STATUS OF INSECTICIDE RESISTANCE ON *Aedes aegypti* (L.) AND *Aedes albopictus* (SKUSE) IN KAMPAR, PERAK, MALAYSIA

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ABSTRACT

The threat of dengue and Chikungunya transmitted by *Aedes* mosquitoes continues to cause concern to the public resulting to control by the usage of insecticides. However, the heavy dependence on such chemicals has caused the development of resistance towards insecticides. The objective of this study is to determine the insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* in Kampar, Perak, Malaysia. Ovitraps were placed in the urban and suburban region of Kampar, Perak and collected after seven days. Identified *Ae. aegypti* and *Ae. albopictus* females aged from three to five days were exposed to two different chemical classes, pyrethroids (0.75% permethrin & 0.05% deltamethrin) and organophosphates (5% malathion & 0.25% pirimiphos-methyl) using WHO adult bioassay test. For *Ae. aegypti* the knockdown time recorded for 0.75% permethrin was significantly longer at KT_{50} of 222.475 minutes and KT_{95} of 1742.297 minutes as compared to 0.05% deltamethrin and 5% malathion ($F=82.19$, $df=4$, $P<0.05$). Meanwhile the knockdown time for *Ae. albopictus* recorded for 5% malathion was significantly longer at KT_{50} 56.827 minutes of and KT_{95} 105.175 minutes as compared to 0.75% permethrin and 0.05% deltamethrin ($F=968.82$, $df=4$, $P<0.05$). No knockdown was recorded for 0.25% pirimiphos-methyl for both species. *Aedes aegypti* was confirmed to be resistant to 0.75% permethrin, 0.05% deltamethrin and 0.25% pirimiphos-methyl tested after 24 hours, while *Ae. albopictus* was confirmed resistant towards 0.25% pirimiphos-methyl (4 ± 0.20% mortality) and 5% malathion (77 ± 3.85%) and possibly resistant to 0.05% deltamethrin (93 ± 4.65% mortality). No mortality was found in the control group. This study concludes that the constant usage of insecticides in Kampar especially in the urban area has caused the development of resistance in *Ae. aegypti* and *Ae. albopictus.*

Keywords: *Aedes*, adult bioassay, insecticides, mosquito, resistance

ABSTRAK

Ancaman denggi dan Chikungunya yang disebarkan oleh nyamuk *Aedes* menimbulkan kerisauan kepada masyarakat menyebabkan penggunaan racun serangga sebagai kaedah pengawalan. Walau bagaimanapun, kebergantungan terhadap bahan kimia telah menyebabkan pembentukan kerintangan terhadap racun serangga. Objektif kajian ini adalah untuk

menentukan status kerintangan *Aedes aegypti* dan *Aedes albopictus* terhadap racun serangga di Kampar, Perak. Ovitrap dipasang di kawasan bandar dan pinggir bandar di Kampar, Perak dan dikumpul selepas tujuh hari. *Aedes aegypti* dan *Ae. albopictus* betina yang dikenal pasti dan berusia tiga hingga lima hari telah didedahkan kepada dua bahan kelas kimia yang berbeza, piretroid (0.75% permetrin & 0.05% deltametrin) dan organofosfat (5% malation & 0.25% metil-pirimifos mengunakan kaedah bioassaiy dewasa WHO. Masa yang diambil utuk *Ae. aegypti* rebah disebabkan oleh 0.75% permethrin dengan KT_{50} selama 222.475 minit dan KT_{95} selama 1742.297 minit berbanding 0.05% deltametrin dan 5% malation ($F=82.19$, $df=4$, $P<0.05$). Manakala masa yang direkodkan untuk *Ae. albopictus* rebah disebabkan 5% malation jauh lebih lama dengan KT_{50} selama 56.827 minit dan KT_{95} selama 105.175 minit berbanding 0.75% permetrin dan 0.05% deltametrin ($F=968.82$, $df=4$, $P<0.05$). Tiada nyamuk dari kedua-dua species yang rebah akibat 0.25% metil-pirimifos direkodkan. *Aedes aegypti* disahkan rintang terhadap 0.75% permetrin, 0.05% deltametrin dan 0.25% metil-pirimifos yang diuji selepas 24 jam, manakala *Ae. albopictus* disahkan rintang terhadap 0.25% metil-pirimifos (kematian 4 ± 0.20%) dan 5% malation (kematian 77 ± 3.85%) dan kemungkinan rintang terhadap 0.05% deltametrin (kematian 93 ± 4.65%). Tiada kematian dicatatkan dalam kumpulan kawalan. Kajian ini menunjukkan bahawa penggunaan racun serangga secara berterusan di Kampar terutama di kawasan bandar telah menyebabkan perkembangan kerintangan oleh *Ae. aegypti* dan *Ae. albopictus.*

