belonging to the Flaviviridae family. They are closely related but antigenically distinct. Dengue viral infection is known to cause either dengue fever (DF), dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). The virus is transmitted to humans by the bite of infected female *Aedes (Ae.) aegypti* and *Ae. Albopictus* (Lee et al. 1997; Rohani et al. 2014; Rohani et al. 1997). Female mosquitoes remain infectious for their entire lives and have the potential to transmit virus during each human feeding.

To date, with effective dengue vaccines and effective antiviral treatments still not yet available, dengue control relies heavily on vector control activities (Lee at al. 2015). For many years, the usage of insecticide is considered as the primary measure to control mosquito vectors (Dusfour et al. 2019). Basically, there are four classes of insecticide used in dengue vector control program namely organochlorine, organophosphates, pyrethroids and carbamates (WHO 2011). According to Phang and Loh (2016), understanding the seasonal cycles of disease transmission provides a fundamental basis to guarantee a success of such a vector control program. Nevertheless, the rapid application of an insecticide has shown to cause

development of insecticide resistance in mosquito through improper application such as under dose and overdose, bad coverage, extended spraying intervals and spraying frequency in operational field (IRAC 2011). Therefore, it is crucial to monitor the development of insecticide resistance in mosquito that inhabit the hotspot areas to avoid ineffective vector control measures and wastage.

In Malaysia, monitoring and screening for existence of resistance in mosquitoes against numerous insecticides have been long comprehensively implemented, however, they have not been systematically documented or reviewed. This study therefore aims to determine the resistance status of *Ae. aegypti* and *Ae. albopictus* mosquito of dengue hotspot areas in Kuala Lumpur and Selangor towards permethrin (pyrethroids type 1class), lambda-cyhalothrin (pyrethroids type 2 class), dichlorodiphenyltrichloroethane (DDT) (organochlorine class), malathion (organophosphates class) and propoxur (carbamates class). The activity of several enzymes was also examined in order to determine the resistance mechanism involved, followed by mapping of the resistance status and enzyme activity. It is hope that information gathered from this study able to not only improve the efficacy of the current control measure in the fight to combat dengue but increase cost effectiveness.

MATERIALS AND METHODS

Study Sites

The study was carried out at 12 highest repeated outbreak sites recorded from 2011-2016 by Vector Borne Disease Research Centre, Ministry of Health Malaysia. All study sites are situated in 2 states, namely Kuala Lumpur (KL) and Selangor. Table 1 shows the twelve sites selected, coded according to the name of its location.

Table 1. The areas selected for the study

No.	State	District	Name of the Site	Code	Coordinate
1	Selangor	Petaling	Damansara PJU5 Sect. 6	P	3.15952, 101.587915
2	Selangor	Petaling	Sri Nilam, Shah Alam.	S	3.066817001, 101.484778
3	Selangor	Petaling	Jalan Bandar Kinrara 2	K	3.052313961, 101.646058
4	Selangor	Hulu Langat	Taman Teknologi	T	2.966325022, 101.827166
5	Selangor	Hulu Langat	Bandar Rinching	R	2.928491961, 101.854371
6	Selangor	Hulu Selangor	Taman Bunga Raya	B	3.436147962, 101.545038
7	Selangor	Sepang	Taman Permata Dengkil	D	2.863363987, 101.681992
8	Selangor	Klang	Jalan Hulubalang	H	3.000909, 101.473635
9	Selangor	Kuala Langat	Taman Aman Banting	A	2.806742974, 101.50199
10	Selangor	Kuala Selangor	Jalan CakeraPurnama,	Z	3.238923959, 101.424971
11	Kuala Lumpur	Gombak	Taman Gombak Setia	G	3.220175989, 101.718231
12	Kuala Lumpur	Bandar Tun Razak	Flat Sri Labuan Cheras	C	3.090491015, 101.720972

Mosquito Collection
Ovitrap surveillance were performed from January 2017 to December 2018. Standard ovitraps containing 200 ml of tap water were used to collect field strains *Ae. aegypti* and *Ae. albopictus*. For each study site, two trips to deploy 40 ovitraps randomly outside 40 houses were conducted. Mosquito larvae were collected from the traps the following week. The two trips were a week apart.

Mosquito Colonization
The collected mosquito larvae were further reared in the Insectarium of Institute for Medical Research, Kuala Lumpur (IMR). In the insectary, the paddles were transferred into labelled plastic containers. Half-cooked local cow liver was given daily as the larval food until they pupated. The emerged mosquito adults were identified morphologically (Jeffery 2012; IMR 2000). They were then placed in separated cages according to the species. The number of *Ae. aegypti* and *Ae. albopictus* mosquito from each container were recorded. Both species were supplied with white mice for blood-feeding to obtain eggs of the 1st and 2nd generation. The larvae were routinely fed on liver powder (BD Difco™, USA) and bovine liver chunk diet. Adult mosquitoes were provided with 10% sucrose solution. The insectarium was set up to 26±2 ^{o}C temperature and 60±10% relative humidity.

Adult Mosquito Resistant Study
Resistant study for the field strains' colony were conducted according to WHO adult bioassay standard procedure. WHO-impregnated papers were obtained from the Vector Control Research Unit, Universiti Sains Malaysia (USM), Penang. Established diagnostic dosage by WHO (2016) were applied for this study as follows: permethrin 0.25% and lambda-cyhalothrin 0.03% (pyrethroids), DDT 4% (organochlorine), malathion 0.8% (organophosphates) and propoxur 0.1% (carbamates). Unfed female mosquitoes aged 3 to 5days from the 1st or 2nd generation were used for the test. Twenty mosquitoes were introduced into seven WHO holding tubes (five tests and two control) and held for 1 hour. They were then gently blown into the exposure tubes containing the insecticide-impregnated papers. The same field strains of *Ae. aegypti* and *Ae. albopictus* were exposed into impregnated paper with olive oil, risella oil and silicone oil as the control for organophosphates and carbamates, organochlorine and pyrethroids, respectively. The number of knocked down mosquitoes were recorded every 1, 3 or 5 minutes after exposure. After one-hour of exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% sucrose solution and mortalities at 24 hours were recorded. Survived mosquitoes were killed by freezing and stored at -80 °C in an individual, clearly labelled 1.5ml micro centrifuge tubes for further analysis.

Biochemical Assay
Biochemical assays were performed in order to detect insecticide resistances in *Ae. aegypti* and *Ae. albopictus* population by examining the enzymatic activities. The survived field-collection strains of mosquitoes previously exposed and unexposed to insecticide were individually assayed for nonspecific α-esterases, Mixed Function Oxidase (MFO), Glutathione S-transferase (GST) and insensitive Acetyl cholinesterase (AChE) enzymatic activities. Lab strain unexposed to any insecticides aged 3 to 5days old was used as the susceptible reference.

The modified microplate methods described by Lee et al. (1992), Nazni et al. (2000), Lee and Chang (1995) and Brogdon et al. (1988) was used to perform the biochemical assay for α-esterases, MFO, GST and AChE, respectively. A standard equation curve developed from the known serial concentration of α-naphthol for the α-esterases assay and cytochrome C for the MFO assay were used. The protein concentration for each mosquito was determined by

Bradford (1976) method with bovine serum albumin (Sigma, United States) used as standard. For each experiment, at least 30 mosquitoes per species were assayed. In order to avoid enzyme degradation, the preparation of the mosquito homogenate was performed on ice. All assays were conducted in quadruplicates using 96-well microplates. The absorbance values were measured using immunoassay reader (Dynatech, Model MR 5000) at the wavelength indicated for each enzyme (450 nm for α-esterases, 630 nm for MFO, 405 nm for GST, 414 nm for AChE and 595 nm for protein). Determination of the α-esterases enzyme activities was expressed in mole of α-naphthol equivalent/minutes/mg of protein, the MFO enzyme activities was expressed in mole of Cytochrome-C equivalent/minutes/mg of protein, while the GST enzyme activities was expressed in mole of 1-chloro-2,4-diniteobenzene conjugated/minutes/mg of protein. For the AChE enzyme, percentage residual activity was determined by dividing the mean absorbance value of the well inhibited with propoxur by the mean absorbance value without propoxur (uninhibited) times 100%.

Data Analysis

Data were analysed as follow:

i. $$\text{Mortality percentage} = \frac{\text{Total number of dead mosquitoes}}{\text{Total number of exposed mosquito}} \text{ X } 100\%.$$

If the control mortality is above 20%, the test should be discarded and if the control is below 5% the test can be ignored, and no correction is necessary. If the tests are greater than 5% but less than 20% the observed, mortality had to be corrected using Abbots formula (Abbott 1925):

ii. $$\text{Abbots formula} = \frac{(\text{\% Observed mortality- \% Control mortality})}{(100 - \text{\% Control mortality})} \text{ X } 100\%.$$

Mortality results were interpreted into susceptible, low resistance, moderate resistance and high resistance. Mortality range between 98 to 100% indicates susceptible. Mortality more than 97% but less than 98% indicates that low resistance suggested. Further tests are needed to verify. If at least two additional tests consistently showed mortality below 98%, then resistance is confirmed. For mortality 90% to 97%, it shows moderate resistance, thus, mortality below 90% indicates high resistance.

iii. The mean enzyme activities of the unexposed to insecticide field-collected mosquito divided by the susceptible lab strain, enzyme increase/decrease fold was determined. All results were then evaluated by statistical analysis using SPSS version 23.

***Ae. aegypti* and *Ae. albopictus* Resistance Status Geodatabase**

Administrative borders (state and districts) and data on demographical characteristics were retrieved from Department of Survey and Mapping Malaysia (JUPEM) with the copyright license serial number 0930. The resistance and enzymatic level status data were converted into feature layers in a GIS database and overlaid over raster image using ArcGIS 9.3. All digital data in the geodatabase was displayed in the WGS 1984 Coordinate system.

