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REVEALING EFFECTIVE ADULTICIDES FOR OUTDOOR DENGUE VECTOR CONTROL AT SELANGOR HOT SPRINGS

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ABSTRACT

The susceptibility levels of an outdoor dengue vector; *Aedes albopictus* adult populations from four hot springs in Selangor, Malaysia towards two organochlorine, two organophosphate, two carbamate and eight pyrethroid adulticides were determined in this study. *Aedes albopictus* adult populations from selected hot springs were exposed to each adulticide for an hour following the procedures of adult bioassays outlined by the World Health Organization (WHO). The mortalities throughout the exposure period and at 24 h post-exposure to each adulticide were noted. The *Ae. albopictus* laboratory strain which represented the reference strain was fully susceptible towards all tested adulticides, except for the exposure to pyrethroid permethrin 0.25% and bifenthrin 0.2%. As for the field adult populations of *Ae. albopictus*, various levels of resistance against organochlorine DDT 4% and dieldrin 4%; organophosphate fenitrothion 1%; carbamate propoxur 0.1% and bendiocarb 0.1%; as well as pyrethroid permethrin 0.25%, alpha-cypermethrin 0.05% and bifenthrin 0.2% were exhibited. Complete mortalities were recorded at 24 h post-treatment among all *Ae. albopictus* adult populations from hot springs that were exposed to organophosphate pirimiphos-methyl 0.25% as well as pyrethroid permethrin 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.05%, cyfluthrin 0.15% and etofenprox 0.5% which suggested that these adulticides are potentially effective in controlling *Ae. albopictus* populations at selected hot springs if needed in the near future. Despite zero case of mosquito-borne infection reported at selected hot springs so far, more studies on the mosquito densities at these hot springs and continuous monitoring of insecticide susceptibility among mosquito vectors from these hot springs are still vital so that effective future vector control strategies for these hot springs could be planned ahead.

Keywords: Adulticides, Dengue, Hot springs, Selangor, Malaysia.

INTRODUCTION

Dengue is a mosquito-borne infection that is endemic in tropical and subtropical climates. Dengue is transmitted by *Aedes aegypti* and *Ae. albopictus* (Kobayashi *et al.*, 2023). The incidence of dengue has been greatly increasing worldwide with significant morbidity and mortality. In Malaysia, for the year of 2023, until 4th November 2023, a total of 100,936 dengue cases with 78 deaths has been reported (Ministry of Health Malaysia, 2023).

As the development of the dengue vaccine is still ongoing, the spread of dengue infection is managed through the control of mosquito vectors. The World Health Organization (WHO) has recommended various vector control strategies comprising the environmental, mechanical, biological and chemical control methods (World Health Organization, 2012). Among all vector control methods suggested, the chemical control using insecticides remains the most selected control approach due to their quick actions and less costly. Nevertheless, frequent and massive application of insecticides lead to the development of insecticide resistance among mosquito vectors which eventually causes failures in the vector control activities conducted. Hence, it is important to clarify the susceptibility status of mosquito vectors from the target sites before the selection of insecticides to be used in the vector control operations is made.

Hot springs are natural geothermal water spots which are also a public attraction especially for illness treatment and relaxation. Besides human residential areas, recreational areas like hot springs are also at risk of mosquito-borne infection transmission due to the high human traffic and conducive ecology for mosquito vectors. Dense vegetation that surrounds most hot springs are idyllic resting and breeding sites for mosquitoes including the outdoor dengue vector; *Ae. albopictus* (Samson *et al.*, 2013). Thus, there is a need to formulate effective vector surveillance and control methods for hot springs in order to prevent the occurrence of any mosquito-borne infection including dengue. This study was carried out with the aim to determine the susceptibility status of the outdoor dengue vector; *Ae. albopictus* from selected hot springs in Selangor, Malaysia, against various public health insecticides. Four *Ae. albopictus* adult populations from Selayang (SEL), Hulu Tamu Batang Kali (HTBK), Kuala Kubu Bharu (KKB) and Kerling (KERL) hot springs in Selangor, Malaysia were exposed to different adulticides via the WHO adult bioassays which covered four insecticide classes, namely organochlorines, organophosphates, carbamates and pyrethroids.

MATERIALS AND METHODS

2.1 Study localities

Four hot springs in Selangor, Malaysia have been selected for this study, namely Selayang (SEL) hot spring in Gombak district, as well as Hulu Tamu Batang Kali (HTBK), Kuala Kubu Bharu (KKB), and Kerling (KERL) hot springs in Hulu Selangor district.

2.2 Samples of *Aedes albopictus*

Samples of an outdoor dengue vector; *Aedes albopictus*, were collected at each study locality via the ovitrapping technique. Ovitrap containing hay infusion water for the mosquito egg-laying were placed at the study localities for five days. All mosquito immatures found in the ovitraps were nurtured to adult stage in the insectarium. Only *Ae. albopictus* adults were maintained while other mosquito species were discarded.

