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Original article

Organochlorines and organophosphates susceptibility of *Aedes albopictus* Skuse larvae from agricultural and non-agricultural localities in Peninsular Malaysia

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A B S T R A C T

Aedes albopictus larvae obtained from different types of agricultural and non-agricultural localities in Peninsular Malaysia were subjected to several larvicides at World Health Organization (WHO) recommended dosages. Upon 24 h of WHO larval bioassay using two organochlorines and six organophosphates, high resistance against dichlorodiphenyltrichloroethane (DDT), temephos, chlorpyrifos and bromophos were demonstrated among all larval populations. *Aedes albopictus* larvae from both paddy growing areas (92.33% mortality) and rubber estates (97.00% mortality) were moderately resistant to dieldrin while only *Ae. albopictus* larvae from dengue prone residential areas (89.00% mortality) showed high resistance against dieldrin. All *Ae. albopictus* larval populations also developed either incipient or high resistance to both malathion (33.67%–95.33% mortality) and fenitrothion (73.00%–92.67% mortality). Only *Ae. albopictus* larvae from fogging-free residential areas that were tolerant to fenthion (97.33% mortality), whereas *Ae. albopictus* larvae from dengue prone residential areas were highly resistant to the same organophosphate (88.33% mortality). Cross resistance between intraclass and interclass larvicides of organochlorines and organophosphates were also exhibited in this study. The present study provided baseline data on various susceptibility levels of *Ae. albopictus* larval populations from different types of agricultural and non-agricultural localities against organochlorines and organophosphates at WHO recommended dosages. Nevertheless, further susceptibility investigations are suggested using revised doses of larvicides established from the local reference strain of *Ae. albopictus* to prevent the underestimation or overestimation of insecticide resistance level among *Ae. albopictus* field strains of larvae.

1. Introduction

Aedes albopictus Skuse is one of the primary mosquito vectors for human threatening diseases like dengue, yellow fever, chikungunya and Zika virus throughout the world, including Malaysia. Even though the vectorial competence of *Ae. albopictus* in transmitting arboviruses is lesser than its closely related species of *Ae. aegypti* (Hussain et al., 2018), it has gained greater attention worldwide due to its capability to inhabit various types of breeding grounds (Wan-Norafikah et al., 2018). Immatures of *Ae. albopictus* are usually found in natural breeding habitats like tree holes (Paul et al., 2018) as well as in artificial breeding containers such as plastic containers and discarded tires (Mohan et al., 2014; Abilio et al., 2018).

Among many mosquito control tools, source reduction has been evidenced to be the most useful method in destroying mosquito breeding habitats. Nevertheless, larviciding is the most appropriate method in line whenever these breeding grounds are not easily accessible or discarded (Koou et al., 2014). However, the heavy reliance and miscellaneous use of chemical compounds have proffered

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an enormous challenge in the management of mosquito control. Long-lasting use of chemical compounds has directed to the development of insecticide resistance in mosquitoes which later heading to failures in vector control activities (Barnes et al., 2017).

Development of insecticide resistance in mosquito species is due to the massive use of chemical compounds in both vector control and agricultural pest management (Yang et al., 2017). However, numerous former work on insecticide susceptibility carried out locally has been concentrating only on mosquito immatures or adults from human dwellings in cities especially with previous records of dengue or chikungunya outbreaks. Therefore, the objective of this study was to determine the susceptibility of *Ae. albopictus* larvae from fogging-free agricultural and non-agricultural areas which consisted of both fogging-free and dengue prone residential areas against diagnostic dosages of larvicides recommended by the World Health Organization (WHO).

2. Materials and methods

2.1. Study localities

The collection of *Aedes albopictus* samples was carried out in fifteen study localities across Peninsular Malaysia. These study localities consisted of human habitations within the agricultural and non-agricultural localities. The agricultural areas and three fogging-free residential areas that represented the non-agricultural localities are free from any history of mosquito control activities while another three dengue prone residential localities which also represented the non-agricultural localities experienced regular mosquito control activities by Ministry of Health Malaysia and local authorities following the reported dengue cases (Table 1). The agricultural areas encompassed three oil palm plantations, rubber estates, and paddy growing areas, each with a routine application of agricultural pesticides to manage the crop pest attacks. Oil palm plantations, rubber estates and paddy growing areas have been identified as the topmost widely planted industrial crops in Malaysia (Department of Agriculture Peninsular Malaysia, 2015). All results of experiments for each study locality were analysed as clusters according to their types of area.