RESULTS

Mapping of Insecticide Mortality Profiles of *Aedes* Mosquito

The results of WHO assay tests on *Ae. aegypti* and *Ae. albopictus* collected were illustrated in Figure 1. For *Ae. aegypti*, low mortality rate (range 0% to 70.44%) was observed against established diagnostic dosage of permethrin, lambda-cyhalothrine, DDT, malathion and propoxur for all study sites (Table 2). In contrast, *Ae. albopictus* displayed slightly different mortality profile (Table 2). Kruskal-Wallis H test confirmed that the mortality of *Ae. aegypti* and *Ae. albopictus for* each study site were found to be significantly different for all the insecticides tested ($\chi^2_{(4)} = 11.84$, $p < 0.05$) ($\chi^2_{(4)} = 39.38$, $p < 0.05$). Mortality of adult mosquito from 12 different sites were found to be significantly different for *Ae. aegypti* ($\chi^2_{(11)} = 20.36$, $p < 0.05$) but not significantly different for *Ae. albopictus* ($\chi^2_{(11)} = 9.35$, $p > 0.05$).

Table 2. Mortality percentage of *Ae. aegypti* and *Ae. albopictus* from 12 study sites against insecticides

Strain	Mortality (%)				
	Permethrin 0.25%	**Lambda-cyhalothrin 0.03%**	**DDT 4%**	**Malathion 0.8%**	**Propoxur 0.1%**
Ae. aegypti[a]					
P	29.17	32.95	1.33	7.04	3.95
S	70.44	48.41	31.79	28.42	12.60
K	7.61	7.00	2.00	6.32	8.11
T	7.69	17.44	1.43	15.22	16.85
R	17.65	35.63	10.47	5.97	23.71
B	15.29	13.21	3.09	60.07	9.51
D	8.99	62.96	3.09	3.00	4.55
H	5.88	22.35	3.03	6.19	27.91
A	8.89	13.28	23.61	3.38	3.38
Z	8.42	10.00	2.67	1.03	0.00
G	1.89	9.39	21.15	4.35	7.45
C	65.43	86.30	7.72	27.85	20.00
Ae. albopictus[b]					
P	93.97	92.93	87.37	6.54	20.87
S	95.87	98.95	96.91	3.43	10.80
K	50.00	97.00	71.43	15.89	67.39
T	91.00	81.00	96.00	0.00	6.00
R	66.67	98.95	71.70	16.00	71.11
B	81.93	73.24	60.34	1.25	5.00
D	94.58	100.00	96.39	59.85	78.01
H	88.66	93.62	77.03	12.50	17.20
A	93.90	95.45	61.18	32.10	61.45
Z	88.54	76.84	26.79	0.00	1.00
G	74.83	80.98	63.89	4.88	0.00
C	85.22	80.58	47.46	2.00	12.50

Kruskal-Wallis H test: a) The mortality of *Ae. aegypti* against temephos 0.012mg/L and 1mg/L were found not to be significantly different between all the study sites ($\chi^2_{(11)} = 11.00$, $p > 0.05$) and ($\chi^2_{(11)} = 11.00$, $p > 0.05$) respectively. b) The mortality of *Ae. albopictus* against temephos 0.012mg/L and 1mg/L were found not to be significantly different between all the study sites ($\chi^2_{(11)} = 11.00$, $p > 0.05$) and ($\chi^2_{(11)} = 0.00$, $p > 0.05$) respectively.

Mapping of Insecticides Resistant Status of Aedes Mosquito

Figures 2 and 3 show the geographical spread of the insecticide resistance status of *Ae. aegypti* and *Ae. albopictus* against selected insecticides for all study sites. Map of insecticide resistant status of *Ae. aegypti* (Figure 2) indicated high resistance towards permethrin, lambda-cyhalothrin, DDT, malathion and propoxur for all 12 dengue hotspots outbreak areas study sites. Map of insecticide resistant status of *Ae. albopictus* (Figure 3) displayed high resistance for R, B, H, Z, G and C strains; and moderate resistance for P, S, K, T, D and A strains towards permethrin. *Ae. albopictus* displayed high resistance for T, B, Z, G and C strains; moderate resistance for P, K, H and A strains; and susceptible for S, R and D strains towards lambda-cyhalothrin. High resistance towards DDT was displayed by *Ae. albopictus* from 9 out of 12 strains (P, K, R, B, H, A, Z, G and C) whereas moderate resistance was displayed by 3 out of 12 strains (S, T and D). *Ae. albopictus* from all 12 sites displayed high resistance towards malathion and propoxur.

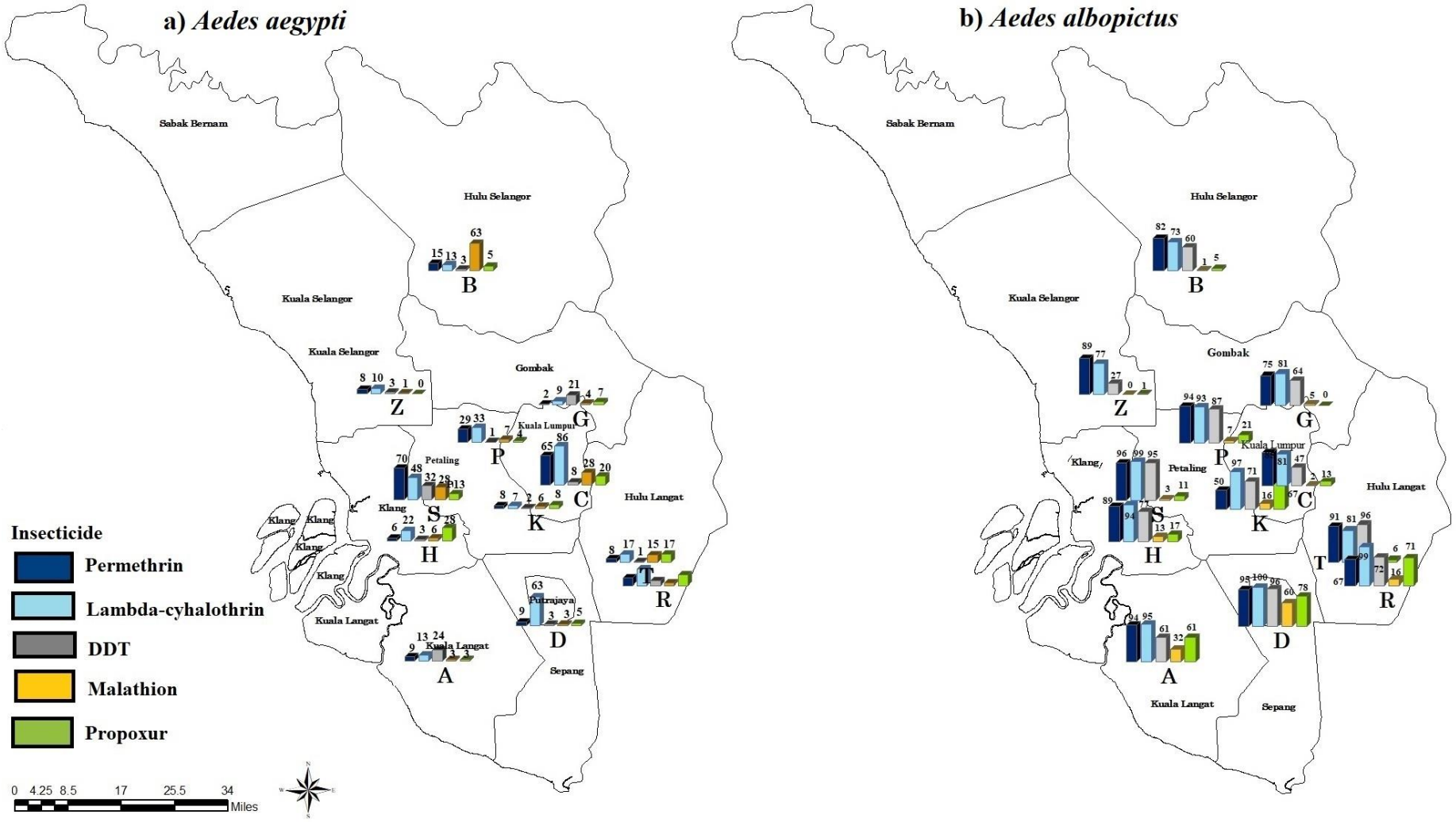

Figure 1. Mortality rates of adult female a) *Ae. aegypti* and b) *Ae. albopictus* against selected insecticides

