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PYRETHROID AND ORGANOPHOSPHATE SUSCEPTIBILITY STATUS OF *AEDES AEGYPTI* (LINNAEUS) AND *AEDES ALBOPICTUS* (SKUSE) IN PENANG, MALAYSIA

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A B S T R A C T

Dengue is a serious problem in Malaysia, particularly in high-density urban communities. Many methods are used for mosquito control but the most important procedure usually includes chemical insecticides. Dengue control relies on insecticides to control the vectors, *Aedes aegypti* and *Aedes albopictus*. Using standard World Health Organization (WHO) adult bioassay tests, this study evaluated the susceptibility to lambda-cyhalothrin and pirimiphos-methyl of local populations of both species in a traditional community at Bagan Dalam, Penang, Malaysia. Unfed female mosquitoes aged 3-5 days post-emergence were exposed to WHO recommended dosages of insecticides over fixed time periods with results presented as knock-down time (KT_{50}) for each strain. The insecticide susceptible Vector Control Research Unit (VCRU) laboratory strains of both species were used as controls. Both species from Bagan Dalam were highly resistant to lambda-cyhalothrin with less than 10% mortality recorded 24 hours after exposure. However, both *Ae. aegypti* and *Ae. albopictus* from Bagan Dalam were susceptible to pirimiphos-methyl, showing 100% mortality recorded 24 hours after treatment. The results indicated that organophosphates such as pirimiphos-methyl might be used as an alternative to pyrethroid for dengue vector control in this highly dengue-prone area.

Keywords: Vector control, *Ae. aegypti*, *Ae. albopictus*, resistance, pyrethroid, organophosphate.

INTRODUCTION

Aedes mosquitoes correspond to a major mosquito borne diseases including dengue fever, chikungunya and yellow fever. These diseases collectively infect over 2.5 billion people live in areas where these diseases can be transmitted (WHO, 2008). *Ae. aegypti* is mostly found in tropic and subtropic regions such as South America, South East Asia and Africa (WHO, 2008) while *Ae. albopictus* was considered as endemic species in South East Asia but now has dramatically increased to temperate areas (Gratz, 2004). In Malaysia, control of the mosquito vector population currently depends heavily on insecticides (Chen *et al.,* 2005a; Nazni *et al.,* 2005; WHO, 2006). However, insecticide resistance is widespread and has been responsible for failures in control (Selvi *et al.,* 2006) and routine surveillance of the susceptibility status of field mosquito population is essential in order to ensure effectiveness. Resistant populations of dengue vectors have been reported in Malaysia (Chen *et al.,* 2005a; Chen *et al.,* 2005b; Nazni *et al.,* 2005; Wan-Norafikah *et al.,* 2008; Wan-Norafikah *et al.,* 2010; Rong *et al.,* 2012; Low *et al.,* 2013) but there are no recent data on the insecticide susceptibility status of mosquitoes on the mainland of Penang. This study investigated two locally abundant mosquito species, *Ae. aegypti* and *Ae. albopictus* to determine their susceptibility status to lambda-cyhalothrin (pyrethroid) and pirimiphos-methyl (organophosphate) in Bagan Dalam, Penang.

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MATERIALS AND METHODS

Study area: The study was conducted in the area of Ujong Batu, Bagan Dalam which is located on the mainland of Penang state (05°23.289”N 100°22.397”E; altitude 11 m, November, 2011). The study site was an unregulated ‘squatter’ housing area mainly comprising small single or double-storied detached houses.