2.2. Sample collection

The laboratory strain and the field strains of *Ae. albopictus* were tested in this study. *Aedes albopictus* laboratory strain (F69) which acted as the reference strain throughout this study was obtained from the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. This population was initially collected from Selangor, Malaysia and has been retained in the insectarium of the IMR for more than ten years. *Aedes albopictus* laboratory strain is free from any previous exposure of insecticides.

On the other hand, *Ae. albopictus* field strains were captured from fifteen study localities by ovitrapping method. An ovitrap surveillance was carried out once for five sequential days in each study locality which took place between October 2015 and May 2016. Standardized ovitraps (Lee, 1992) were sited in each study locality. An ovitrap is comprised of a 300 ml black plastic container with its opening and base diameter of 6.8 cm and 9.1 cm tall. An informative label is glued on the exterior body of ovitrap. A hardboard paddle (10.0 cm × 2.5 cm × 0.3 cm) was also placed diagonally into each ovitrap with the rough surface upwards to assist in

Table 1
Geographical description of study areas.

State	District	Study areas	Geographical description (coordinates and elevation)
Agricultural area : Oil palm plantations			
Johor	Kota Tinggi	University of Malaya Oil Palm Research Plantation, Jementah **(Kota Tinggi OP)**	02°01.727′N, 103°51.924′E; 28 m
Selangor	Klang	Jalan Paip Kiri, Meru **(Klang OP)**	03°09.201′N, 101°27.535′E; 5 m
Pahang	Temerloh	Taman Paya Pulai **(Temerloh OP)**	03°27.642′N, 102°28.098′E; 42 m
Agricultural area : Paddy growing areas			
Selangor	Kuala Selangor	Parit 3, Ban 3, Tanjung Karang**(Kuala Selangor PD)**	03°29.770′N, 101°09.288′E; −25 m
Kedah	Kulim	Kg. Terat Batu, Mukim Sidam Kanan **(Kulim PD)**	05°32.741′N, 100°32.350′E; 9 m
Negeri Sembilan	Kuala Pilah	Kg. Padang Lebar Terachi, Tanjong Ipoh **(Kuala Pilah PD)**	02°44.520′N, 102°07.787′E; 81 m
Agricultural area : Rubber estates			
Selangor	Sungai Buloh	Sungai Pelong **(Sungai Buloh RB)**	03°12.549′N, 101°32.436′E; 39 m
Pahang	Temerloh	Taman Jaya 8 **(Temerloh RB)**	03°27.423′N, 102°27.638′E; 43 m
Johor	Kota Tinggi	Malaysian Rubber Board, Desaru **(Kota Tinggi RB)**	01°33.844′N, 104°14.267′E; 23 m
Residential area : Fogging-free residential areas			
Selangor	Shah Alam	Alam Nusantara, Setia Alam**(Shah Alam FF)**	03°06.692′N, 101°28.134′E; 34 m
Kedah	Padang Serai	Taman Serai Wangi, Mukim Kulim **(Padang Serai FF)**	05°31.301′N, 100°32.673′E; 3 m
Pahang	Temerloh	Taman Seberang Temerloh **(Temerloh FF)**	03°26.985′N, 102°26.743′E; 19 m
Residential area : Dengue prone residential areas			
Johor	Kota Tinggi	Felda Air Tawar 2 **(Kota Tinggi DEN)**	01°40.552′N, 104°01.340′E; 5 m
Selangor	Shah Alam	Kg. Padang Jawa, Seksyen 17 **(Shah Alam DEN)**	03°03.000′N, 101°29.200′E; 1 m
Federal Territory of Kuala Lumpur	Cheras	Kg. Cheras Baru**(Cheras DEN)**	03°06.630′N, 101°45.101′E; 89 m

Kg. = Kampung

mosquito egg-laying. All ovitraps were poured with 10% hay infusion water (Reiter et al., 1991) until up to 5.5 cm level. These ovitraps were used as defined by the guidelines of the Ministry of Health Malaysia (1997) and positioned randomly inside and outside premises, within the proximity of human habitations. Ovitraps were collected and brought back to the laboratory after five days of deployment.