Kata kunci: *Aedes,* bioesei dewasa, racun serangga, nyamuk, kerintangan

INTRODUCTION

Aedes mosquitoes are identified as important vectors responsible for transmitting yellow fever disease, dengue disease as well as Chikungunya. *Aedes aegypti* is known to be the primary vector in transmitting the dengue virus while *Ae. albopictus* is known to be the secondary vector. However, since it is difficult to stop the virus, it is best to control the spread of the vector instead, which are the *Aedes* mosquitoes. There are plenty of techniques in controlling these mosquitoes, primarily environmental management, chemical and biological control and personal protection through the most common method in controlling mosquito is chemical control (WHO 2003). The major type of control being used in controlling the *Aedes* mosquitoes is chemical which includes chemical larviciding, mosquito coil, aerosol and thermal fogging (WHO 2003).

However, the excessive usage of insecticides has caused the development of resistance in *Aedes* mosquitoes towards the insecticides. Past researches further supported this statement. Rosilawati et al. (2017) stated that *Ae. aegypti* in Johor, Melaka and Selangor were resistant towards permethrin, while a study from Elisa-Amira et al. (2018) stated that *Ae. albopictus* in Sabah is resistant towards malathion, temephos and DDT, and recent study by Ali et al. (2020) stated that both *Aedes* species have developed resistance towards permethrin, lambda-cyhalothrin, DDT, malathion and propoxur in dengue hotspots of Kuala Lumpur and Selangor. The increase in the number of cases of Chikungunya in the district of Kampar, Perak has raised some concern due to the development of resistance towards insecticides in the *Aedes* mosquitoes in Kampar, Perak. In an official statement issued by the Ministry of Health, the number of Chikungunya cases in Kampar, Perak has reached 137 cases, representing the highest district with Chikungunya cases in the state of Perak in May 2020 (Manjit 2020).

The resistance of *Aedes* mosquitoes towards these insecticides can be categorized into four categories which are the behavioural resistance, target-site resistance, metabolic resistance

and reduced penetration. Behavioural resistance associates with the modification in the insect behaviour to avoid the lethal effects of insecticides while target site resistance involves mutations at a genetic level to alter the target site of action of an insecticide (IRAC 2011). One of the most common types of resistance developed by an insect is metabolic resistance. The enzyme system in an insect is often enhanced to help metabolize or detoxify the insecticide before exerting its toxic effects on the insect. Finally reduced penetration resistance is defined by the modifications in the insect cuticle or digestive tract lining to prevent or reduce the absorption of insecticides into the insect (IRAC 2011).

Resistance monitoring should be an important aspect in the vector and public health control to possibly delay or avoid the development of resistance with the constant threat of dengue and Chikungunya (Karunaratne et al. 2018). Hence, the knowledge of vector susceptibility to insecticides and resistance trend changes will help vector-borne disease control programs. The objective of this study to determine the resistance of *Ae. aegypti* and *Ae. albopictus* in Kampar, Perak towards pyrethroids and organophosphates using adult bioassay test.