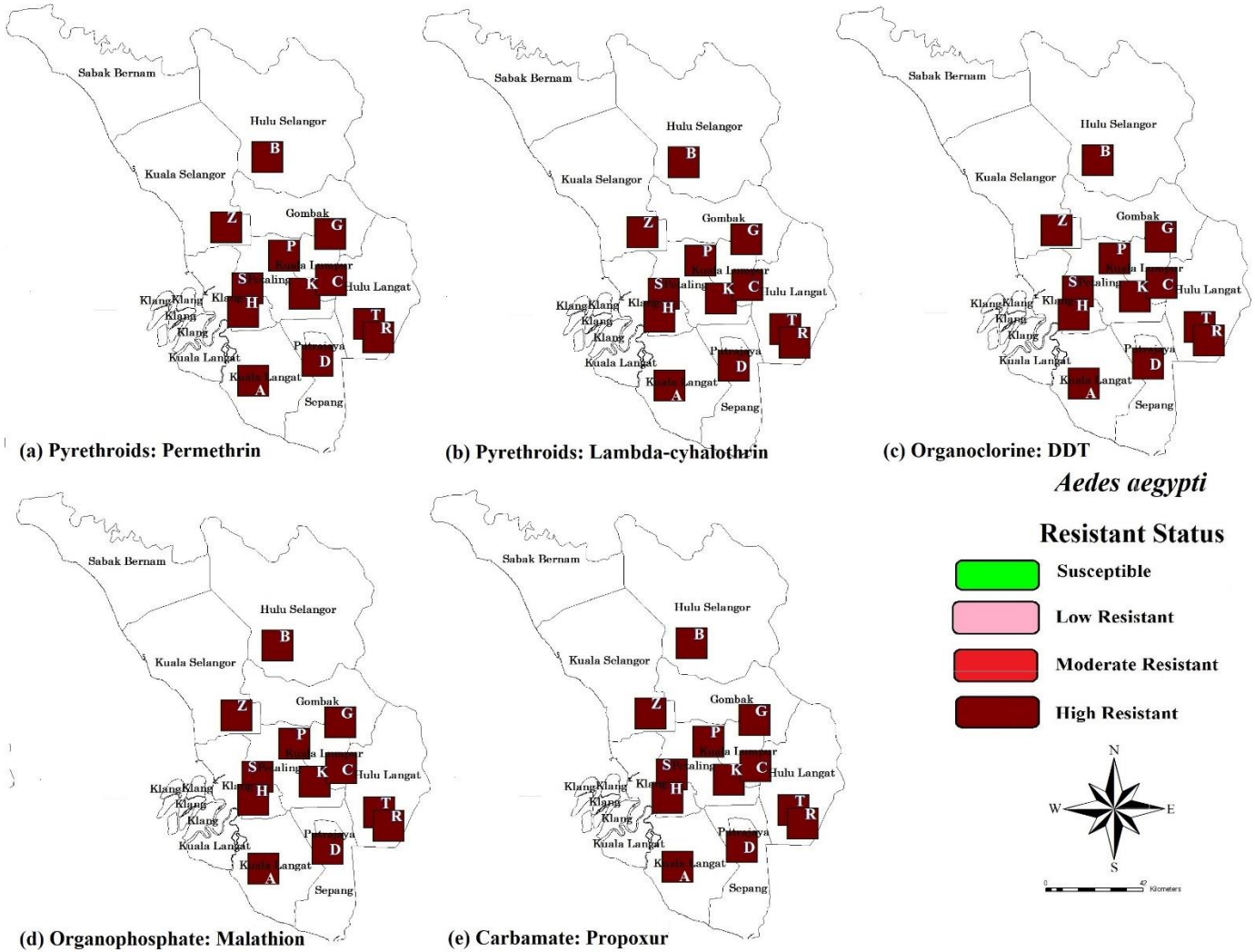

Figure 2. Insecticide resistant status of *Ae. aegypti* towards pyrethroids (Permethrin and Lambda-cyhalothrin), organochlorine (DDT), organophosphate (Malathion) and carbamate (Propoxur). The map is zoomed to the state of Kuala Lumpur and Selangor, Malaysia

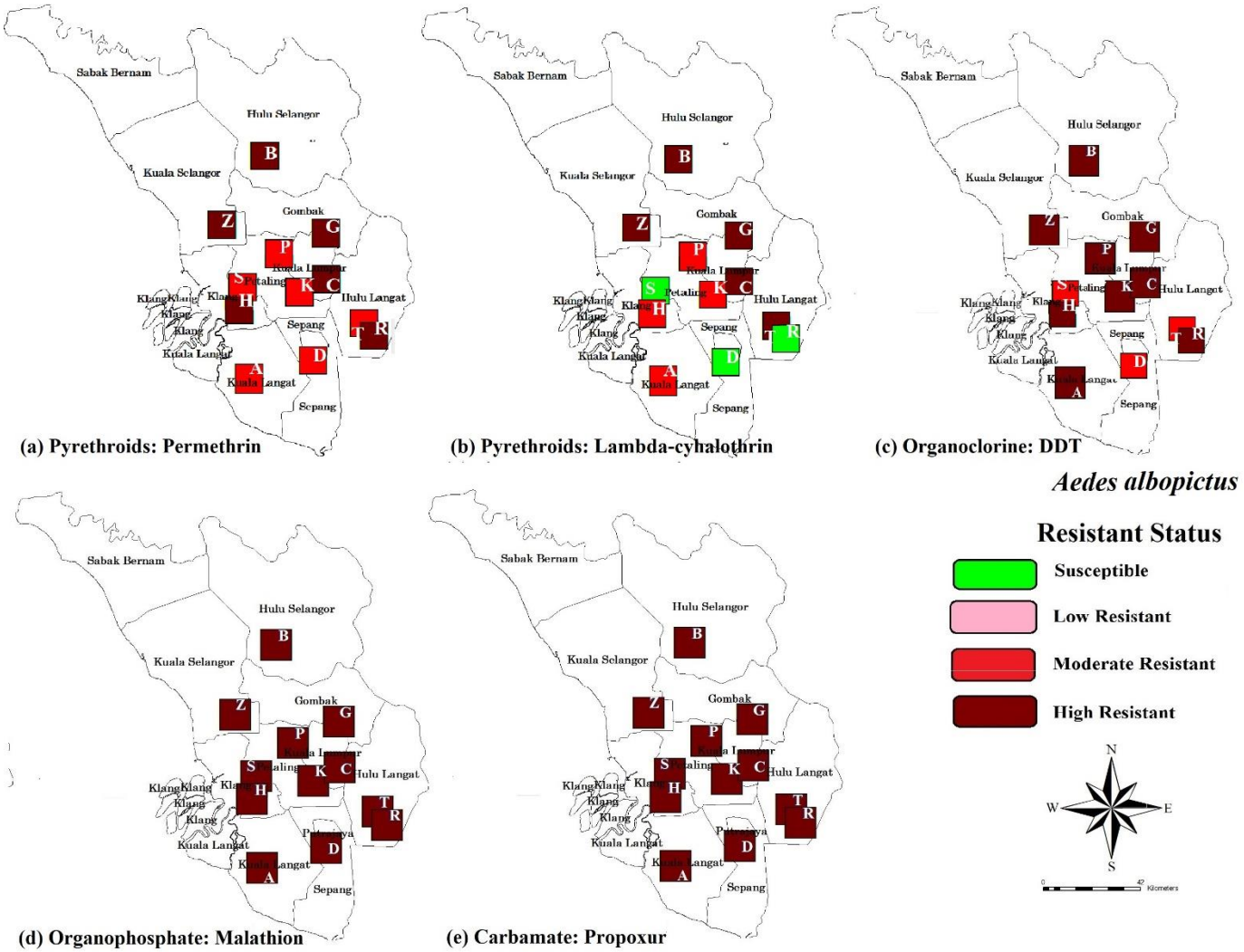

Figure 3. Insecticide resistant status of *Ae. albopictus* towards pyrethroids (Permethrin and Lambda-cyhalothrin), organochlorine (DDT), organophosphate (Malathion) and carbamate (Propoxur). The map is zoomed to the state of Kuala Lumpur and Selangor, Malaysia

Biochemical Assays Profiles of Aedes Mosquito

Figure 4 represent the α-Esterase, mixed function oxidase, glutathione S-transferase and acetylcholinesterase enzymatic activities of field population *Ae. aegpyti* and *Ae. albopictus*. The enzymatic activities of 12 field strains were compared to susceptible laboratory strain using Mann-Whitney U-test (Table 3). All field strains of *Ae. aegpyti* and *Ae. albopictus* exhibited significant elevation of α-esterase activity ($P < 0.05$). Significant increase in MFO activities were also detected in both *Aedes* species from all the field population except for *Ae. albopictus* from H strain ($p > 0.05$).

Table 3. Mann-Whitney U test for enzymes activities of *Ae. aegypti*and *Ae. Albopictus* strain from 12 study sites

Strain	N	α-Esterase	MFO	GST	AChE
Ae. aegypti					
P	30	49.00**	3.00**	0.00**	0.50**
S	30	18.50**	0.00**	0.00**	139.50**
K	30	20.00**	48.00**	1.00**	187.00**
T	30	0.00**	3.00**	59.00**	156.50**
R	30	0.00**	172.00**	18.00**	345.00
B	30	3.50**	104.00**	0.00**	355.50
D	30	0.00**	15.00**	8.50**	79.50**
H	30	0.00**	0.00**	4.00**	120.00**
A	30	0.00**	83.50**	5.00**	313.00*
Z	30	0.00**	0.00**	17.00**	398.00
G	30	0.00**	0.00**	233.00**	231.00**
C	30	1.00**	204.50**	7.00**	425.00
Ae. albopictus					
P	30	0.00**	22.00**	23.00**	110.50**
S	30	1.00**	17.50**	34.00**	0.00**
K	30	0.00**	18.00**	19.00**	112.00**
T	30	28.00**	30.50**	12.00**	65.50**
R	30	0.00**	205.50**	401.00	111.50**
B	30	0.00**	0.00**	0.00**	30.50**
D	30	0.00**	166.50**	26.00**	0.00**
H	30	0.00**	328.00**	0.00**	6.00**
A	30	0.00**	47.50**	0.00**	0.00**
Z	30	0.00**	3.00**	0.00**	0.00**
G	30	0.00**	6.50**	205.50**	94.50**
C	30	38.000**	0.00**	29.00**	310.00*

**$p < 0.001$ *$p < 0.05$

Most of the field strain demonstrated significant increase of GST enzymatic activities compared to susceptible laboratory strain except in *Ae. albopictus* from R strain ($p > 0.05$). For AchE with propoxur inhibition, field strain *Ae. aegpyti* from P, K, T, D, H, A, and G exhibited significant elevation ($p < 0.05$). In contrast, AChE with propoxur inhibition for *Ae. aegpyti* from S was significantly demoted ($p < 0.05$). AChE with propoxur inhibition was found elevated significantly ($p < 0.05$) in *Ae. albopictus* strains from P, S, K, T, R, B, D, H, Z, G and

C. On the contrary, for *Ae. albopictus* C strain, AChE with propoxur inhibition was found demoted significantly ($p < 0.05$).

The mean enzymatic activities within all field strain were compared using ANOVA test (Table 4). The analysis of α-esterase enzyme activity showed that *Ae. aegypti* of D strain demonstrated the highest activity (131.70 nmole α-napthol/min/mg protein) which was significantly higher than other field strains except A strain and was 5.6 folds higher compared to the laboratory strain. As for *Ae. albopictus*, the B strain was found to be the strain with the highest NSE enzyme activity (167.90 nmole α-napthol/min/mg protein) and significantly different than S, K, R, T, A, G and C strains. It was 5.7 folds higher compared to the laboratory strain.

The analysis of MFO enzyme activity showed that, among all the field strains of *Ae. aegypti* mosquito tested, H strain exhibited the highest MFO activity of 2.00 nmole cyto-c/min/mg protein which was significantly higher than other field strains. It was 4.7folds higher than the laboratory strain. *Ae. albopictus* of the A strain showed the highest MFO activity of 1.34 nmolecyto-c/min/mg protein. It was significantly different compared to other field strains except P and B strains and was 3.1 folds higher than the laboratory strain.

