RESISTANCE STATUS OF *Aedes aegypti* TOWARDS DIFFERENT INSECTICIDES IN SELECTED DENGUE OUTBREAK AREA IN PETALING DISTRICT (DIPTERA: CULICINAE)

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ABSTRACT

Four field strains of *Aedes aegypti* from dengue outbreak areas in Petaling, Selangor were evaluated using two groups of insecticide; pyrethroid class II (deltamethrin) and organophosphate (malathion and pirimiphos-methyl). This study was conducted according to the WHO adult mosquito bioassay procedure (WHO/ZIKV/VC/16.1). Results of two-way ANOVA indicated that there was a significant difference resistant levels in type of insecticides used against different localities which were TUDM, Brunsfield, Gugusan Semarak and Apartment Sri Indah ($F=57.985$, df 6, 48, $p<0.05$). From the Tukey post-hoc test, it revealed that across different type of insecticides tested, the percentage of mortality in *Ae. aegypti* was significantly difference ($p<0.05$) amongst insecticides, viz., deltamethrin, malathion and pirimiphos-methyl. Factor of locality shows significant difference towards percentage of mortality in *Ae. aegypti* in TUDM and Apartment Sri Indah ($p<0.05$), but there were no significant difference of percentage of *Aedes aegypti* mortality in Brunsfield and Gugusan Semarak ($p>0.05$). Resistance ratio for all the *Ae. aegypti* from localities selected ranged from 20 fold to 128 fold (moderate resistance and highly resistance categories). Results of this research showed that resistance presence in mosquito populations in the selected dengue outbreak areas in Petaling and that it could the reason controlling dengue is ineffective. We suggest to rotate insecticides with different mode of action and safer insecticides other than pyrethroids plus public awareness to eliminate the mosquitoes breeding places.

Keywords: Resistance ratio, *Aedes aegypti*, dengue, malathion, pirimiphos-methyl, deltametrhin.

ABSTRAK

Empat strain nyamuk *Aedes aegypti* dari kawasan wabak denggi di Petaling, Selangor telah diuji menggunakan dua kumpulan insektisid; kelas pirethroid II (deltamethrin) dan organofosfat (malathion dan pirimiphos-metil). Kajian ini dijalankan mengikut prosedur bioassay nyamuk dewasa WHO (2016) (WHO/ZIKV/VC/16.1). Keputusan ANOVA dua hala menunjukkan terdapat perbezaan yang ketara dalam jenis insektisid yang digunakan terhadap kawasan yang berbeza iaitu TUDM, Brunsfield, Gugusan Semarak dan Pangsapuri Sri Indah ($F=57.985$, $df=6, 48$, $p<0.05$). Dari ujian post-hoc Tukey, ia menunjukkan pelbagai jenis racun serangga yang diuji; terdapat perbezaan yang ketara bagi peratusan kematian *Ae. aegypti* iaitu ($p<0.05$) terhadap deltamethrin, malathion dan pirimiphos-metil. Faktor lokaliti menunjukkan perbezaan yang ketara ke atas peratusan kematian di *Ae. aegypti* di TUDM dan Apartment Sri Indah ($p<0.05$), tetapi tidak terdapat perbezaan peratusan kematian *Aedes aegypti* di Brunsfield dan Gugusan Semarak ($p>0.05$). Nisbah kerintangan untuk semua kawasan yang dipilih adalah antara 20 kali ganda hingga 128 kali ganda (kategori kerintangan sederhana dan tinggi). Keputusan kajian ini menunjukkan kehadiran kerintangan dalam populasi nyamuk di kawasan wabak denggi yang dipilih di daerah Petaling dan merupakan salah satu penyebab mengapa aktiviti kawalan vektor yang dijalankan tidak berkesan. Kami mencadangkan agar melakukan pengiliran racun serangga mengikut jenis tindakbalas, penggunaan racun serangga yang lebih selamat daripada pirethroid sediada dan meningkatkan tahap kesedaran orang ramai bagi menghapuskan tempat-tempat pembiakan nyamuk.

Kata kunci: Nisbah kerintangan, *Aedes aegypti*, denggi, malathion, pirimiphos-metil, deltametrhin.