MATERIALS AND METHODS

Aedes Mosquitoes Sampling and Culture

An urban region (4° 18’ 41.175” N, 101° 09’ 09.675” E) for the collection of *Ae. aegypti* and sub-urban region (4° 18’ 28.6” N, 101° 09’ 03.8” E) for collection of *Ae. albopictus* samplings in Kampar, Perak was chosen for this study. Ovitraps were prepared by spray painting aluminium cans black and obtaining wooden paddle that is smooth on one side and coarse of the other. The ovitraps were then filled with seasoned water, and the wooden paddles were placed in the ovitraps coarse side up. Seasoned water is tap water that is left in a container for several days to allow the chlorine in the water to evaporate. A total of 30 ovitraps were placed two metres apart at locations suspected to be *Aedes* breeding site per locality. The ovitraps were then be collected after seven days and brought back to the laboratory. The water in the ovitraps was poured in separate enamel trays, and the collected wooden paddles were submerged in separate enamel trays containing seasoned water. The trays were labelled to know the location of the sample. Once the eggs hatched, the larvae were transferred into new enamel trays containing seasoned water and were cultured at a temperature of 26±2^{o}C and 70-80% of relative humidity. The larvae were fed with 10mg of larval food daily. Food consisted of dog biscuit, beef liver, yeast and milk at a ratio of 2:1:1:1 by weight. When the larvae pupate, the pupae were transferred into a small plastic cup and placed in the mosquito cage containing cotton soaked in sucrose solution as a food source for the emerging adult mosquitoes. The pupae were in separate mosquito cages based on the location of the sample obtained. Once emerged, the mosquitoes were identified and sorted out by species. *Aedes aegypti* and *Ae. albopictus* were used in this study.

Adult Bioassay Test

The insecticides classes chosen for this study were pyrethroids (0.75% permethrin and 0.05% deltamethrin) and organophosphate (5% malathion and 0.25% pirimiphos-methyl). The bioassay test was conducted following WHO (2018) procedure using insecticides doses recommended for *Anopheles* mosquitoes, which is higher than the recommended dose used to test for *Aedes* mosquitoes. Thus, to test the *Aedes* mosquitoes’ resistance status against higher doses of insecticides. A total of 25 female *Aedes* mosquitoes aged 3-5 days were aspirated into the holding tube (green dotted) of the bioassay kit. After an hour, any moribund or dead mosquitoes were removed and replaced with healthy ones. The mosquitoes were then blown

into the exposure tube (red dotted) consisting of each insecticide-impregnated paper rolled in it. The knockdown of the mosquitoes was recorded for an hour at 5 minutes' interval. After an hour, the exposed mosquitoes were transferred back into the holding tube. Cotton soaked in sucrose was placed on top of the holding tube, and the holding tube was left for 24 hours. The mortality of the mosquitoes was recorded after 24 hours to determine the susceptible status of each species. A total of four replicates were conducted for each insecticide and species. For the control treatment, silicone oil-impregnated paper was rolled into the exposure tube (yellow dotted) for pyrethroid control, and olive oil-impregnated paper was used for organophosphate control. A total of two replicates of control were conducted for both pyrethroid and organophosphate control, as recommended by WHO (2018).

Data Analysis

All data were analysed using probit analysis in SPSS version 20.0 to obtain the knockdown 50 (KT_{50}) and knockdown time 95 (KT_{95}). Data were log-transformed prior to statistical analysis to fulfil the assumption of probit analysis. KT_{50} was defined as the time required to knockdown 50% of the mosquitoes, whereas KT_{95} is required to knockdown 95% of the mosquitoes. The knockdowns were analyzed using two-way ANOVA in order to check for significant effects among time and insecticides used (IBM 2011). The susceptibility status of each species to each insecticide was determined using WHO (2018) guidelines. The species is susceptible when the mortality rate is more than 98% while mortality rates between 90-97% are suspected of having resistance and mortality rate below 90% is confirmed resistant.

RESULTS

When exposed to all insecticides in this study, *Ae. aegypti* was found to have fewer knockdown readings compared to *Ae. albopictus* as shown in Figure 1. However, both species did not record any knockdown for 0.25% pirimiphos-methyl in the study at one-hour interval. Thus, the KT_{50} and KT_{95} value cannot be calculated (Table 1). Significantly longest knockdown time was recorded for *Ae. aegypti* by 0.75% permethrin with KT_{50} of 222.475 minutes and KT_{95} of 1742.297 minutes ($F=82.19$, $df=4$, $P<0.05$) while 5% malathion recorded the longest knockdown time for *Ae. albopictus* with KT_{50} of 56.827 minutes and KT_{95} of 105.175 minutes ($F=968.82$, $df=4$, $P<0.05$) which can be seen in Table 1 and Table 2.