The analysis of GST enzyme activity showed that P strain of *Ae. aegypti* exhibited the highest GST activity of 12.71 mmole CDNB/min/mg protein and significantly different compared to other field strains. It was 2.4 folds higher compared to the laboratory strain. As for *Ae. albopictus*, the H strain was found to be the strain with the highest GST enzyme activity of 13.79 mmole CDNB/min/mg protein and significantly higher than other strains with 4.0 folds higher compared to the laboratory strain.

Comparing the findings within all the field strains, *Ae. aegypti* from P strain showed the highest AChE inhibition of 66.80% and it was significantly different. It was 1.7 folds higher than the laboratory strain. *Ae. albopictus* of the Z strain was the strain that showed the highest AChE inhibition of 72.10% and was 3.1 folds higher than the laboratory strain.

Table 4. Mean±SD of α-Esterase, MFO, GST and AChE enzyme value of *Ae. aegypti* and *Ae. albopictus.*

	α-Esterase (Mean±SD)	**increase/ decrease fold**	**MFO (Mean±SD)**	**increase/ decrease fold**	**GST (Mean±SD)**	**increase/ decrease fold**	**AChE (Mean±SD)**	**increase/ decrease fold**
Ae. aegypti								
Lab strain	23.70±6.58	-	0.43±0.13	-	5.20±1.08	-	39.93±6.14	-
P	40.77±9.79^{a}	1.7	1.08±0.30^{ef}	2.5	12.71±1.72^{j}	2.4	66.80±9.31^{k}	1.7
S	59.60±17.45^{ab}	2.5	0.98±0.19^{cdf}	2.3	9.08±1.63^{bcde}	1.7	29.03±7.93^{a}	0.7
K	69.80±26.95^{bc}	2.9	0.68±0.13^{a}	1.6	10.59±4.10^{efghi}	2.0	52.87±14.019^{gi}	1.3
T	93.33±21.03^{cdeg}	3.9	1.532±0.42^{g}	3.6	7.75±1.50^{ab}	1.5	49.77±8.82^{cdefgh}	1.2
R	78.37±21.84^{be}	3.3	0.60±0.14^{a}	1.4	8.52±0.99^{ad}	1.6	43.33±5.87^{be}	1.1
B	74.07±27.80^{bd}	3.1	0.72±0.19^{ac}	1.7	10.14±2.32^{dh}	2.0	43.03±9.54^{bd}	1.1
D	131.70±47.53^{k}	5.6	0.74±0.11^{ad}	1.7	7.99±0.98^{ac}	1.5	52.97±7.98^{gj}	1.3
H	100.17±46.49^{dei}	4.2	2.00±0.76^{h}	4.7	9.70±2.10^{bcdg}	1.9	57.27±15.04^{hij}	1.4
A	113.63±42.20^{fghijk}	4.8	0.96±0.42^{bcde}	2.2	10.54±3.50^{efghi}	2.0	44.20±8.06^{bf}	1.1
Z	101.87±20.87^{ej}	4.3	1.47±0.32^{g}	3.4	10.17±2.85^{di}	2.0	42.17±11.23^{bc}	1.1
G	99.97±35.59^{deh}	4.2	0.69±0.15^{ab}	1.6	6.60±1.66^{a}	1.3	46.63±9.52^{bg}	1.2
C	93.17±29.07^{cdef}	3.9	0.58±0.14^{a}	1.3	9.14±2.15^{bcdf}	1.8	39.43±6.94^{b}	0.9
Ae. albopictus								
Lab strain	29.67±5.57	-	0.44±0.14	-	3.43±0.73	-	22.97±4.01	-
P	164.23±51.37^{gh}	5.5	1.27±0.519^{d}	3.0	9.89±3.47^{f}	2.9	33.40±7.54^{b}	1.5
S	91.97±29.68^{bcd}	3.1	0.91±0.20^{c}	2.0	10.53±3.69^{fg}	3.1	54.00±12.81^{c}	2.4
K	93.17±30.04^{bce}	3.1	0.86±0.15^{bc}	2.0	5.69±0.79^{cd}	1.7	31.70±9.42^{b}	1.3
T	53.90±15.26^{a}	1.8	0.96±0.30^{c}	2.2	6.84±1.57^{ce}	2.0	37.57±8.94^{b}	1.6
R	73.20±17.97^{ab}	2.5	0.63±0.18^{ab}	1.4	3.29±0.63^{a}	0.9	33.40±6.55^{b}	1.5
B	167.90±73.79^{h}	5.7	1.31±0.30^{d}	3.1	11.80±2.60^{gh}	3.4	38.33±8.19^{b}	1.7
D	146.00±38.81^{fh}	4.9	0.75±0.28^{ac}	1.7	6.46±1.82^{bde}	1.9	48.33±8.55^{c}	2.1
H	167.70±33.41^{h}	5.7	0.56±0.20^{a}	1.3	13.79±1.83^{i}	4.0	49.80±16.30^{c}	2.2
A	130.43±53.98^{fg}	4.4	1.34±0.65^{d}	3.1	12.32±1.47^{h}	3.6	50.00±8.99^{c}	2.2
Z	163.97±37.97^{gh}	5.5	0.94±0.19^{c}	2.1	10.00±2.18^{f}	2.9	72.10±12.48^{d}	3.1
G	125.13±62.08^{def}	4.2	0.94±0.15^{c}	2.1	4.80±1.32^{abd}	1.4	34.43±8.95^{b}	1.5
C	87.77±33.36^{ac}	3.0	1.00±0.16^{c}	2.3	5.44±1.48^{bc}	1.6	20.90±5.07^{a}	0.9

Values followed by different letters within a column for each are significantly different (one-way ANOVA followed by Tukey test, $P < 0.05$).

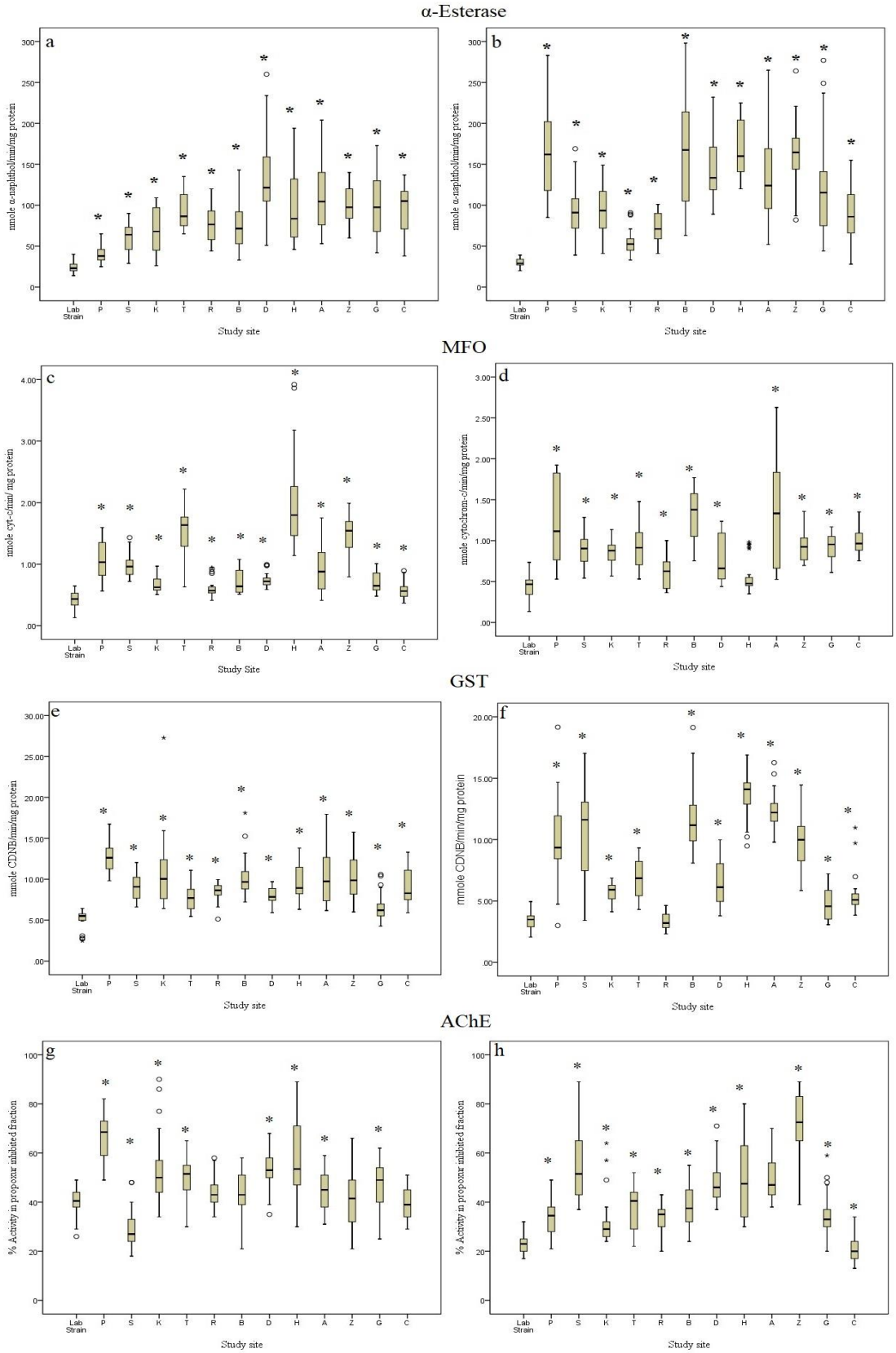

Figure 4. Activity profiles of α-esterase, mixed function oxidase, glutathione S-transferase and acethyl chlorinesterase with propoxur inhibition in *Ae. aegypti* (a, c, e, g) and *Ae. albopictus* (b, d, f, h). Asterisks indicate significant differences compared to susceptible laboratory strain ($p < 0.05$, Mann-Whitney tests)

Map of Enzymatic Activity of Aedes Mosquito

Figures 5 and 6 displayed distribution of α-esterases, MFO, GST and AChE fold activity for 12 study sites. The map illustrated that α-esterase in D strain of *Ae. aegypti* and P, B, H, and Z strains of *Ae. albopictus* expressed enzyme activity ranging from 5 to 6 folds. P strain of *Ae. aegypti* and T strain of *Ae. albopictus* expressed lowest α-Esterase enzyme activity (1 to 2 folds) compared to other field strains. For MFO enzyme activity, H strain of *Ae. aegypti* expressed the highest (4 to less than 5 folds) compared to other strains. MFO enzyme activity 3- < 4 folds expressed in T and Z strains of *Ae. aegypti* and from B and A strains of *Ae. albopictus*.