2.3. Mosquito cultivation

All strains of *Ae. albopictus* were nurtured simultaneously in a designated room in the insectarium. They were managed similarly through all manipulations and free from any exposure of insecticides. The temperature of the insectarium was retained at 27 ± 2 °C and 75 ± 10% relative humidity (R.H.).

All *Ae. albopictus* adults of each strain were cultured separately in wooden cages (32 cm × 32 cm × 32 cm) and covered with fine mosquito netting. They were daily fed with 10% sucrose solution mixed with vitamin B complex for their liveliness. Mice were supplied as blood meal once a week for egg production of *Ae. albopictus* offsprings. *Aedes albopictus* female adults laid eggs at about three to six days after a blood meal in small, round, black-coated plastic containers measuring 4 cm deep and 7 cm diameter containing chlorine-free water. Contents of these egg-laying containers were daily and individually strained using funnels lined with Whatman No. 1 filter papers. These *Ae. albopictus* eggs were then air-dried at room temperature and later kept in well saturated, sealed and labelled plastic bags and stored in plastic containers. Once the testings were about to be carried out, the filter papers with *Ae. albopictus* eggs were soaked into chlorine-free water in a plastic tray (25.5 cm × 30.5 cm × 5 cm) to allow these eggs to hatch. The commercial liver powder and small chunks of half-cooked beef liver were provided in the same plastic tray as a larval food source. Larvae of *Ae. albopictus* of all strains were allowed to grow, and only third instar were subjected to the testings of this study.

Meanwhile, for the field strains of *Ae. albopictus*, the contents of ovitraps, including oviposition paddles, were individually poured into plastic containers and topped up with chlorine-free water. The liver powder and small chunks of half-cooked beef liver were also provided in all containers for feeding of larvae. All these containers with modified tiny air holes on their lids were kept covered to prevent egg-laying of other mosquitoes from the surroundings. All hatched larvae (F0) were nurtured before being morphologically identified at fourth instar larvae using standard taxonomic keys by Division of Medical Entomology (2000a, 2000b) and Jeffery et al. (2012). Only *Ae. albopictus* larvae from all study localities were further reared to the adult stage in the insectarium to produce their descendants (F1). The late third (3rd) instar larvae of these *Ae. albopictus* (F1) were then used in the testings. The selection of the late third instar larva is based on the physical observation on the size, length and darker colouration of the entire structure of the larva which is due to the enhancement in the complexity of its internal and external body structures.

2.4. Larvicides

All strains of *Ae. albopictus* larvae were tested against two organochlorines and six organophosphates listed by WHO (1992), namely organochlorines DDT (0.012 mg/L) and dieldrin (0.050 mg/L), as well as organophosphates malathion (0.125 mg/L), fenitrothion (0.020 mg/L), fenthion (0.025 mg/L), temephos (0.012 mg/L), chlorpyrifos (0.012 mg/L) and bromophos (0.050 mg/L). Three of these diagnostic dosages of larvicides (dieldrin, temephos and chlorpyrifos) adhered to WHO diagnostic dosages for *Ae. albopictus* (World Health Organization, 1992) while the rest followed the WHO recommended diagnostic dosages for *Ae. aegypti*. All these larvicides were originally in the form of 0.25 g/ 50 ml solution per bottle which were obtained from the WHO Collaborating Centre; Vector Control Research Unit (VCRU) in Universiti Sains Malaysia (USM), Penang, Malaysia.

2.5. WHO larval bioassay

The WHO larval bioassay was performed according to the WHO standard procedure of larvicide testing (World Health Organization, 2016). This bioassay was carried out in the laboratory and free from any insecticide exposure and extreme exposures of temperature, relative humidity, wind and illumination. The condition of the laboratory was maintained at 27 ± 2 °C and 75 ± 10% relative humidity throughout this study.