Based on WHO (2018) guideline, *Ae. aegypti* was confirmed to be resistant to three insecticides (0.75% permethrin, 0.05% deltamethrin & 0.25% pirimiphos-methyl) tested as the mortality rates did not exceed 90% except for 5% malathion as it is suspected to be resistant with the mortality of 93 ± 4.65% (Figure 2). 0.75% permethrin recorded the lowest mortality rate for *Ae. aegypti* with a mortality rate of 13 ± 0.65% compared to other insecticides ($F=78.349$, $df=4$, $P<0.05$). Meanwhile, *Ae. albopictus* was confirmed resistant towards 0.25% pirimiphos-methyl and possibly resistant to 0.05% deltamethrin (Figure 2). 0.25% pirimiphos-methyl recorded the lowest mortality rate for *Ae. albopictus* with a mortality rate of 4 ± 0.2% as shown in Table 2. The 100% mortality when *Ae. albopictus* was tested with 0.75% permethrin indicated that they are still susceptible to 0.75% permethrin. No mortality was found in control.

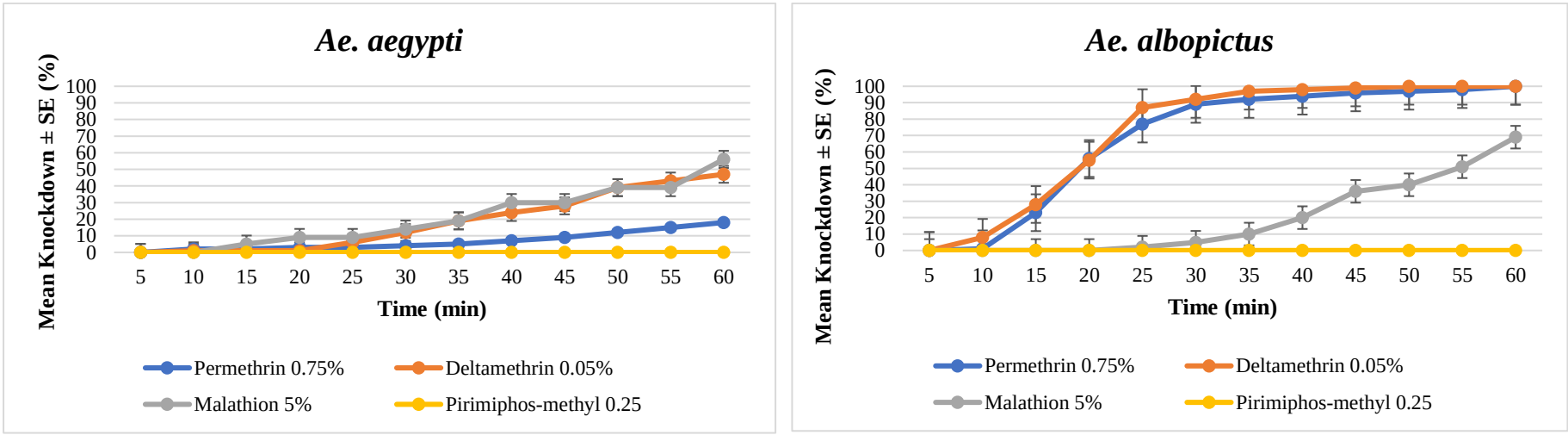

Figure 1. *Aedes aegypti* and *Ae. albopictus* knockdown period by four tested insecticides recorded at 5 minutes' intervals for 60 minutes

Table 1. Mean knockdown time, KT_{50} and KT_{95} (in minutes) of *Ae. aegypti* and *Ae. albopictus* when exposed with pyrethroids and organophosphates for 60 minutes

Species	Insecticides	KT_{50}	KT_{95}	Regression equation
Ae. aegypti	Permethrin 0.75%	222.475 (141.552-538.034)	1742.297 (671.811-11639.451)	Y=-4.313+0.498X
	Deltamethrin 0.05%	61.801 (57.102-68.512)	167.925 (135.983-225.616)	Y=-6.786+0.538X
	Malathion 5%	62.073 (56.632-69.944)	210.357 (163.432-299.308)	Y= -5.56+0.430X
	Pirimiphos-methyl 0.25%	-	-	-
Ae. albopictus	Permethrin 0.75%	19.824 (19.434-22.272)	38.979 (35.797-43.337)	Y=-7.266+0.417X
	Deltamethrin 0.05%	17.991 (17.110-18.844)	32.851 (30.876-35.347)	Y=-7.895+0.476X
	Malathion 5%	56.827 (54.187-60.324)	105.175 (92.934-124.816)	Y=-11.770+0.873X
	Pirimiphos-methyl 0.25%	-	-	-