Mosquito strains: Eggs of *Aedes* mosquitoes were collected using ovitraps. The mosquitoes were reared to adults in an insectary (25ºC ± 2ºC with 80 ± 10% relative humidity). Standard ovitraps containing 200 ml of tap water were used to collect field strains of *Ae. aegypti* and *Ae. albopictus*. Two ovitraps were placed randomly in each of the 123 houses in the study area. Both ovitraps were placed outdoors, at about 1.2 metres above ground. Ovitraps were collected after 6 days from the houses. In insectary, the paddles that contained mosquito eggs were transferred into plastic containers and topped up with fresh water. Larval food (1 finely ground fish flakes: 1 brewers' yeast) were given daily until they pupated. The collected mosquito larvae were further reared until they emerged as adults, which were identified morphologically. Both species were supplied with white mice for blood-feeding to obtain eggs of the F1 progeny. These were provided with 10% sucrose solution and female mosquitoes (3 to 5 days old) from this or the next generation (F1 and F2) were used in the bioassays. Susceptible laboratory strains of *Ae. aegypti* and *Ae. albopictus* from Vector Control Research Unit (VCRU), Universiti Sains Malaysia (USM) were used as controls in the bioassay tests.

Insecticides: WHO-impregnated papers were obtained from the VCRU, USM, Penang. Diagnostic concentrations have been widely used for monitoring insecticide resistance in mosquitoes and other disease vectors (WHO, 1992; WHO, 1998). WHO (1981a) has defined diagnostic concentration as double the lowest concentration that gave 100% mortality for a period of 60 minutes of exposure and 24 hours of recovery periods on a susceptible population. Diagnostic concentrations have been established for four insecticides namely pyrethroids, organophosphates, organochlorines and carbamates under standardized laboratory conditions (WHO, 1981a; WHO, 1981b). According to WHOPES (2007), *Ae. aegypti* and *Ae. albopictus* were tested against 0.03% lambda-cyhalothrin (VCRU, USM, 2012) and 0.25% pirimiphos-methyl (VCRU, USM, 2012).

WHO adult bioassays: The bioassay tests were conducted according to the WHOPES tube bioassays protocol (WHO, 2013). Unfed female mosquitoes (3 to 5 days) were introduced into the control tubes (insecticide-free) and held for 20 minutes. After this pre-test period, they were transferred into the test tubes lined with a piece of WHO test paper (12 cm x 15 cm). After exposure to 0.03% lambda-cyhalothrin and 0.25% pirimiphos-methyl, female mosquitoes were left inside the holding chamber with sucrose solution provided, and maintained in a climatic room for 24 hours at 25°C ± 2°C and 80 ± 10% RH. For pyrethroids, observation of the number of knock-down mosquitoes was recorded at regular intervals of 60 minutes. After completing the exposure period, the mortality rate after 24 hours was also recorded for each test. Five replicates were conducted for each test and control, and each replicate consisted of 20 mosquitoes.

Data analysis: Both mortality and knock-down rates were analysed by regression log time-probit, using statistical model IBM SPSS Statistic 20.0 version. For pyrethroid insecticide, the results obtained were presented as knock-down time (KT50). Based on KT50 values, resistance ratio (RR) was determined by the ratio of field strain to the ratio of susceptible laboratory strain. The mortality of mosquito samples were calculated by total up the number of dead mosquitoes across all five exposure replicates and the results were expressed as a percentage of the total number of exposed mosquitoes:

$$\text{Observed Mortality} = \frac{\text{Total No. of Dead Mosquitoes}}{\text{Total Sample Size}} X\ 100$$

If the control mortality is above 20%, the test should be discarded while if the control is below 5%, the test can be ignored and no correction is necessary. For the control tests, similar calculations were made in order to obtain a value for control mortality. If the tests are greater than 5% but less than 20%, the observed mortality have to be corrected using Abbots formula as follows:

$$\frac{(\%\ \text{observed Mortality} - \%\ \text{control Mortality})}{(100 - \%\ \text{control Mortality})}\ \text{X}\ 100$$

RESULTS

The results of pyrethroid susceptibility tests are illustrated in Table 1 and 2. The control tests were conducted simultaneously and showed that laboratory strains of *Ae. aegypti* and *Ae. albopictus* remained completely susceptible throughout the study, with mortality ranging between 98 to 100% after 24 hours for both lambda-cyhalothrin and pirimiphos-methyl. *Ae. aegypti* from Bagan Dalam was highly resistant to lambda-cyhalothrin with 0% mortality, 24 hours after exposure to the diagnostic concentration. For *Ae. aegypti* Bagan Dalam, the values of KT_{50} ranged from 22.11 to

30.49 in this species. *Ae. albopictus* Bagan Dalam strain also was highly resistant to lambda-cyhalothrin with 0% mortality after 24 hours' exposure to the diagnostic concentration.