INTRODUCTION

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years. The global problem of dengue virus is well documented (Pinheiro & Corber 1997) and transmitted by female mosquitoes mainly of the species *Aedes aegypti* (Thaikruea et al. 1997) and to a lesser extent, *Aedes albopictus*. Dengue is widespread throughout the tropics in Asia, the Pacific, the Americas and Caribbean with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization (Holstein 1967). In 2017, there were 83,849 reported dengue cases with 177 deaths in Malaysia (Ministry of Health Malaysia 2017) while in 2016, 101,357 dengue cases were recorded with 237 deaths (Ministry of Health Malaysia 2016). The number of dengue cases in 2017 decreased by 17.3% compared to 2016 total dengue cases in Malaysia (Ministry of Health Malaysia 2017). Selangor recorded the highest dengue cases in Malaysia since 2012 (Ministry of Health Malaysia 2017) with 45,290 dengue cases (67 deaths) in 2017.

Multiple vector control approaches are required to combat dengue virus in the absence of dengue vaccine and specific treatment (Eisen et al. 2009). Space spraying is one of the common chemical approach in vector control programmed. The use of various insecticides such as pyrethroids and organophosphates, is the main method to control major disease vectors in Malaysia (Nazni et al. 2000). Insecticides are widely used in Malaysia not only by the Ministry of Health (MoH) vector control programmed but also by private sector and the community to control mosquitoes as well as others household pests (Rohani et al. 2011). The extensive and probably inappropriate application of insecticides has led to develop resistance in mosquitoes to all major insecticides especially pyrethroids (Mebrahtu et al. 1997) and organophosphates. Finally, this problem will lead to failure in vector borne disease control programmed especially in the highest dengue cases area. Therefore, the main objective of this study was to determine the pyrethroids and organophosphates resistant status from four selected dengue hotspots area in Petaling District, Selangor.

MATERIALS AND METHODS

Study Site

Mosquito eggs were collected from January to March 2017 in Petaling District, Selangor. This study was conducted at four selected dengue outbreak areas; more than one case in 200m radius within two weeks of period (Anon 2019) TUDM (N03∞118751', E101∞541857), Brunsfield (N03∞079837', E101∞554012), Gugusan Semarak (N03∞153335', E101∞581985) and Apartment Sri Indah (N02∞'997336, E101∞664334). All of the localities were apartment type from four subdivisions in Petaling.

Sampling Method

300 ovitrap cups with paddles were placed randomly indoors and outdoors at selected houses each site. Modified ovitraps were used for surveillance as described by Lee (1992). The traps were left for four to five days then collected and brought to the laboratory. The ovitrap contents and the paddles were transferred to the new container that contained de-chlorinated water. The eggs were allowed to hatch and the larval were fed with larval food comprises chicken liver powder. Upon the larval emergence into third and fourth instar, *Aedes* species were identified morphologically under compound microscope. Only *Ae. aegypti* were reared and maintained under laboratory conditions for further testing.

Bioassay Procedure

140 of non-blood fed adult female mosquitoes aged 3-5 days were used for WHO insecticide susceptibility testing following WHO guidelines (2016) (WHO/ZIKV/VC/16.1) (Ministry of Health Malaysia 2016) for each test. The *Ae. aegypti* populations from all four localities and laboratory strain (F 1064) were tested against pyrethroid class II, deltamethrin (0.03%) and organophosphate, malathion (0.8%) and pirimiphos-methly (0.21%). The selection of the type for insecticide tested is based on the history of insecticide usage in the dengue hotspots areas throughout 2016 (Anon 2019). During exposure to the insecticides, the knockdown number of the mosquitoes were recorded every five minutes for one hour. After one-hour exposure, the mosquitoes were transferred to a clean plastic cup and were fed with 10% sucrose solution. The mortality of mosquitoes was recorded after 24-hours post-exposure.

Data Analysis

Susceptibility test data were analyzed according to WHO guidelines (2016) (WHO/ZIKV/VC/16.1) (Ministry of Health Malaysia 2016): 98–100% mortality is indicated susceptibility; less than 98% mortality is indicated possibility of resistance that needed further tests to verify and less than 90% mortality indicated resistance. Mortality rates were corrected using Abbott’s formula when control mortality was between 5% and 20% (Abbott 1925). The results were then evaluated by probit analysis (SPSS software version 24) to compute the KT_{50} and KT_{99} values. The resistance ratio, RR_{50} was calculated by dividing KT_{50} value of field strains with the corresponding KT_{50} value of susceptible/ laboratory strain. RR_{50} was scaled as follows: RR_{50}<1 (susceptible), RR_{50}=1 to 10 (low resistance), RR_{50} =11 to 30 (moderate resistance), RR_{50}=31 to 100 (high resistance), and RR_{50}>100 very high resistances (Khan et al. 2011). Non-normal data were arcsine log transformed to stabilize the variance. Two-way ANOVA and t-test test were applied to test for differences in the mortality between four field strain and laboratory strain.