For GST, 7 out of 12 of the *Ae. aegypti* strains expressed enzyme activity ranging from 1 to less than 2 folds except P, K, A, B and Z strains which expressed enzyme activity ranging from 2 to less than 3 folds. H strain of *Ae. albopictus* expressed GST enzyme activity ranging from 4 to less than 5 folds. S, B, and A strains expressed GST enzyme activity ranging from 3 to less than 4 folds. Meanwhile, R strain showed decline in the enzyme activity compared to the susceptible laboratory strain.

For AChE, 10 out of 12 *Ae. aegypti* field strains expressed inhibition activity ranging from 1 to less than 2 folds. The rest of the strains (S and C) expressed inhibition activity less than 1fold. In *Ae. albopictus*, the highest reading was obtained from Z strain (3 to less than 4 folds), followed by S, D, H and A strains with a reading of 2 to less than 3 folds, P, K, T, R, B and G strains revealed a reading of 1 to less than 2 folds. C strain indicated less than 1fold inhibition activity.

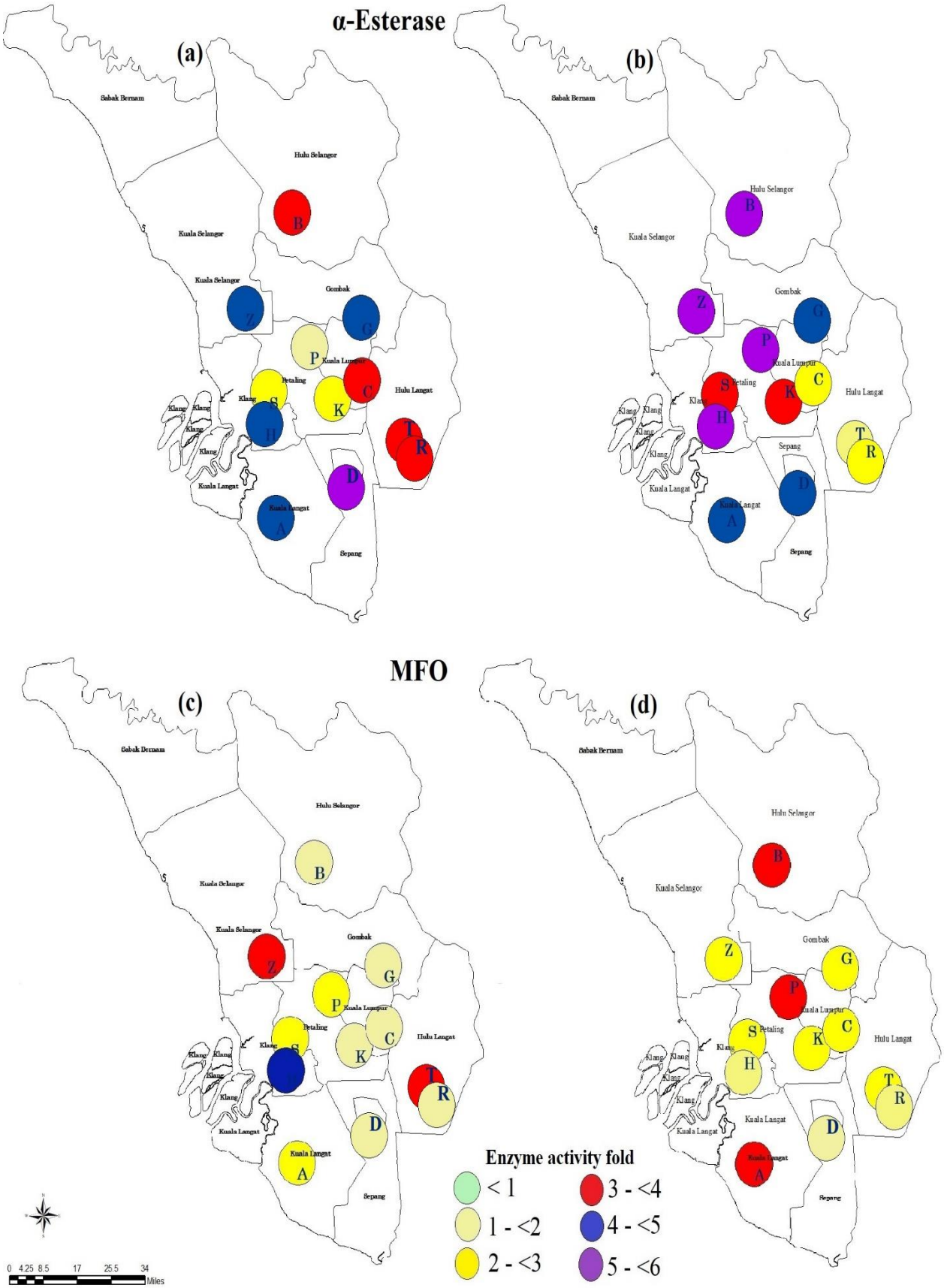

Figure 5. Mapping of enzyme activity (increase/decrease fold) of α-esterase and MFO in *Ae. aegypti* (a, c) and *Ae. albopictus* (b, d).

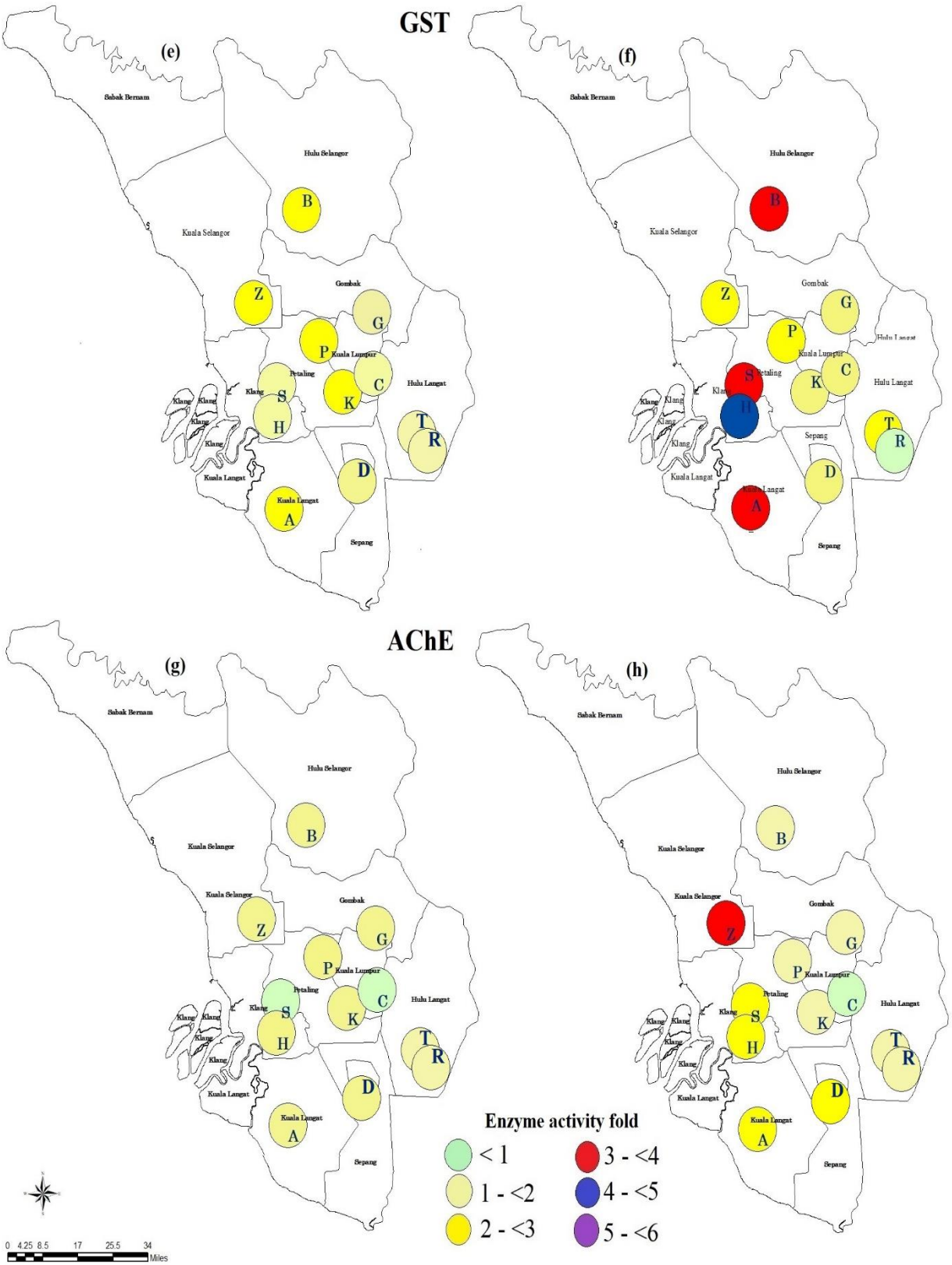

Figure 6. Mapping of enzyme activity (increase/decrease fold) of GST and AChE inhibition in *Ae. aegypti* (e, g) and *Ae. albopictus* (f, h).