Two hundred and fifty (250) ml of test solution containing an appropriate volume of the respective larvicide diluted in chlorine-free tap water to obtain the WHO diagnostic dosage was prepared in a 300 ml paper cup and left for at least half an hour. Twenty-five (25) healthy late third instar larvae were then supplied into each paper cup. A total of 4 replicates were prepared for each concentration of larvicide. Similar stage and number of larvae were applied for each control paper cup consisting of 1 ml of absolute ethanol in 249 ml chlorine-free tap water.

Cumulative larval mortality was scored after 24 h post-exposure. Both moribund and dead larvae were counted to determine the mortality percentage. As indicated by WHO (2016), larvae that failed to move when they were probed with a needle in the siphon or cervical region were considered dead while larvae that were incapable of appearing at the water surface or not showing any sign of diving behaviour when the water was disturbed were considered as moribund larvae.

2.6. Data analysis

Mortality percentage was calculated based on the number of dead and moribund larvae after 24 h post-exposure. As defined by WHO (2016), larval bioassay of the respective larvicide was discarded and repeated when>10% of the larvae of the control population pupated during the testing. If the mortality of control population was between 5% and 20%, the mortality percentage of field strains were corrected using Abbott's formula (1925) as follows:

$$\frac{\%\ \text{Test Mortality} - \%\ \text{Control Mortality}}{100 - \%\ \text{Control Mortality}} \times 100$$

Data of mortality percentage were interpreted based on guidelines by World Health Organization (2016): 98–100% mortality indicated susceptibility; 90–97% mortality suggested moderate resistance which is also defined as incipient resistance or tolerance that required confirmation by conducting additional bioassay testings; and < 90% mortality confirmed the presence of high resistance.

Furthermore, Normality Test using Shapiro-Wilk test was performed to confirm that the data of mortality percentage for *Ae. albopictus* larval populations against WHO diagnostic dosages of organochlorines and organophosphates were normally distributed. One-way ANOVA and Post Hoc Test were then carried out to establish any significant difference between populations from different types of area exposed to each organochlorine and organophosphate employed. The correlation test using Pearson Correlation Test was also performed to discover any significant cross resistance between two larvicides of organochlorines and organophosphates based on the data of mortality percentage of *Ae. albopictus* larval populations against WHO diagnostic dosages. The significant correlation value (r) of>0.4 ($r > 0.4$, $P \leq 0.05$) implied a significant cross resistance between two tested larvicides. The significant cor-

Table 2
Percent mortality of *Aedes albopictus* larvae from different types of area against WHO diagnostic dosage (mg/L) of organochlorines and organophosphates for larval bioassay at 24 h post-treatment.