Table 2. Analysis of variance on *Ae. aegypti* and *Ae. albopictus* knockdown comparing insecticides and time

Source of variation	*Ae. Aegypti*				*Ae. albopictus*			
	df	MS	*F*-value	*P*-value	df	MS	*F*-value	*P*-value
Insecticides	4	292.900	82.186	**0.000**	4	3917.000	968.822	**0.000**
Time	11	81.305	22.813	**0.000**	11	441.050	109.088	**0.000**
Insecticides*Time	44	19.827	5.563	**0.000**	44	111.900	27.677	**0.000**

df, degree of freedom; MS, mean-squared value.
Significant values are given in bold.

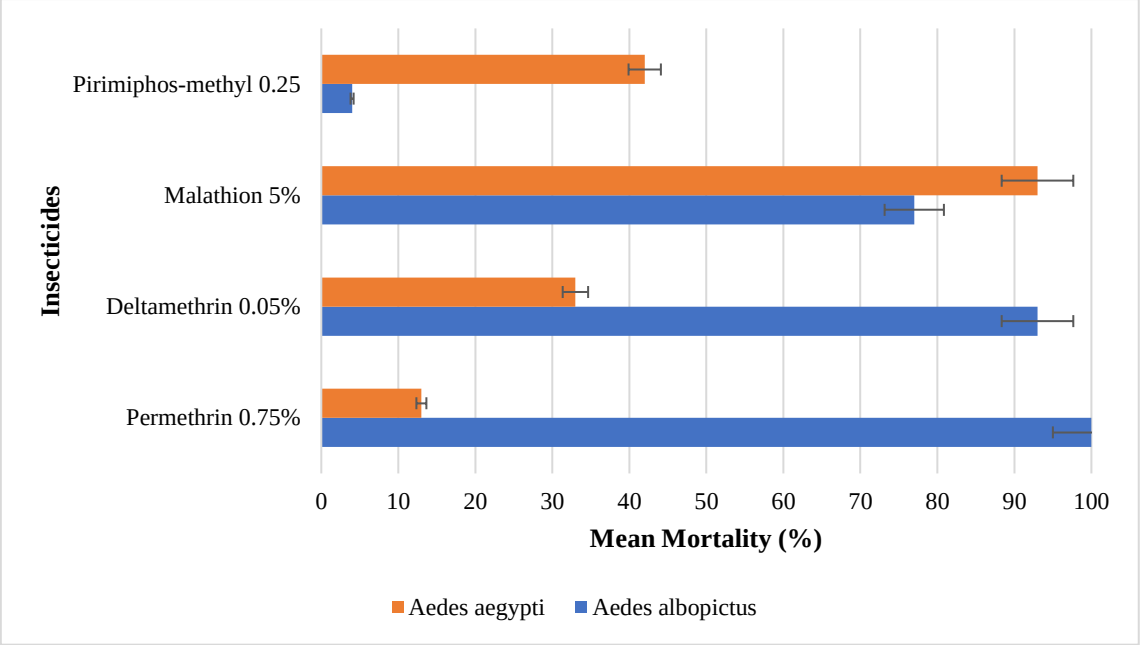

Figure 2. Mean mortality of *Ae. aegypti* and *Ae. albopictus* 24 hours' post exposure by four tested insecticides

DISCUSSION

The mortality rate of *Ae. aegypti*, which was sampled in the urban region of Kampar, Perak was less than 90% when exposed to three insecticides (0.75% permethrin, 0.05% deltamethrin & 0.25% pirimiphos-methyl) suggested that resistance had developed. Thus, this indicates that these chemicals might have been used frequently for an extended period in controlling mosquitoes in urban regions of Kampar and the resistance had occurred on this *Aedes* mosquito population. Similarly, chemical control involving pirimiphos-methyl, malathion and deltamethrin might have been frequently used in the sub-urban region of Perak, causing the development of resistance in *Ae. albopictus.* This has proven with research made in 2014 by Ho et al. (2014) that showed that *Ae. albopictus* have started to develop resistance towards organophosphate insecticides in another locality (Taman Kampar Jaya and Taman Juloong) in Perak. This study showed that *Ae. albopictus* tested with 5% malathion recorded KT_{50} of 48.46 minutes and KT_{95} of 87.72 minutes at Taman Kampar Jaya and KT_{50} of 62.69 minutes and KT_{95} of 141.04 minutes at Taman Juloong. The prolonged use of chemical insecticides for as short as two years can create selection pressure, resulting in the development of resistance in *Aedes* mosquitoes (Besar et al. 2019).