RESULTS

The percentage of *Ae. aegypti* mortality after 24-hours exposure to the insecticides against type of insecticides tested in four different localities and Selangor laboratory strain (susceptible strain) obtained from WHO adults bioassay are illustrated in Figure 1. As expected, the Selangor laboratory mosquito strain showed the highest mortality (100%) for the three type of insecticides tested. For the pyrethroid group, deltamethrin (0.03%), the highest percentage of mosquito mortality was 85% which is from Apartment Sri Indah, followed by mosquito strain from Gugusan Semarak (22%), Brunsfield (17%) and the lowest mosquito mortality was from TUDM with 2% of mosquito mortality. Meanwhile, organophosphate group the malathion (0.8%), highest percentage of mortality detected from Brunsfield strain (20%) and the lowest from Apartment Sri Indah (4%) meanwhile, pirimiphos-methyl (0.21%) shows the highest percentage of mosquito mortality from Apartment Sri Indah (46%) and the lowest from TUDM (0%). Overall, mosquito strain from TUDM shows low percentage of mortality for all the insecticides test.

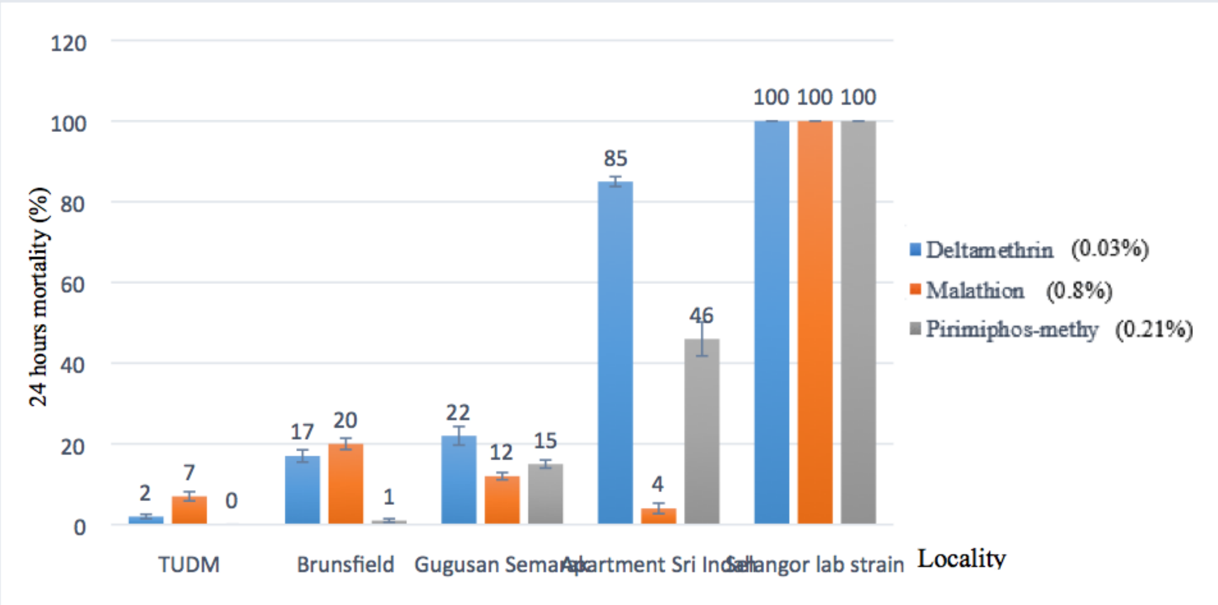

Figure 1. Percentage of *Aedes aegypti* mortality ~~rate~~ against type of insecticides tested in four different localities.

Table 1 shows the two-way ANOVA between the percentages of *Ae. aegypti* mortality against type of insecticides used at different localities in Petaling district. Results of two-way ANOVA indicated that there was a statistically significant interaction in type of insecticides used against different localities (TUDM, Brunsfield, Gugusan Semarak and Apartment Sri Indah), ($F=57.985$, df = 6.48, $p<0.05$). Tukey post-hoc test revealed that, across different type of insecticides tested, the percentage of mortality in *Ae. aegypti* is significantly difference ($p<0.05$) towards deltamethrin, malathion and pirimiphos-methyl. Different localities shows a significant difference towards percentage of *Ae. aegypti* mortality at TUDM and Apartment Sri Indah ($p<0.05$), but there were no significant difference on percentage of *Ae. aegypti* mortality between Brunsfield and Gugusan Semarak ($p>0.05$).