Table 1. Susceptibility status of *Ae. aegypti* to 0.03% lambda-cyhalothrin.

Ae. aegypti						
Strain	Time	Mean % ± SD 24 h Mortality	Mean % ± SD KD after 1 h exposure	KT_{50} in min (95% Cl)	RR50	Slope ± SE
Bagan Dalam	Baseline	0 (3)	100 ± 0.00	22.11 (21.38 - 22.77)	2.37	13.79 ± 1.27
	1 month	0 (3)	100 ± 0.00	23.51 (22.72 - 24.25)	1.90	11.69 ± 0.96
	3 months	0 (3)	100 ± 0.00	26.47 (24.42 - 28.37)	1.26	10.06 ± 0.76
	6 months	0 (3)	100 ± 0.00	30.49 (29.67 - 31.31)	1.34	12.08 ± 0.92
VCRU	Baseline	100 ± 0.00 (1)	100 ± 0.00	34.32 (32.34 - 36.21)	-	13.86 ± 0.92
	1 month	100 ± 0.00 (1)	100 ± 0.00	36.64 (34.98 - 38.28)	-	14.09 ± 0.95
	3 months	100 ± 0.00 (1)	100 ± 0.00	33.36 (31.64 - 35.00)	-	12.56 ± 0.84
	6 months	100 ± 0.00 (1)	100 ± 0.00	33.43 (32.43 - 34.40)	-	9.73 ± 0.69

(1): Susceptible, (2): Resistance suspected, (3): Resistant. Slope ± SE: Slope of regression line ± standard error.
RR: Resistance ratio KT field strain/KT laboratory strain.

Table 2. Susceptibility status of *Ae. albopictus* to 0.03% lambda-cyhalothrin.

Ae. albopictus						
Strain	Time	Mean % ± SD 24 h Mortality	Mean % ± SD KD after 1 h exposure	KT_{50} in min (95% Cl)	RR50	Slope ± SE
Bagan Dalam	Baseline	0 (3)	100 ± 0.00	36.62 (35.39 - 37.85)	1.06	6.82 ± 0.39
	1 month	0 (3)	100 ± 0.00	32.92 (31.79 - 34.03)	0.91	7.16 ± 0.41
	3 months	0 (3)	100 ± 0.00	32.64 (31.57 - 33.68)	0.93	7.87 ± 0.44
	6 months	0 (3)	100 ± 0.00	31.88 (30.79 - 32.94)	0.99	7.52 ± 0.43
VCRU	Baseline	100 ± 0.00 (1)	100 ± 0.00	34.68 (31.54 - 37.68)	-	9.22 ± 0.66
	1 month	100 ± 0.00 (1)	100 ± 0.00	36.11 (33.76 - 38.52)	-	9.27 ± 0.67
	3 months	100 ± 0.00 (1)	100 ± 0.00	35.25 (32.63 - 37.84)	-	10.0 ± 0.70
	6 months	100 ± 0.00 (1)	100 ± 0.00	32.10 (31.12 - 33.02)	-	10.14 ± 0.72

(1): Susceptible, (2): Resistance suspected, (3): Resistant. Slope ± SE: Slope of regression line ± standard error.
RR: Resistance ratio KT field strain/KT laboratory strain.

Table 3. Susceptibility status of *Ae. aegypti* and *Ae. albopictus* to 0.25% pirimiphos-methyl.

Strain	Time	*Aedes aegypti*	*Aedes albopictus*
		Mean % ± SD 24 h Mortality	Mean % ± SD 24 h Mortality
Bagan Dalam	Baseline	100 ± 0.00 (1)	100 ± 0.00 (1)
	1 month	100 ± 0.00 (1)	100 ± 0.00 (1)
	3 months	100 ± 0.00 (1)	100 ± 0.00 (1)
	6 months	100 ± 0.00 (1)	100 ± 0.00 (1)
VCRU	Baseline	100 ± 0.00 (1)	100 ± 0.00 (1)
	1 month	100 ± 0.00 (1)	100 ± 0.00 (1)
	3 months	100 ± 0.00 (1)	100 ± 0.00 (1)
	6 months	100 ± 0.00 (1)	100 ± 0.00 (1)

(1): Susceptible, (2): Resistance suspected, (3): Resistant.