Types of area	Study areas	Insecticides Organochlorines		Organophosphates					
		DDT 0.012 mg/L*	**Dieldrin 0.050 mg/L**	**Malathion 0.125 mg/L***	**Fenitrothion 0.020 mg/L***	**Fenthion 0.025 mg/L***	**Temephos 0.012 mg/L**	**Chlorpyrifos 0.012 mg/L**	**Bromophos 0.050 mg/L***
Reference	Laboratory	^{R}0.00 ± 0.00	^{S}100.00 ± 0.00	^{M}90.00 ± 3.46	^{M}92.00 ± 2.31	^{S}100.00 ± 0.00	^{R}1.00 ± 1.00	^{R}0.00 ± 0.00	^{R}3.00 ± 1.91
Oil palm plantations	Kota Tinggi OP Klang OP Temerloh OP	^{R}0.33 ± 0.33	^{S}98.33 ± 1.67^{a}	^{M}95.33 ± 1.76^{a}	^{R}73.00 ± 7.55	^{S}99.33 ± 0.33^{a}	^{R}1.33 ± 0.67^{a}	^{R}0.00 ± 0.00	^{R}0.00 ± 0.00
Paddy growing areas	Kuala Selangor PD Kulim PD Kuala Pilah PD	^{R}0.00 ± 0.00	^{M}92.33 ± 4.26	^{R}50.33 ± 21.87	^{R}74.33 ± 9.74^{b}	^{S}98.67 ± 0.88^{b}	^{R}0.33 ± 0.33^{b}	^{R}0.00 ± 0.00	^{R}0.00 ± 0.00
Rubber estates	Sungai Buloh RB Temerloh RB Kota Tinggi RB	^{R}0.00 ± 0.00	^{M}97.00 ± 2.52	^{R}79.67 ± 8.65^{c}	^{R}83.00 ± 11.59	^{S}100.00 ± 0.00^{c}	^{R}11.00 ± 2.52^{abc}	^{R}0.33 ± 0.33	^{R}0.33 ± 0.33
Fogging-free residential areas	Shah Alam FF Padang Serai FF Temerloh FF	^{R}0.00 ± 0.00	^{S}98.33 ± 1.20^{d}	^{R}79.67 ± 6.69^{d}	^{M}92.67 ± 3.48^{b}	^{M}97.33 ± 2.19^{d}	^{R}2.67 ± 2.67	^{R}0.00 ± 0.00	^{R}0.00 ± 0.00
Dengue prone residential areas	Kota Tinggi DEN Shah Alam DEN Cheras DEN	^{R}0.00 ± 0.00	^{R}89.00 ± 1.53^{ad}	^{R}33.67 ± 15.19^{acd}	^{R}77.00 ± 9.71	^{R}88.33 ± 0.88^{abcd}	^{R}0.00 ± 0.00^{c}	^{R}0.00 ± 0.00	^{R}0.00 ± 0.00
One way ANOVA		F = 0.812 df = 15 P = 0.567	F = 2.489 df = 15 P = 0.103	F = 3.205 df = 15 P = 0.055	F = 0.776 df = 15 P = 0.589	F = 14.807 df = 15 P = 0.000	F = 6.022 df = 15 P = 0.008	F = 0.812 df = 15 P = 0.567	F = 25.000 df = 15 P = 0.000

Percent mortality after 24 h (%) = Mean of mortality for larvae + Standard Error (S.E.)
S = susceptible (98–100% mortality), M = moderate resistance/incipient resistance/tolerance (90–97% mortality), R = high resistance (<90% mortality), as determined by WHO (2016).
Percent mortality followed by different letter indicated significant difference between one another ($P \leq 0.05$) (Post Hoc Tukey HSD Test): [a] = Significantly different with oil palm plantations population, [b] = Significantly different with paddy cultivation areas population, [c] = Significantly different with rubber estates population, [d] = Significantly different with fogging-free residential areas population.
* WHO diagnostic dosages (mg/L) for *Aedes aegypti*

Table 3
Cross resistance between larvicides based on the correlation of percent mortality of *Aedes albopictus* larvae from different types of area between organochlorines and organophosphates utilized in WHO larval bioassay using WHO diagnostic dosages (mg/L) at 24 h post-treatment.

Insecticides		Organochlorines		Organophosphates				
		DDT 0.012 mg/L	Dieldrin 0.050 mg/L	Malathion 0.125 mg/L	Fenitrothion 0.020 mg/L	Fenthion 0.025 mg/L	Temephos 0.012 mg/L	Chlorpyrifos 0.012 mg/L
Organochlorines	Dieldrin 0.050 mg/L	r = -0.016 P = 0.954						
Organophosphates	Malathion 0.125 mg/L	r = 0.207 P = 0.441	r = 0.527 P = 0.036					
	Fenitrothion 0.020 mg/L	r = 0.131 P = 0.629	r = 0.258 P = 0.335	r = 0.369 P = 0.160				
	Fenthion 0.025 mg/L	r = 0.118 P = 0.663	r = 0.590 P = 0.016	r = 0.628 P = 0.009	r = 0.176 P = 0.514			
	Temephos 0.012 mg/L	r = -0.165 P = 0.541	r = 0.126 P = 0.642	r = 0.296 P = 0.265	r = 0.251 P = 0.348	r = 0.396 P = 0.129		
	Chlorpyrifos 0.012 mg/L	r = -0.067 P = 0.806	r = -0.167 P = 0.535	r = 0.207 P = 0.441	r = 0.294 P = 0.270	r = 0.175 P = 0.516	r = 0.622 P = 0.010	
	Bromophos 0.050 mg/L	r = -0.086 P = 0.751	r = 0.175 P = 0.516	r = 0.250 P = 0.350	r = 0.292 P = 0.273	r = 0.227 P = 0.399	r = 0.095 P = 0.725	r = 0.258 P = 0.334