Table 1. Two-way ANOVA between percentages of mortality in *Ae. aegypti* against type of insecticides used in different localities in Petaling .

Parameter	df	ss	F	Sig.
Type of insecticides	2	94.550	62.685	$P<0.05$
Locality	3	195.794	129.808	$P<0.05$
Type of insecticides × Locality	6	87.461	57.985	$P<0.05$

Table 2 shows the resistance status of pyrethroid group (deltamethrin) insecticides against *Ae. aegypti* adults collected from four localities in dengue outbreaks area. The RR_{50} value for TUDM strain (128.49 fold) was the highest among all of the localities tested and categorized as very high resistance as well as Gugusan Semarak (116.64 fold). RR_{50} were moderate resistance for two localities tested; Brunsfield (23.79 fold) and Apartment Sri Indah (20.06 fold). All four localities are dengue outbreak areas in Petaling and has high insecticide pressure. Interestingly, there is no significant difference in resistance among all population from dengue outbreak areas.

Table 2. Deltamethrin resistance status against *Ae. aegypti* (adults) collected from four localities in Petaling District

Strain	KT_{50} (95% CL) (min)	KT_{99} (95% CL) (min)	Slope	χ^2 (df)	Sig.	RR_{50}
TUDM	1389.776 (268.674-7.789×10^{11})	53281.667 (1976.811-1.692×10^{21})	4.505±1.007	2.648 (10)	0.989	128.49
Brunsfield	257.329 (126.459-13115.378)	1754.050 (394.410-7477965.512)	6.727±1.667	2.545 (10)	0.990	23.79
Gugusan Semarak	1261.596	38044.104	4.876±1.332	14.640 (10)	0.146	116.64
Apartment Sri Indah	216.983 (130.789-779.909)	2021.269 (621.159-41614.325)	5.608±0.886	4.132 (10)	0.941	20.06
Selangor lab strain	10.816 (10.143-11.467)	24.629 (22.278-28.002)	6.731±0.523	8.731 (10)	0.558	-

CL: Confidence limits,
RR_{50}: Resistance ratio values are based on KT_{50} levels of the field strain divided by KT_{50} levels of the reference strain (Selangor).
Chi square (χ^2) indicates the goodness of fit of the regression line.

DISCUSSION

Field strain from Petaling District exhibited less than 90% mortality which indicated resistance happened in *Ae. aegypti* against all type of insecticides used (deltamethrin, malathion and pirimiphos-methyl). All of these insecticides were used for 2 years continuously due to high dengue cases reported in the selected areas. The prolonged use of insecticides to control dengue vectors has created selection pressure resulting in the emergence of resistant populations especially in *Ae. aegypti*. Neighboring country, Thailand has reported resistance to deltamethrin and permethrin due to frequency of usage in vector control program. Resistance studies throughout the year 2003 till 2005 detected that most mosquito population were resistance to deltamethrin and permethrin in Thailand (Ponlawat et al. 2005; Jirakanjanakit et al. 2007). Thus, it is not uncommon that deltametrin resistance occurred in Petaling because of the intensive exposure of vector to deltamethrin use in space spraying in dengue outbreaks areas. Pyrethroids are broadly categorized into three groups based on their structure and toxicology: type I, type II and non-ester pyrethroid. Type II pyrethroids, which contain α-cyano group, are more toxic than type I pyrethroids (Schleier & Peter 2012; Davies et al. 2007). Malathion was replaced with pyrethroid formulation in 1996 in the vector control program in Malaysia (Ang & Singh 2001). Malathion resistance among *Aedes aegypti* is rarely recorded in this country but it was reported in *Aedes albopictus* (Brown 1986) and *Culex quinquefasciatus* (Nazni et al. 2005) in Malaysia

CONCLUSION

In the current study, the populations of *Aedes aegypti* mosquitoes in selected dengue outbreak areas in Petaling, Selangor, Malaysia were found resistant against three type of insecticide used. It is urgent to identify alternative insecticides that are effective and less harmful to the environment that could be used in a vector control strategy based on a rotation and/or combination of insecticides which lead to slow resistance development, as recommended by the WHO 2010.

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