DISCUSSION

Basically, mosquitoes are slowly becoming resistant to most insecticides used to control them. WHO describes resistance as the ability of mosquitoes to survive exposure to a standard dose of insecticide; caused by either physiological or behavioral change (WHO 2016). Being resistant allow mosquitoes to survive even large doses of chemicals that would be lethal to them normally. Left unchecked, insecticide resistance could lead to a substantial increase in incidence of mosquito borne diseases and mortality caused by mosquito borne diseases. As insecticide resistance continues to develop and spread, there is a real danger that these valuable tools will be lost. Moyes et al. (2017) reviewed on the available evidence for the geographical distribution of insecticide resistance on *Ae. aegypti* and *Ae. albopictus* and reported that resistance has occurred towards the 4 commonly used insecticide classes – pyrethroids, organochlorines, carbamates and organophosphates. It is widespread in both dengue vectors across the regions of the Africa, Americas and Asia. In Malaysia, evidence of resistance towards permethrin, lambda-cyhalothrin, DDT, malathion, temephos, bendiocarb, propoxur and cyfluthrin has been recorded in both species all over the country (Chen et al. 2013; Elia-Amira et al. 2018; Farah Ayuni et al. 2012; Hadura et al. 2015; Leong et al. 2019; Loke et al. 2012; Noor Aslinda et al. 2019; Rohani et al. 2001). Leong et al. (2019) reported that *Ae. aegypti* field strains of Gombak, Hulu Langat, Kuala Langat, Hulu Selangor, Kuala Selangor, Petaling and Sepang districts were resistant to five pyrethroid insecticides (cyfuthrin, deltamethrin, etofenprox, lambda-cyhalothrin and permethrin). A study conducted by Ishak et al. (2015) determined that *Ae. aegypti* and *Ae. albopictus* strains from Kuala Lumpur, Johor, Kelantan and Pulau Pinang showed resistance towards DDT and bendiocarb. DDT resistance has also been observed in *Ae. aegypti* strain of Kuala Lumpur from the study conducted by Hidayati et al. (2011), Nazni et al. (2009) and Rohani et al. (2001), and Selangor's strain by Loke et al. (2012). In Sabah, a few populations of *Ae. albopictus* was reported to be resistant towards DDT, malathion and temephos (Elia-Amira et al. 2018).

The 12 hotspot areas in this study were chosen in order to determine their insecticide resistance status and the mechanism involved and the possibly to have an insight in relation to the effectiveness of control method applied in these areas. By knowing the resistance status of the area, suitable and more effective control measures can be applied to help bring down number of cases in the areas. Results obtained from this study demonstrated that all *Ae. aegypti* population in all study sites has developed insecticide resistance towards all insecticides tested. *Ae. albopictus* population however showed development of resistance to all insecticides in most study sites with some areas remain susceptible to lambda-cyhalothrin.

One of the main reasons for the development of insecticide resistance is mutation where it caused mosquitoes to overproduce certain enzymes, which then absorb the insecticide before it can get to their nervous system and kill them. So, the insecticide is no longer harmful to the mosquitoes once they basically adapted to that point (Brogdon et al. 1997). Mutation happen when mosquitoes are exposed for too long to the same insecticide causing simultaneous increase in the number of copies of one or two corresponding genes resulting in overproduction of the enzymes (Faucon et al. 2015; Fouet et al. 2018).

Pyrethroids is a major class of insecticides in the pest control industry and is widely used in dengue and malaria control programmes (Yap et al. 2000) in many countries including Malaysia. Example of insecticides under this class is permethrin and lambda-cyhalothrin. In Malaysia permethrin has been used since 1999 (Rosilawati et al. 2017). DDT, an insecticide under the organochlorines class meanwhile has never been used for dengue control in Malaysia,

but has been utilized since the late 1950s until the 1980s for malaria eradication (Sandosham & Thomas 1983) until its usage was eventually stopped in 1998 (Yap et al. 2000) due to their toxicity (WHO 2012). Malathion which is classified under organophosphates' class on the other hand is one of the common insecticides use in Malaysia to control the vector of mosquito borne diseases especially during the outbreak session since 1970s (Lam & Tham 1988). Last but not least, propoxur, an insecticide under carbamates class has never been used as an active ingredient in vector control programmes or public health activities in Malaysia (Leong et al. 2019) but it was used as a common household pest control product in the early 1970s. Like DDT, its utilization was also terminated in the 1990s (Low et al. 2013b) due to their toxicity as well (ATSDR 2003)**.** Clearly, these insecticides used for one purpose or another have been applied for a very long period of time and thus, it is not surprising to find that mosquito population in hotspot areas in this study is highly resistant towards these insecticides most likely due to the intensive exposure during fogging operations for many dengue or other mosquito borne diseases outbreaks.

It is interesting though to note that resistance to lambda-cyhalothrin was detected in all *Ae. aegypti* and some *Ae. albopictus* populations although this compound was no longer used in the vector control programme. Similar finding regarding lambda-cyhalothrin has been discovered, resistance has been discovered in both *Aedes* species from Selangor (Leong et al. 2019) and Pulau Pinang (Hadura et al. 2015) as well. Development of resistance to lambda-cyhalothrin may be due to cross-resistance among the pyrethroids. Cross-resistance is defined as development of resistance by a population to one insecticide, exhibits resistance to one or more insecticide(s) that it has never encountered (Corbel & N'Guessan 2013). Such resistance development has been reported to occur in Colombia (Chaverra-Rodriguez et al. 2012), Mexico (Flores et al. 2013) and Malaysia (Leong et al. 2019; Rasli et al. 2018) where resistance is detected in permethrin resistant populations of *Ae. aegypti.* Since lambda-cyhalothrin and permethrin are insecticides of the same class, the cross-resistance could be described as "within class cross-resistance".

Similarly, although DDT was also no longer used these days, the study showed that resistance towards DDT was detected in *Ae. aegypti* and *Ae. albopictus* population and this could be due to cross-resistance to the pyrethroids as well. DDT was once used for indoor residual spray to combat malaria and it has been reported to cause a legacy of cross-resistance to pyrethroids (Brengues et al. 2003). Hemingway et al. (1989) who reviewed numerous reports indicated that DDT has conferred cross resistance to pyrethroids. Cross-resistance between DDT and pyrethroids (permethrin, cypermethrin and cyfluthrin) was also detected in the German cockroach, *Blattella germanica* (L.) (Limoee et al. 2006). Since this resistance involved insecticides of a different classes, such cross-resistance could be described as "between classes cross-resistance".

Insecticide resistance in *Aedes* mosquito is generally based on two main mechanisms; metabolic mechanism which occurs when enhanced levels or modified activities of enzymes such as esterases, oxidases, or glutathione S-transferases (GST) is produced, hence prevent the insecticide from reaching its site of action. While target-site mechanism occurs when the insecticide is no longer able to bind to its target-sites that became modified by the presence of an enzyme such as AChE thus causing the insecticide to be less effective or even ineffective. This target-site mechanism usually occurs in the voltage gated sodium channel, AChE and GABA receptors (Brogdon et al. 1997; Hemingway & Ranson 2000; WHO 1998).

Both mechanisms can be determined by performing biochemical enzyme assay. Based on maps of resistance status and maps of enzyme activity developed, it can be concluded that the resistance shown by *Ae. aegypti* and *Ae. albopictus* populations, is most likely associated with the high production of the α-esterase primarily, followed by MFO, and GST. This study strongly suggested that elevation of α-esterase among *Ae. aegypti* and *Ae. albopictus* population must have contributed to permethrin, lambda-cyhalothrin, malathion and propoxur resistance and therefore confirmed the type of mechanism involved as the metabolic mechanism. Resistance in *Ae. aegypti* and/or *Ae. albopictus* was previously validated by Rohaniet al. (2001), Low et al. (2013a), Frances et al. (2016) and Rosilawati et al. (2017) towards pyrethroids; Budi et al. (2017) towards carbamates; and Budi et al. (2017), Chen et al. (2008) and Lima et al. (2003) towards organophosphates. Study by Low et al. (2013b) in addition showed alteration of the α-esterases activity was associated with malathion resistance in *Culex quiquefasciatus*.

There were few studies that reported *Ae. aegypti* and *Ae. albopictus* pyrethroid resistance were associated with MFOs activity (Brogdon et al. 1997; Farouk et al. 2019; Pethuan et al. 2007; Wan Norafikah et al. 2010; Wan Norafikah et al. 2011). Study by Leong et al. (2019) reported that the MFO activity contributed to the DDT (organochlorine) resistance as well. Similarly, Brazillian *Ae. aegypti* population resistance towards organophosphate was associated to enhanced MFO activity (Diogo et al. 2016). Although this study concurred with those studies, the enhancement of MFO activity was not as high as α-esterases activity indicating that enhancement of MFO activity may not act as the only enzyme that caused resistance. Many studies have indicated that resistance development in individual mosquito species involved multiple enzymes. Resistance caused by multiple enzymes in populations of *Ae. aegypti* has been reported in Madeira Island Portugal (α- esterases, β-esterases and MFO) (Seixas et al. 2017) and Martinique Island (MFO, AChE and GST) (Marcombe et al. 2012) and in populations of *Culex quinquefasciatus* in Chennai India (α- esterases, β-esterases, GST and MFO) (Anju-Viswan et al. 2016).

Correlation between elevated GST levels and DDT resistance in *Ae. aegypti* population was reported from Brazil and in *Anopheles gambiae* populations from West Africa (Aïzoun et al. 2014). In this current study, the GST activity for *Ae. aegypti* and *Ae. albopictus* population from all sites demonstrated enhancement of GST activity but it was also not as high as α-esterases activity, again indicating that resistance shown could not be directly due to GST activity alone.

As for the target-sites mechanism, only AChE is amenable to develop resistance via this mechanism (Hemingway et al. 1986) since AChE is an important enzyme required for hydrolysis of acetylcholine at the cholinergic nerve synapses that caused modification (mutation) of target-site resulted in inhibition of organophosphate and carbamate insecticides. In this, inhibition activity of AChE was only detected in a very small number of study sites indicating that AChE activity may not play a big role in all resistance observed. This finding was very similar to other studies that reported activity of AChE is associated with propoxur inhibition in *Ae. aegypti* and *Ae. albopictus* which only involve small number of localities (1 out of 7 localities) in Thailand (Pethuan et al. 2007) and in *Ae. aegypti* (2 out of 5 localities) in Central Africa Republic (Ngoagouni et al. 2016). Study by Pinto et al. (2019) even reported that alteration of AChE activity in *Ae. aegypti* did not have association to DDT's resistance strain from Peru.