Another explanation of the resistance development of *Ae. aegypti* in the urban region of Kampar, Perak could possibly due to the proximity they live to human residences. This might have increased the risk of *Ae. aegypti* being exposed to different forms of insecticides such as aerosol spray from household insecticide or fogging conducted by the Ministry of Health, thus causing them to develop resistance towards the exposed insecticides. The wide-ranging breeding habitat from natural to man-made container probably would have led to having less exposure to the insecticides, making them more susceptible to certain insecticides (Rattanam et al. 2020). The dose used in this study is *Anopheles* diagnostic dose, which is higher than the recommended dose *Aedes* diagnostic dose (WHO 2016, 2018). However, resistant is still prominent in *Ae. aegypti* even when the higher dose was used. Several studies

have further proved that *Ae. aegypti* was resistant towards anopheline mosquito's diagnostic dose such as 0.75% permethrin than the WHO recommended diagnostic dose for *Aedes* which is 0.25% permethrin (Hamid et al. 2017; Iwani 2019). This could point towards suggesting that the recommended diagnostic dose for *Aedes* mosquitoes by WHO is too low to be viable to control *Aedes* mosquitoes here in Malaysia especially in Kampar, Perak thus, need to be re-evaluated.

The two possible and most common type of resistance developed by these *Aedes* mosquitoes towards pyrethroid and organophosphate insecticides could be target-site resistance or metabolic resistance. Constant exposure of pyrethroids might have caused amino acid substitution in the voltage-gated sodium channel (VGSC), which is the target site of DDT and pyrethroids (Rinkevich et al. 2013; Shono 1985). This mechanism is called "knockdown resistance". Meanwhile, constant exposure towards organophosphate based insecticides might have caused point mutation at the acetylcholinesterase (AChE) gene, which is the target-site for organophosphate and carbamate insecticides (Hidajat et al. 2019). Another explanation could be the constant exposure to pyrethroids and organophosphates might have made have caused an increase on the detoxifying enzymes which are Esterases, Glutathione-*S*-transferases (GST) and Mixed-Function Oxidases (MFOs or P450) for the *Aedes* mosquitoes to be metabolically resistant to these insecticides (Martins et al. 2009). Therefore, further study can be conducted on the type of resistance that has been developed by *Aedes* mosquitoes especially *Ae. aegypti* in Kampar, Perak as it is important to determine the type of resistance the insects have developed before taking necessary actions.

CONCLUSIONS

This study concludes that urban region of *Ae. aegypti* has developed resistance to both pyrethroid class insecticides (0.75% permethrin and 0.05% deltamethrin) and organophosphate class insecticides (5% malathion and 0.25% pirimiphos-methyl), while the sub-urban region of *Ae. albopictus* is resistant to organophosphates and may have started to develop resistance toward deltamethrin in Kampar, Perak. Results that are found in this study is beneficial in vector control and management program. The use of biocontrol agent can be synergistically added into the vector control program alongside the use of insecticides as a measure to reduce the resistance issue. Constant monitoring and surveillance on the status of resistance developed by the mosquito populations will help towards stopping disease outbreaks and reducing the number of dengue and Chikungunya case in Perak.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of Vector Control Research Unit and School of Biological Sciences, USM for field assistance. We would like to thank the Ministry of Health Perak for their support during the study. This project was funded by Research University Grant Universiti Sains Malaysia (1001/PBIOLOGI/8011066) and Fundamental Research Grant Scheme by Ministry of Higher Education Malaysia (203/PBIOLOGI/6711629).

REFERENCES

Ali, W.N.W.M., Ahmad, R., Nor, Z.M. & Hassan, A.F. 2020 Spatial distribution, enzymatic activity and insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* from dengue hotspot areas in Kuala Lumpur and Selangor, Malaysia. *Serangga* 25(3): 65-92.