Other than the field populations of *Ae. albopictus*, the laboratory strain of the same species (F80) was also utilized as a reference strain in this study. All *Ae. albopictus* colonies were nurtured in the same manner in the insectarium. *Aedes albopictus* female adults aged 3-5 days old were employed in this study.

2.3 Adulticides

Fourteen adulticides from four insecticide classes were tested in this study: organochlorine dichlorodiphenyltrichloroethane (DDT) 4%, and dieldrin 4%, organophosphate fenitrothion 1% and pirimiphos-methyl 0.25%; carbamate propoxur 0.1% and bendiocarb 0.1%; and pyrethroid permethrin 0.25% and 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.05%, cyfluthrin 0.15%, etofenprox 0.5%, alpha-cypermethrin 0.05% and bifenthrin 0.2%. All adulticides used were in the form of impregnated papers, bought from the WHO Collaborating Centre, Vector Control Research Unit (VCRU), Universiti Sains Malaysia, Penang, Malaysia.

2.4 Adult bioassays

The adult bioassays were conducted following the World Health Organization guidelines (2016). Twenty-five healthy sugar-fed *Ae. albopictus* female adults aged 3-5 days old were exposed to each adulticide impregnated paper in the exposure tube for 1 h. A total of four replicates per population were utilized in the adult bioassay. Two control tubes, each containing either the olive oil-impregnated paper (as an organochlorine and carbamate control) or the silicone oil-impregnated paper (as a

pyrethroid control), and similar number of sugar-fed female mosquitoes were also prepared and run. The mortality rates of mosquitoes were noted for every minute throughout the exposure time and at 24 h post-treatment.

2.5 Analysis of data

The mortality results of each population at 24 h post-treatment of all adulticides were converted into mortality percentages. Based on these mortality percentages, the susceptibility status of each *Ae. albopictus* population against all tested adulticides were then classified following the World Health Organization guidelines (2016): A 98-100% mortality showed susceptibility to the tested adulticide; 90-97% mortality revealed possible resistance against the tested adulticide which later had been confirmed by additional adult bioassays conducted; and <90% mortality signified confirmed resistance against the tested adulticide among the respective *Ae. albopictus* population.

RESULTS AND DISCUSSION

At 24 h post-exposure, full susceptibility was demonstrated among *Ae. albopictus* laboratory strain towards almost all organochlorine, organophosphate, carbamate and pyrethroid adulticides, except for permethrin 0.25% (51.00%) and bifenthrin 0.2% (67.00%) exposures (Table 1). Full susceptibility was also observed among all four hot springs populations of *Ae. albopictus* towards organophosphate pirimiphos-methyl 0.25% and also pyrethroid permethrin 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.05%, cyfluthrin 0.15% and etofenprox 0.5%. Possible resistance was displayed among all hot spring's populations against organochlorine DDT 4% (82.00 – 96.00%) and dieldrin 4% (93.00 – 100.00%) as well as carbamate propoxur 0.1% (83.00 – 95.00%). All hot springs populations exhibited confirmed resistance against organophosphate fenitrothion 1% (63.00 – 79.00%) and carbamate bendiocarb 0.1% (58.00 – 77.00%). *Aedes albopictus* adult populations from SEL, HTBK and KKB hot springs showed possible resistance against pyrethroid permethrin 0.25%, whereas, KERL hot spring population displayed confirmed resistance against the same adulticide. *Aedes albopictus* populations from HTBK, KKB and KERL hot springs were susceptible to pyrethroid alpha-cypermethrin 0.05% while possible resistance was displayed among *Ae. albopictus* population from SEL hot spring against the same pyrethroid. In contrast, full susceptibility towards bifenthrin 0.2% was demonstrated only among *Ae. albopictus* population from SEL hot spring while confirmed resistance was observed among other hot springs populations against bifenthrin 0.2%.

Among four insecticide classes tested in this study, the utilization of organochlorines in both agriculture and public health sectors has been banned worldwide including in Malaysia since the 1970s due to their persistent presence in the environment (Sharip *et al.*, 2017). However, resistance against organochlorines were detected among nearly all hot springs populations of *Ae. albopictus* tested in this study, suggesting that organochlorine elements were still persisted in these hot spring's environments.

On the other hand, organophosphates like fenitrothion, pirimiphos-methyl, malathion, fenthion and temephos have been used in the space treatment for dengue control activities in Malaysia (Ong, 2016). Pirimiphos-methyl is the recommended organophosphate adulticide to be applied at all hot springs if needed in the future based on the full susceptibility towards pirimiphos-methyl observed among *Ae. albopictus* hot springs populations.

Meanwhile, the utilization of carbamate adulticides in the selected hot springs should be carefully considered, monitored and rotated with adulticides from other insecticide classes due to the resistance development detected among *Ae. albopictus* populations from these hot springs against both carbamate propoxur and bendiocarb. The use of carbamate propoxur and bendiocarb in the local mosquito control activities are unknown. However, propoxur was formerly used as an active ingredient in the household aerosol locally before it was replaced by pyrethroids in 1990s (Low *et al.*, 2013).