Cross resistance between two larvicides (Pearson Correlation Test) based on the correlation of percent mortality at 24 h post-treatment for two tested larvicides: r > 0.4 = Correlated (Two tested larvicides showed cross resistance between one another); r > 0.8 = Highly correlated (Two tested larvicides showed strong cross resistance between one another). ▭ $P \leq 0.05$ = Significant

relation value (r) of>0.8 (r > 0.8, $P \leq 0.05$) indicated a significantly strong cross resistance between two tested larvicides.

The calculation of mortality percentage, Normality Test, One-way ANOVA, Post Hoc Test and Pearson Correlation Test were carried out using the computer-aided statistical programme (IBM SPSS Statistics version 23.0). All levels of statistical significance were determined at P = 0.05.

3. Results

The susceptibility study of *Ae. albopictus* larvae from different types of area against two organochlorines (DDT; dieldrin) and six organophosphates (malathion; fenitrothion; fenthion; temephos; chlorpyrifos; bromophos) larvicides listed by WHO were performed at WHO recommended dosages (World Health Organization, 2016). At 24 h post-exposure, *Ae. albopictus* larvae from all types of area, including the reference strain showed high resistance against DDT, temephos, chlorpyrifos and bromophos (Table 2). Diverged results were demonstrated upon the exposure to dieldrin in which *Ae. albopictus* larvae of the reference strain, oil palm plantations and fogging-free residential areas were susceptible to dieldrin while *Ae. albopictus* larvae from paddy growing areas and rubber estates developed tolerance to dieldrin. Simultaneously, *Ae. albopictus* larvae from dengue prone residential localities were resistant to dieldrin.

In addition, a similar trend of susceptibility was demonstrated in the exposure of malathion and fenitrothion. *Aedes albopictus* larvae of both the reference strain and the oil palm plantations showed moderate resistance against malathion, whereas *Ae. albopictus* larvae of both the reference strain and fogging-free residential areas developed tolerance to fenitrothion. The rest of the field strains displayed high resistance against malathion and fenitrothion, respectively. On the other hand, only *Ae. albopictus* larvae from fogging-free residential areas were tolerance to fenthion while *Ae. albopictus* larvae from dengue prone residential areas were highly resistant to the same larvicide.

The Normality Test performed has confirmed that data of mortality percentage of *Ae. albopictus* larval populations from different types of area against WHO diagnostic dosages were normally distributed (P > 0.05). One-way ANOVA displayed significant differences in the susceptibility status of *Ae. albopictus* larvae from different agricultural and non-agricultural localities only in the exposure of fenthion, temephos and bromophos ($P \leq 0.05$). Significant differences were also demonstrated in the mortality percentage against WHO diagnostic dosages between several *Ae. albopictus* larval populations exposed to dieldrin, malathion, fenitrothion, fenthion and temephos ($P \leq 0.05$) through the Post Hoc Tukey HSD Test.

Based on the Pearson Correlation Test conducted, cross resistance between intraclass larvicides was discovered between malathion and fenthion (r = 0.628, P = 0.009) as well as temephos and chlorpyrifos (r = 0.622, P = 0.010) (Table 3). Furthermore, cross resistance between organochlorines and organophosphates was revealed involving dieldrin with malathion (r = 0.527, P = 0.036) and fenthion (r = 0.590, P = 0.016).