Besar, N.A.U.A., Sulaiman, A., Asri, L.N. & Khairuddin, 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.

Elisa-Amira, N.M.R, Chen, C.D., Lau, K.W., Low, V.L., Norma-Rashid, Y. & Sofian-Azirun, M. 2018. Organophosphate and organochlorine resistance in larval stage of *Aedes albopictus* (Diptera: Culidae) in Sabah, Malaysia. *Journal of Economic Entomology* 111(5): 2488-2492.

Hamid, P.H., Prastowo, J., Widyasari, A., Taubert, A. & Hermosilla, C. 2017. Knockdown resistance (*kdr*) of the voltage-gated sodium channel gene of *Aedes aegypti* population in Denpasar, Bali, Indonesia. *Parasit. Vectors* 10: 283.

Hidajat, M.C., Dharmana, E., Prihatin, M.T., Martini & Ambargarjito, T. 2019. Molecular resistance status of *Aedes aegypti* to the organophosphate and pyrethroid insecticides in central Sulawesi and East Nusa Tenggara provinces, Indonesia. *Advances in Health Sciences Research* 24: 122-127.

Ho, L.Y., Loh, T.S. & Yam, L.A. 2014. Surveillance and resistance status of *Aedes* population in two suburban residential areas in Kampar town, Perak, Malaysia. *Tropical Biomedicine* 31(3): 441-448.

IBM. 2011. IBM SPSS Advanced Statistics 20. New York: IBM Corporation.

Insecticide Resistance Action Committee (IRAC). 2011. Prevention and management of insecticide resistance in vectors of public health importance. A manual produced by Insecticide Resistance Action Committee (IRAC).

Iwani, J. 2019. Investigating insecticide resistance mechanisms in dengue vectors population; *Aedes aegypti* and *Aedes albopictus* in Penang Island. Master of Science Thesis, Universiti Sains Malaysia.

Karunaratne, S.H.P.P., Silva, W.A.P.P.D, Weeratne, T.C. & Surendran, S.N. 2018. Insecticide resistance in mosquitoes: Development mechanisms and monitoring. *Ceylon Journal of Science* 47(4): 299-309.

Manjit, K. 2020. Perak sees 30 times more chikungunya cases compared with last year. *The Star*, 30 May: https://www.thestar.com.my/news/nation/2020/05/30/perak-sees-30-times-more-chikungunya-cases-compared-with-last-year (10 June 2020).

Martins, A.J., Lins, R.M.M.A.A., Linss, J.G.B., Peixoto, A.A. & Valle, D. 2009. Voltage-gated sodium channel polymorphism and metabolic resistance in pyrethroid-resistant *Aedes*

aegypti from Brazil. *The American Journal of Tropical Medicine and Hygiene* 81(1): 108-115.

Rattanam, A., Ishak, I.H., Yahya, Z.S. & Zuharah, W.F. 2020. Knockdown resistance (*kdr*) in dengue vectors, *Aedes aegypti* and *Aedes albopictus*: A post-flood risk assessment. *Genetics and Molecular Research* 19(2): 1-16.

Rinkevich, F.D., Du, Y. & Dong, K. 2013. Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids. *Pesticide Biochemistry and Physiology* 106: 93–100.

Rosilawati, R., Lee, H.L., Nazni, W.A., Nurulhusna, A.H., Roziah, A., Asuad, K.M., Fahaininajua, S.F.F., Farihan, M.M.Y. & Ropiah, J. 2017. Pyrethroid resistance status of *Aedes (Stegomyia) aegypti (Linneaus)* from dengue endemic areas in Peninsular Malaysia. *IIUM Medical Journal Malaysia* 16(2): 73-78.

Shono, T. 1985. Pyrethroid resistance: importance of the *kdr*-type mechanism. *Journal of Pesticide Science* 10: 141–146.

WHO. 2003. Guidelines for Dengue Surveillance and Control. Manila: World Health Organisation.

WHO. 2016. Monitoring and Managing Insecticide Resistance in *Aedes* Mosquito Populations. Geneva: World Health Organisation.

WHO. 2018. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. Geneva: World Health Organization.