The fact that mosquito population from majority of the study sites developed resistance to all insecticide tested, it is very possible that the insecticide currently used for control measures is no longer effective in killing the vectors in these areas. For that reason, there is a dire need to change or modify the currently used insecticide for improved efficacy and cost-effective control measure.

CONCLUSION

Based on the evaluation criteria of WHO (2016), it can be concluded that most of the *Aedes* strains from dengue hotspots outbreak areas in Kuala Lumpur and Selangor tested has developed resistance towards permethrin, lambda-cyhalothrin, DDT, malathion and propoxur. Insecticide resistance detected shown to be localized in nature and thus, detailed investigation is required to confirm the insecticide resistant level for each dengue hotspot outbreak areas. This study also demonstrated the presence of cross-resistance within and between the insecticide classes. The resistance shown in this study is most likely developed mainly via metabolic mechanism although target-site mechanism might be involved. To the best of our knowledge this is the first report of geodatabase distribution of insecticide resistant and biochemical enzyme activity in Malaysia. It is hope that this information will assist in the betterment of an insecticide resistance surveillance programme and resistance management strategies aimed at combating the spread of dengue effectively in Malaysia. The data can also serve as important reference for further monitoring and planning of counter measures to ensure the continued effectiveness of chemical insecticides used in dengue vector control.

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REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J Econ. Entomol.* 18: 265-267.

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. *Toxicological Profile for Malathion.* Georgia: U.S. Department of Health and Human Services. https://www.atsdr.cdc.gov/toxprofiles/tp154.pdf [8th December 2019]

Aïzoun, N., Aïkpon, R., Gnanguenon, V., Azondekon, R., Oke-Agbo, F., Padonou, G.G., et al. 2014. Dynamics of insecticide resistance and effect of synergists piperonyl butoxide (PBO), SSS-tributylphosphorotrithioate (DEF) and ethacrynic acid (ETAA or EA) on permethrin, deltamethrin and dichlo- rodiphenyltrichloroethane (DDT) resistance in two *Anopheles gambiae s.l.* populations from southern Benin, West Africa. *J Parasit Vector Biol.* 6: 1–10.

Alexander, N., Balmaseda, A., Coelho, I.C.B., Dimaano, E., Hien, T.T., Hung, N.T., et al. 2011. Multicentre prospective study on dengue classification in four Southeast Asian and three Latin American countries. *Tropical Medicine and International Health* 16 (8): 936-48.

Anju-Viswan, K., Pushapalatha, E. & Azhahianambi, P. 2016. Application of synthetic insecticide and change in detoxifying enzyme levels in *Culex quinquefasciatus* Say. *International Journal of Mosquito Research* 3(6): 31-35.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72(1): 248-254.

Brengues, C., Hawkes, N.J., Chandre, F., McCarroll, L, Duchon, S, Guillet, P, et al. 2003. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Medical and Veterinary Entomology* 17(1): 87-94.

Brogdon, W.G., Beach, R.F., Stewart, J.M. & Castanaza, L. 1988. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan *Anopheles albimanus*. *Bulletin of World Health Organization* 66(3): 339-346.

Brogdon, W.G., McAllister, J.C. & Vulule, J. 1997. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. *J. Am. Mosq. Control Assoc*. 13: 233-237.

Budi, M., Siti Rahmah, U. & Tridjoko, H. 2017. Detection of nonspecific esterase activity in organophosphate resistant strain of *Aedes albopictus* Skuse (Diptera: Culicidae) larvae in Yogyakarta, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* 48(3): 552-560.

Chaverra-Rodriguez, D., Jaramillo-Ocampo, N. & Fonseca-Gonzales, I. 2012. Artificial selection of resistance to lambda-cyhalothrin in *Aedes aegypti* and cross-resistance to other insecticides. *Revista Colombiana de Entomología* 38(1): 100-107.

Chen, C.D., Nazni, W.A., Seleena, B. & Sofian-azirun, M. 2008. Biochemical detection of temephos resistance in *Aedes* (Stegomyia) *aegypti* (Linnaeus) from dengue-endemic areas of Selangor state, Malaysia. *Proc ASEAN Congr Trop Med Parasitol* 3: 6-20.

Chen, C.D., Nazni, W.A., Lee, H.L., Norma-Rashid, Y., Lardizabal, M.L. & Sofian-Azirun, M. 2013. Temephos resistance in field *Aedes* (Stegomyia) *albopictus* (Skuse) from Selangor, Malaysia. *Tropical Biomedicine* 30(2): 220–230.

Corbel, V. & N'Guessan, R. 2013. Distribution, mechanisms, impact and management of insecticide resistance in malaria vectors: A pragmatic review. In Sylvie, M. (ed.). *Anopheles Mosquitoes - New Insights into Malaria Vectors*, pp. 579-633. Crotia: InTech.

Diogo, F.B., Viana-medeiros, P.F., Araújo, S.C., Martins, A.J., Bento, J., Lima, P., et al. 2016. Resistance status to the insecticides temephos, deltamethrin, and diflubenzuron in Brazilian *Aedes aegypti* populations. *BioMed Research International* 2016: 1-12.

Dusfour, I., Vontas, J., David, J.P., Weetman, D., Fonseca, D.M., Corbel, V., et al. 2019. Management of insecticide resistance in the major Aedes vectors of arboviruses: Advances and challenges. *PLOS Neglected Tropical Diseases* 13(10): e0007615.

Elia-Amira, N.M.R., Chen, C.D., Lau, K.W., Lee, H.L., Low, V.L., Norma-Rashid, Y., et al. 2018. Organophosphate and organochlorine resistance in larval stage of *Aedes albopictus* (Diptera: Culicidae) in Sabah, Malaysia. *Journal of Economic Entomology* 56(6): 1715–1725.

Farah Ayuni, S., MohdPozi, M.T. & Nor Muniza, S. 2012. Aedes mosquitoes resistance in urban community setting. *Procedia - Social and Behavioral Sciences* 36: 70-76.

Farouk, S.A., Sarah, A.R. & Siti Nasuha, H. 2019. Effect of xenobiotic challenge on enzymatic activity of detoxification enzymes at different exposure periods in *Aedes albopictus* (Skuse) (Diptera: Culicidae). *Serangga* 24(2): 119-133.

Faucon, F., Dusfour, I., Gaude, T., Navratil, V., Boyer, F., Chandre, F., et al. 2015. Identifying genomic changes associated with insecticide resistance in the dengue mosquito *Aedes aegypti* by deep targeted sequencing. *Genome Res.* 25(9): 1347-59.

Flores, A.E., Ponce, G., Silva, B.G., Gutierrez, S.M., Bobadilla, C., Lopez, B., et al. 2013. Wide spread cross resistance to pyrethroids in *Aedes aegypti* (Diptera: Culicidae) from Veracruz state Mexico. *J Econ Entomol*. 106(2): 959-69.

Fouet, C., Atkinson, P. & Kamdem, C. 2018. Human Interventions: Driving forces of mosquito evolution. *Trends Parasitol*. 34(2):127-139.

Frances, S.P., Morton, C.J. & Pettit, W.J. 2016. Studies of the susceptibility of *Aedes aegypti* (Diptera: Culicidae) from Timor-Leste to pyrethroid and organophosphate insecticides. *Austral Entomology* 55: 3–3 03.

Garg, P., Nagpal, J., Khaimar, P. & Seneviratne, S.L. 2008. Economic burden of dengue infections in India. *Trans Royal Society of Tropical Medicine and Hygiene* 102: 570-7.

George, R. & Lam, S.K. 1997. Dengue virus infection--the Malaysia experience. *Ann Acad Med Singapore* 26(6): 815-9.

Hadura, A.H., Zairi, J., Ranson, H. & McCall, P. 2015. Pyrethroid and organophosphate susceptibility status of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Penang, Malaysia. *Int. J. Entomol. Res.* 03(03): 91-95.

Hemingway, J., Smith, C., Jayawardena, K.G.I. & Herath, P.R.J. 1986. Field and laboratory detection of the altered acetylcholinesterase resistance genes which confer organophosphate and carbamate resistance in mosquitoes (Diptera: Culicidae). *Bull. Ent. Res.*76: 559–565.

Hemingway, J., Boddington, R.G., Harris, J. & Dunbar, S.J. 1989. Mechanisms of insecticide resistance in *Aedes aegypti* (L.) (Diptera:Culicidae) from Puerto Rico. *Bull. Entomol. Res.* 79: 123–30.

Hemingway, J. & Ranson, H. 2000. Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology* 45: 371-391.

Hidayati, H., Nazni, W.A., Lee, H.L. & Sofian-Azirun, M. 2011. Insecticide resistance development in *Aedes aegypti* upon selection pressure with malathion. *Tropical Biomedicine* 28: 425-437.

Institute for Medical Research Kuala Lumpur (IMR). 2000. Pictorial key to common genera of mosquito. In Abdullah, A.G. (ed.). *Medical Entomology III.* pp. 6–13. Kuala Lumpur: Division of Medical Entomology, Institute for Medical Research.

Insecticide Resistance Action Committee (IRAC). 2011. Prevention and management of insecticide resistance in vectors of public health importance. http://www.irac-online.org/content/uploads/VM-layout-v2.6_LR.pdf. [7th March 2015]

Ishak, H.I., Jaal, Z., Ranson, H. & Wondji, C.S. 2015. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasites & Vectors* 8:181.

Jahan, N.K., Mohtar, P.A., Amreeta, D., Cheong, Y.M., Lau, W.M., Daniel, D.R., et. al. 2016. A community-based prospective cohort study of dengue viral infection in Malaysia. *Infectious Diseases of Poverty* 5: 76.

Jeffery, J. 2012. *Illustrated Keys: Some Mosquitoes in Peninsula Malaysia*. Kuala Lumpur: University of Malaya Press, Malaysia.

Lam, W. & Tham, A. 1988. A field evaluation of the effectiveness of ULV application of malathion 96% technical grade and sumithion L-40S against *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Ipoh Municipality, Perak. Malaysia. *Trop Biomed.* 5: 81–8.

Lee, H.L., Abimbola, O. & Singh, K.I. 1992. Determination of insecticide susceptibility in *Culex quinquefasciatus* Say adults by rapid enzyme microassays. *SouthestAsean J Trop Med Pub Health* 23(3): 458-463.