In addition, the application of pyrethroid permethrin, deltamethrin, lambda-cyhalothrin, cyfluthrin and etofenprox in the future vector control activities at selected hot springs are also recommended based on the full susceptibility demonstrated among *Ae. albopictus* adult populations from these localities. In fact, some of these pyrethroids have been applied by the Ministry of Health Malaysia in their vector control programmes (Ong, 2016). Pyrethroid alpha-cypermethrin and bifenthrin could also be utilized in several hot springs selected but their effectiveness against *Ae. albopictus* population would require some time to be evidenced.

In general, all four selected hot springs are supported with common facilities like concrete benches surrounding the hot springs, resting huts and the lavatories. KERL hot spring is located marginally far from any human residential areas and surrounded by denser vegetation than other selected hot springs. On the other hand, SEL, HTBK and KKB hot springs are situated nearer to human residential areas which indirectly increase the chance of getting the insecticide exposure from the vector control activities conducted in the residential areas towards the hot spring's mosquitoes. The presence of human visitors, mosquito vectors as well as physical facilities and vegetation that could serve as prospective mosquito resting and breeding habitats at the hot springs are important factors that could mediate the transmission of mosquito-borne infections at these localities. Hence, it is crucial to continuously monitor the density and susceptibility of the mosquito vectors from the hot springs against insecticides so that effective mosquito control strategies could be planned and conducted at these hot springs when needed in the near future.

Table 1: Mortality Percentage at 24 h Post-Treatment for Five Populations of *Aedes albopictus* Female Adults Against Organochlorine, Organophosphate, Carbamate and Pyrethroid Adulticides.

Insecticide class	Populations	24 h mortality (%)				
		Laboratory strain (Reference)	Selayang hot spring (SEL)	Hulu Tamu Batang Kali hot spring (HTBK)	Kuala Kubu Bharu hot spring (KKB)	Kerling hot spring (KERL)
Organochlorines	DDT 4%	^S 100.00 ± 0.00	^M 91.00 ± 4.12	^R 87.00 ± 5.74	^R 82.00 ± 5.77	^M 96.00 ± 2.83
	Dieldrin 4%	^S 100.00 ± 0.00	^M 93.00 ± 1.00	^S 99.00 ± 1.00	^M 97.00 ± 1.91	^S 100.00 ± 0.00
Organophosphates	Fenitrothion 1%	^S 100.00 ± 0.00	^R 74.00 ± 8.08	^R 66.00 ± 10.13	^R 63.00 ± 4.43	^R 79.00 ± 4.73
	Pirimiphos-methyl 0.25%	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00
Carbamates	Propoxur 0.1%	^S 100.00 ± 0.00	^M 92.00 ± 2.83	^M 90.00 ± 3.46	^R 83.00 ± 3.00	^M 95.00 ± 3.00
	Bendiocarb 0.1%	^S 100.00 ± 0.00	^R 58.00 ± 3.46	^R 74.00 ± 3.46	^R 69.00 ± 6.61	^R 77.00 ± 5.26
Pyrethroids	Permethrin 0.25%	^R 51.00 ± 1.91	^M 96.00 ± 2.31	^M 90.00 ± 1.15	^M 91.00 ± 1.91	^R 83.00 ± 5.74
	Permethrin 0.75%	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00
	Deltamethrin 0.05%	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00
	Lambda-cyhalothrin 0.05%	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00
	Cyfluthrin 0.15%	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00
	Etofenprox 0.5%	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 100.00 ± 0.00
	Alpha-cypermethrin 0.05%	^S 99.00 ± 1.00	^M 97.00 ± 1.91	^S 100.00 ± 0.00	^S 100.00 ± 0.00	^S 99.00 ± 0.49
Bifenthrin 0.2%	^R 67.00 ± 7.55	^S 100.00 ± 0.00	^R 90.00 ± 3.46	^R 86.00 ± 2.58	^R 97.00 ± 3.00	

Percent mortality after 24 h (%) = Mean of mortality percentage of adult mosquitoes ± standard error (S.E.).

S = susceptible, M = possible resistance, R = confirmed resistance, as determined by WHO (2016).

CONCLUSION

In essence, findings of this study indicate organophosphate pirimiphos-methyl as well as pyrethroid permethrin, deltamethrin, lambda-cyhalothrin, cyfluthrin and etofenprox as potential adulticides to be applied at all four selected hot springs. Even though there is no case of mosquito-borne infection reported at these hot springs so far, more research on the mosquito density and insecticide susceptibility status among mosquito vectors from hot springs should be carried out following the

current popularity of these hot springs among local and foreign visitors so that the occurrence of any mosquito-borne infection at these hot springs could be prevented.

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