The knockdown rate of both species after one hour exposed to lambda-cyhalothrin was 100% throughout the study. For *Ae. albopictus* Bagan Dalam, the KT_{50} values ranged from 31.88 to 36.62 throughout the study. Both KT_{50} values of *Ae. aegypti* and *Ae. albopictus* populations at Bagan Dalam were not significantly different to the susceptible VCRU strains (Kruskal-Wallis Test, $P > 0.05$). There were also not significantly different in KT_{50} values across time point in both *Ae. aegypti* and *Ae. albopictus* (Friedman Test, $P > 0.05$). For organophosphate insecticide, bioassay results indicated that both *Ae. aegypti* and *Ae. albopictus* from Bagan Dalam strain were susceptible to pirimiphos-methyl (Table 3). Both of these species showed 100% mortality after 24 hours' exposure to this insecticide.

DISCUSSION

Both *Ae. aegypti* and *Ae. albopictus* samples collected from Bagan Dalam were resistant to lambda-cyhalothrin. Information provided by the local Health Department at Seberang Perai Utara, indicated that pyrethroid insecticides have been widely used as mosquito adulticides for more than 10 years in this study area, as is the case for mosquito vector control throughout Malaysia. Following earlier reports on pyrethroid resistance in Malaysia by Chan *et al.* (2011), this study revealed that *Ae. albopictus* has now developed resistance to 0.2% deltamethrin and 0.7% permethrin in two dengue hotspots in Penang Island. Pyrethroid resistance is also known to be widely developed in both *Aedes* species in other at-risk dengue areas in Peninsular and East Malaysia (Nazni *et al.,* 2005; Wan-Norafikah *et al.,* 2008; Wan-Norafikah *et al.,* 2010; Rong *et al.,* 2012; Low *et al.,* 2013). Various mosquito control methods such as ultra-low volume (ULV) fogging, thermal fogging, surface residual spraying and numerous household insecticide products are widely used for the control of adult mosquitoes in Malaysia (Yap *et al.,* 2000). It is likely that these have all combined to contribute to the now widespread pyrethroid resistance in *Aedes* species throughout the country (Rohani *et al.,* 2001; Nazni *et al.,* 2005). Pyrethroids' are neurotoxic to insects. The lethal effects of pyrethroids include the action on both peripheral and central neurons, while the knock-down effect possibly involves peripheral intoxication (Becker *et al.,* 2010). In this study, the effect of lambda-cyhalothrin was rapid, with 100% knock-down rate recorded for both *Ae. aegypti* and *Ae. albopictus*. However, 100% recovery was observed after 24 hours, demonstrating that *Aedes* mosquitoes in the study area were extremely resistant. Organophosphates such as pirimiphos-methyl produce toxic action at synapses by binding to and inhibiting acetylcholinesterase (AChE), leading to accumulation of acetylcholine, which interferes with the neuromuscular junction, generating rapid muscles twitching and killing the insects (Becker *et al.,* 2010). This killing effect has been observed in *Aedes* mosquitoes in Bagan Dalam as the mortality rate after 24 hours was 100%. Indeed, both *Aedes* species were fully susceptibility to pirimiphos-methyl. The results reported here suggest that the application of organophosphates as adulticide in this area could be rotated with pyrethroid during control programmes as this class of insecticide still remains largely effective against both *Aedes* species.

CONCLUSION

Insecticide resistance to a pyrethroid, lambda-cyhalothrin was detected in two local mosquito populations in Bagan Dalam, Penang. Dengue vectors remained susceptible to the organophosphate, pirimiphos-methyl. Organophosphates such as this might be considered for use as an alternative to pyrethroid by local authorities for dengue vector control.

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