4. Discussion

Aedes albopictus larvae from different types of area were exposed to eight larvicides of organochlorines and organophosphates at WHO recommended dosages. All larval populations were highly resistant to DDT, temephos, chlorpyrifos and bromophos while moderate to high resistance was observed among the majority of these larval populations against dieldrin, malathion, fenitrothion and fenthion. Generally, these findings indicated that more volume of larvicides and frequent larviciding activities are required if the WHO recommended dosages are used as the diagnostic dosages in the initial preparation of these larvicides in the laboratory before these values are augmented many times to obtain the operational dosages to be applied in all types of area. However, it is worth noting that the implementation of a higher volume of larvicides and recurrent larviciding activities could worsen the insecticide resistance development among all *Ae. albopictus* larval populations. Furthermore, the use of WHO recommended diagnostic dosages which are very low, in determining the susceptibility status of local mosquito larval populations could also lead to an overestimation and misinterpretation of the susceptibility status of these larval populations. The idea of applying the WHO recommended dosages of larvicides as the diagnostic dosages for local mosquito larval strains should be carefully decided as these recommended dosages are too general, whereas *Aedes* larval populations from different areas in Malaysia and even in other countries undergone a different history of insecticide exposures which then prompted various levels of susceptibility against each larvicide.

Different larvicides are employed for larval control programmes in Malaysia and other countries over time. Both DDT and dieldrin which belong to the organochlorine class of insecticides are persistent organic pollutants (Rahman, 2013) and have been extensively used worldwide in public health and agricultural sector. In the old days, DDT had been used in the control of *Ae. aegypti* in Malaysia until 1957 before it was replaced with dieldrin (Macdonald, 1958; Nazni et al., 2009). However, as both insecticides are slowly degraded in nature (Jorgenson, 2001; Ahmed et al., 2015), they could remain in the environment for such a very long time. Hence, it is not surprising to perceive the presence of resistance phenotype against any of these insecticides among local mosquito species, including *Ae. albopictus*.

Owing to the suspension of organochlorines in the vector control programmes, the era of the application of organophosphates in the mosquito control had taken place. Organophosphates were believed to be safer than organochlorines since their degradation processes in the environment are faster than the latter insecticide class (Hertz-Picciotto et al., 2018). Both temephos and malathion are the recommended organophosphates used for the control of mosquito larvae and adult mosquitoes in Malaysia, respectively (Vythilingam et al., 1992). Although the use of malathion for local dengue control had been substituted by pyrethroids since 1996 (Teng & Singh, 2001), it is still being used in the local space spraying operations in rotation with pyrethroids until now (J. Nor-Jaiza, personal communication, January 15, 2019). Therefore, the resistance against both temephos and malathion among Malaysian *Aedes* larvae and adults should be expected.

Meanwhile, fenitrothion and fenthion exposures have significant effects on both larval and adult stages of mosquitoes (Thomas, 1962; Sulaiman et al., 1999). However, in Malaysia, these insecticides are more frequently used as adulticides (Loke et al., 2015, Ong, 2016). Moreover, chlorpyrifos was observed to be effective in eliminating *Anopheles* larvae from the Malaysian paddy growing areas for at least two to seven days (Yap & Ho, 1977). Other than that, chlorpyrifos is mostly being implemented in the management of agricultural pests and also to counter the infestation of pyrethroid-resistant German cockroaches in local food preparation retailers (Ismail & Ngan, 2005; Chai & Lee, 2010). As for bromophos, no report on its field utilization in Malaysia has been recorded so far. In fact, across the world, only one field trial using bromophos was carried out and reported in Nigeria so far to control *An. gambiae* and *An. funestus* which was useful for at least five months in the Lagos area and only a month in the Kaduna area (Pant & Self, 1966).

The insecticide resistance development among mosquito vectors are not only due to the extensive use of insecticides in the mosquito control operations of public health but also as a result from the pesticide utilization in the agricultural sector. This is because most of these agricultural pesticides possess a similar mode of actions or target sites with public health insecticides. Based on the casual conversation with the staff, supervisors and farmers of the agricultural areas selected for this study, glyphosate of organophosphates is being used as a weed herbicide in all selected oil palm plantations and rubber estates at different dosages and frequency. Furthermore, alphacypermethrin, cypermethrin and lambdacyhalothrin of pyrethroids, as well as malathion, chlorpyrifos and propoxur of organophosphates, are consistently utilized in all oil palm plantations, paddy cultivation areas and rubber estates selected for this study to control agricultural pests like the cotton leafworm (*Spodoptera litura*), the bagworm (*Metisa plana*, *Pteroma pendula* and *Mahasena corbetti*), the brown planthopper (*Nilaparvata lugens*) and the red cotton stainer (*Dysdercus cingulatus*) at various dosages and regularity. The decision on the dosages of agricultural pesticides to be used and the application regularity in these agricultural areas depends on the directive of the plantation management members or supervisors-in-charge as well as the affordability of the farmers in purchasing the pesticides. These scenarios have led to the uneven insecticide exposures between all study areas.