Lee, H.L. & Chang, W.L. 1995. Glutathione S-transferase activity and DDT-susceptibility of Malaysian mosquitos. *Southest Asian Journal of Tropical Medicine and Public Health* 26(1): 164-167.

Lee, H.L., Mustafakamal, I. & Rohani, A. 1997. Does transovarian transmission of dengue virus occur in Malaysian *Aedes aegypti* and *Aedes albopictus*. *Southeast Asian J Trop Med Public Health* 28(1): 230-2.

Lee, H.L., Rohani, A., Khadri, M.S., Nazni, W.A., Rozilawati, H., Nurulhusna, A.H., et al. 2015. Dengue vector control in Malaysia- challenges and recent advances. *The International Medical Journal of Malaysia* 14(1): 11-16.

Leong, C.H., Vythilingam, I., Jonathan, W.K.L., Meng-Li, W., Wan-Yusoff, W.S. & Yee-Ling, L. 2019. Enzymatic and molecular characterization of insecticide resistance mechanisms in field populations of *Aedes aegypti* from Selangor, Malaysia. *Parasites Vectors*12:.236.

Lima, J.B.P., Da-Cunha, M.P., Da-Silva, J.R., Gallardo, A.K.R., Soares, S.D.S, Braga, I.A., et al. 2003. Resistance of *Aedes aegypti* to organophosphates in several municipalities in the state of Rio de Janeiro and Espírito Santo, Brazil. *The American Society of Tropical Medicine and Hygiene* 68(3): 329-333.

Limoee, H., Ladonni, H., Enayati, A.A., Vatandoost, H. & Abdoulhasani, M. 2006. Pyrethroid resistantce and cross resistance to DDT in seven field-collected strains of the German cockroach, *Blatella germanic*a (L.) (Dictyoptera: Blattellidae). *J. Biol. Sc.* 6(2): 382-387.

Loke, S.R., Tan, A.W.A., Ahmad, N.W., Lee, H.L. & Sofian-Azirun, M. 2012. Insecticide susceptibility status of field-collected *Aedes*(Stegomyia) *aegypti* (L.) at a dengue endemic site in Shah Alam, Selangor, Malaysia. *Southeast Asian J Trop Med Public Health* 43: 34–47.

Low, V., Chen, C.D., Lee, H.L., Lim, P.E., Leong, C.S. & Sofian-Azirun, M. 2013a. Current susceptibility status of Malaysian *Culex quinquefasciatus* (Diptera: Culicidae) against DDT, propoxur, malathion, and permethrin. *J. Med. Entomol*. 50: 103–111.

Low, V.L., Chen, C.D., Lee, H.L., Tan, T.K., Chen, C.F., Leong, C.S., et al. 2013b. Enzymatic characterization of insecticide resistance mechanisms in field populations of Malaysian *Culex quinquefasciatus* Say (Diptera: Culicidae). *PLoS ONE* 8: e79928.

Marcombe, S., Mathieu, R.B., Pocquet, N., Riaz, M.A., Poupardin, R., Sélior, S., et al. 2012. Insecticide resistance in the dengue vector *Aedes aegypti* from martinique: Distribution, mechanisms and relations with environmental factors. *PloS ONE* 7(2): e30989.

Ministry of Health Malaysia (MOHM). 2020. Kenyataan akhbar khas Ketua Pengarah Kesihatan Malaysia berkenaan situasi semasa demam denggi, zika dan chikungunya di

Malaysia tahun 2019 dan inisiatif KKM untuk memerangi denggi tahun 2020. https://www.moh.gov.my/index.php/database_stores/store_view_page/21/1290 [25th September 2020]

Mia, M.S., Begum, R.A, Er, A.C., Abidin, R.D., Pereira, J.J. 2013.Trends of Dengue infections in Malaysia, 2000-2010. *Asian Pacific Journal of Tropical Medicine* 6(6): 462-466.

Moyes, C.L., Vontas, J., Martins, A.J., Ng, L.C., Koou, S.Y., Dusfour, I., et al. 2017. Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Negl Trop Dis* 11(7): e0005625.

Murray, C.J.L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., et. al. 2012. Disability-adjusted Life Years (DALYs) for 291 diseases and injuries in 21 Regions, 1990-2010. *Lancet* 380: 2197-223.

Nazni, W.A., Selvi, S., Lee, H.L., Saadiyah, I., Azahari, H., et. al. 2009. Susceptibility status of transgenic *Aedes aegypti (L.)* against insecticides. *Dengue Bull.* 33: 124–129.

Nazni, W.A., Kamaludin, M.Y., Lee, H.L., T. Rogayah, T.A.R. & Sa'diyah, I. 2000. Oxidase activity in relation to insecticide resistance in vectors of public health importance. *Trop Biomed.* 17(2): 69-79.

Ngoagouni, C., Kamgang, B., Brengues, C., Yahouedo, G., Paupy, C., Nakouné, E. et al. 2016. Susceptibility profile and metabolic mechanisms involved in *Aedes aegypti* and *Aedes albopictus* resistant to DDT and deltamethrin in the Central African Republic. *Parasites and Vectors* 9(1): 1-13.

Noor Aslinda, U.A.B., Azman, S., Lailatul-Nadhirah, A., Khadijah, K. 2019. Resistance status of *Aedes aegypti* towards different insecticides in selected dengue outbreak area in Petaling district (Diptera: Culicinae). *Serangga* 24(2): 41-48.

Pethuan, S., Jirakanjanakit, N., Saengtharatip, S., Chareonviriyaphap, T., Kaewpa, D. & Rongnoparut, P. 2007. Biochemical studies of insecticide resistance in *Aedes* (Stegomyia) *aegypti* and *Aedes* (Stegomyia) *albopictus* (Diptera: Culicidae) in Thailand. *Trop Biomed.* 27(1): 7-15.

Phang, E.L. & Loh, H.S. 2016. Current perspective on dengue episode in Malaysia. *Asian Pacific Journal of Tropical Medicine* 9(4): 395-401.

Pinto, J., Palomino, M., Mendoza-Uribe, L., Sinti, C., Liebman, K.A. & Lenhart, A. 2019. Susceptibility to insecticides and resistance mechanisms in three populations of *Aedes aegypti* from Peru. *Parasites and Vectors* 12(1): 1-11

Rasli, R., Lee, H.L., Wasi Ahmad, N., Fikri, S.F.F., Ali, R., Muhamed, K.A., et al. 2018. Susceptibility status and resistance mechanisms in permethrin-selected, laboratory susceptible and field-collected *Aedes aegypti* from Malaysia. *Insects* 9(2): 43.

Rohani, A., Asmaliza, I., Zainah, S. & Lee, H.L. 1997. Detection of dengue virus from *Aedes aegypti* and *Aedes albopictus* adults and larvae. *Southeast Asian J Trop Med Public Health* 28(1): 138-42.

Rohani, A., Abdullah, A., Ong, Y., Saadiyah, I., Zamree, I. & Lee, H.L. 2001. Survey of mosquito larvae distribution in container habitats collected from urban and rural areas in major towns of Malaysia. *Trop. Biomed*. 18(1): 41-49.

Rohani, A., Aidil Azahary, A.R., Malinda, M., Zurainee, M.N., Rozilawati, H., Wan Najdah, W.M.A., et al. 2014. Eco-virological survey of *Aedes* mosquito larvae in selected dengue outbreak areas in Malaysia. *J Vector Borne Dis* 14: 327-32.

Rosilawati, R., Lee, H.L., Nazni, W.A., Nurulhusna, A.H., Roziah, A., Khairul Asuad, M., et al. 2017. Pyrethroid resistance status of *Aedes* (Stegomyia) *aegypti* (Linneaus) from dengue endemic areas in peninsular Malaysia. *International Medical Journal Malaysia* 16(2): 73-78.

Sandosham, A.A. & Thomas, V. 1983. *Malariology: With Special Reference to Malaya*. 1st Edition. Singapore: NUS Press.

Seixas, G., Grigoraki, L., Weetman, D., Vicente, J.L., Silva, AC., et al. 2017. Insecticide resistance is mediated by multiple mechanisms in recently introduced *Aedes aegypti* from Madeira Island (Portugal). *PLOS Neglected Tropical Diseases* 11(7): e0005799.

Skae, M.T. 1902. Dengue Fever in Penang. *The British Medical Journal* 2(2185): 1581-2.

Wan Norafikah, O., Nazni, W.A., Noramiza, S., Heah, S.K., Nor Azlina, A.H., Abdullah, A.G., et al. 2011.Ovitrap surveillance and mixed infestation of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Northern Region and Southern Region of Malaysia. *Health and the Environmental Journal* 2(1): 1-5.

Wan-Norafikah, O., Nazni, W.A., Lee, H.L., Zainol-Ariffin, P. & Sofian-Azirun, M. 2010. Permethrin resistance in *Aedes aegypti* (Linnaeus) collected from Kuala Lumpur, Malaysia. *Journal of Asia-Pacific Entomology* 13(3): 175-182.

World Health Organization (WHO). 1998. *Techniques to Detect Insecticide Resistance Mechanisms (Field and Laboratory Manual).* Geneva: World Health Organization.

World Health Organization (WHO). 2011. *Global Insecticide Use for Vector-Borne Disease Control: A 10-Year Assessment, 2000–2009*. 5th Edition. http://whqlibdoc.who.int/publications/2011/9789241502153_eng.pdf [7th February 2020]

World Health Organization (WHO). 2012. *Global Plan for Insecticide Management in Malaria Vectors*. Geneva: WHO Press.

World Health Organization (WHO). 2016. *Monitoring and Managing Insecticide Resistance in Aedes Mosquito Populations: Interim Guidance for Entomologists.* http://apps.who.int/iris/bitstream/10665/204588/2/WHO_ZIKV_VC_16.1_eng.pdf. [18th July 2018]

Yap, H., Lee, Y. & Zairi, J. 2000. Chemical control of mosquitoes. In Ng, F. & Yong, H. (eds.). *Mosquitoes and Mosquito-Borne Diseases: Biology, Surveillance, Control, Personal*

and Public Protection Measures, pp. 197–210. Kuala Lumpur: Academy of Sciences Malaysia.