Besides that, cross resistance among four larvicides of organophosphates as well as cross resistance between an organochlorine and two organophosphates had been determined. Although not all larvicides tested were utilized in the public health operations, cross resistance involving these larvicides could be due to their selection for agricultural pest management as described earlier. Therefore, it is essential to prevent the utilization of larvicides involved in the cross resistance to minimize the insecticide resistance development against these insecticides among *Ae. albopictus* larvae from all study areas.

There were only limited earlier studies on the susceptibility status of *Ae. albopictus* larvae against WHO recommended diagnostic doses of larvicides in comparison with the same experiments on *Ae. aegypti*. Furthermore, most of the tested *Ae. albopictus* populations were subjected only to temephos at previous WHO recommended dose of 0.020 mg/L and fewer to the rest of WHO recommended larvicides. For example, *Ae. albopictus* larvae collected from four different landscapes in South Andaman were exposed to WHO recommended doses of temephos (0.020 mg/L), malathion (1 mg/L) and fenthion (0.05 mg/L). These larvae were highly resistant to temephos but almost fully susceptible to the other two larvicides (Sivan et al., 2015). In Rawalpindi, Pakistan, *Ae. albopictus* larvae captured from four study sites developed tolerance to temephos at WHO recommended dose (0.020 mg/L). These results were not surprising as temephos was the only larvicide applied in Rawalpindi, Pakistan for the control of malaria and dengue vectors since 1969 (Arslan et al., 2016). The larval bioassays were also carried out by Bharati & Saha (2017) in India to assess the susceptibility status of nine strains of *Ae. albopictus* larvae against temephos at WHO recommended dose (0.020 mg/L) as well as at the dose recommended by the India government (0.0125 mg/L). From the testings conducted, Nagrakata strain was moderately resistant to both recommended doses while Siliguri strain developed tolerance only to the latter recommended dose.

In Malaysia, Chen et al. (2005) demonstrated that at the revised WHO recommended diagnostic dose of 0.012 mg/L, *Ae. albopictus* larvae from four localities within Kuala Lumpur and Selangor showed high resistance against temephos with mortality percentage ranging from 6.40% to 59.50% at 24 h post-treatment which were much higher than the mortality percentage obtained for *Ae. albopictus* larvae in this study. On the other hand, in another local study by Elia-Amira et al. (2018), all strains of *Ae. albopictus* larvae captured from eight districts in Sabah, Malaysia displayed resistance to WHO recommended diagnostic doses of DDT, malathion and temephos with zero mortality recorded for the first two larvicides. Chlorpyrifos and dieldrin were the most effective larvicides for almost all strains of *Ae. albopictus* larvae from Sabah, Malaysia. Selection to both fenitrothion and fenthion exhibited a>70% mortality in *Ae. albopictus* larvae from two divisions of Sabah, Malaysia. Furthermore, a wide range of mortality percentage was observed upon the bromophos selection to *Ae. albopictus* larvae from different districts of Sabah, Malaysia. Therefore, these two local findings revealed that each larvicide utilized at WHO recommended diagnostic dose was not necessarily effective against *Ae. albopictus* populations in all sites even though they are within the same state or country which implement similar procedures of vector control approaches.

5. Conclusion

In summary, most *Ae. albopictus* larvae from different types of area selected in the present study displayed moderate to high resistance level against two organochlorines and six organophosphates tested at WHO recommended diagnostic dosages. Significant differences in the susceptibility levels of *Ae. albopictus* larvae from different types of agricultural and non-agricultural localities were also noted against fenthion, temephos, and bromophos. Hence, the local health authorities must select the most appropriate larvicides to be utilized at each study area in order to avoid further development of insecticide resistance among these *Ae. albopictus* populations.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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