Evaluation of the DDT and Pyrethroid Resistance Status of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) in Kota Bharu, Kelantan

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ABSTRACT The emergence of resistance in vector mosquitoes such as *Aedes*, mainly against pyrethroids group of insecticide, has become a serious threat to vector control management in public health services. This study aims to investigate the susceptibility/resistance status of the dichloro-diphenyl-trichloroethane (DDT), lambda-cyhalothrin, and permethrin and the presence of F1534C and V1016G knockdown *(kdr)* alleles mutations in *Aedes aegypti* populations in Kota Bharu Kelantan Malaysia. The long-lasting resistant effects of DDT against *Ae. aegypti* is evaluated. The F1 adults' of *Ae. aegypti* were collected from Kota Bharu and assayed to the WHO susceptibility test with 4% DDT, 0.05% lambda-cyhalothrin, and 0.75% permethrin. Mortality percentage, knockdown time KT50, and resistance ratio RR values were calculated for susceptibility analysis. All the mosquito survivors from the assay were kept in -80 °C and subjected to the Allele Specific-Polymerase Chain Reaction (AS-PCR) analysis on the presence of *kdr* mutation F1534C and V1016G. Results obtained from the assay show *Ae. aegypti* was resistant phenotypically against type I and type II pyrethroids viz permethrin and lambda-cyhalothrin. AS-PCR analysis however showed a resistance genotypically against pyrethroid type I only. The RR values for all insecticides tested were more than 10, which indicate resistance. The *kdr* mutation alleles frequencies of 1/3 F/C1534 and 2/3 C/C1534 were detected in *Ae. aegypti* population.

KEYWORDS: *Aedes aegypti*, DDT-pyrethroid, *kdr* alleles, F1534C, V1016G
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Original Article

INTRODUCTION

Aedes sp. mosquito is the known vector for arthropod-borne diseases such as chikungunya, dengue, Yellow fever, and Zika in many tropical and subtropical countries worldwide. *Aedes aegypti* has been known as the primary vector for dengue fever (DF) and dengue haemorrhagic fever (DHF) in Malaysia. For over a decade, dengue has continuously become a serious health problem in Malaysia since its first discovery in the 1920s (Skae, 1902). The most effective method to control the dengue outbreak is by eliminating the vector, in the affected area by using chemical insecticide (Rozendaal & WHO, 1997). Prolonged and heavy usage of chemical insecticide in mosquito control has caused resistance development in the population. However, this approach is continuing to be applied due to its immediate impact to prevent the outbreak. Hence, it became a major threat in the vector control program worldwide mainly from the group of the pyrethroid insecticide (Grossman *et al.,* 2018). While there are studies from certain parts of Malaysia indicating pyrethroids resistance in *Aedes* sp. (Leong *et al.,* 2019; Rohani *et al.,* 2019; Amelia-Yap *et al.,* 2018), ongoing data reporting on the resistance specifically at the hotspot/outbreak area is still lacking. In aiding the loopholes, frequent data/information updating on the insecticide resistance would be helpful**.** They should be made available to the vector management team for their control strategic plans.

For mosquitoes, knock-down resistance (*kdr*) is a target site insensitivity mechanism that is associated with single or multiple mutations of the voltage-gated sodium channel (VGSC) protein

(Saingamsook *et al.*, 2017). *Kdr* is the important mechanism for resistance to pyrethroids and DDT (Hemingway *et al.*, 2004). Briefly, two site mutations are involved in the *kdr*. The first mutation is a valine to glycine substitution at position 1016 within domain II of the VGSC (V1016G) is associated with resistance to type I and II pyrethroids, such as permethrin and deltamethrin, respectively (Stenhouse *et al.*, 2013). A second one is associated with resistance type I pyrethroid, involving phenylalanine to cysteine substitution at position 1534 within domain III (F1534C) that many countries in Asia have reported (Sayono *et al.*, 2016; Wuliandari *et al.*, 2015; Yanola *et al.*, 2010). This study aims to evaluate the current susceptibility and resistance status of *Ae. aegypti* against pyrethroid insecticide at the hotspot dengue area in Kelantan, Malaysia. A bioassay method of the insecticide was employed to study the susceptibility and resistance status of the *Ae. aegypti* mosquito, and the polymerase chain reaction (PCR) was applied to detect the occurrence of *kdr* mutations at the site of allele specifics, F1534C and V1016G in the affected *Ae. aegypti* population.

METHODOLOGY

Sampling Area

The sampling location is situated in the Kota Bharu district of Kelantan, Taman Harmoni, Kg Beris Lanjut (6°08'07.1"N 102°17'30.8"E) (Figure 1). It is a residential area that had been identified as a dengue hotspot locality from 2014 to 2018. The area was occasionally treated with ULV insecticides such as Deltamethrin, and Permethrin, and S-bioallethrin throughout the years to control and prevent the outbreak (KKM, 2017; 2014).

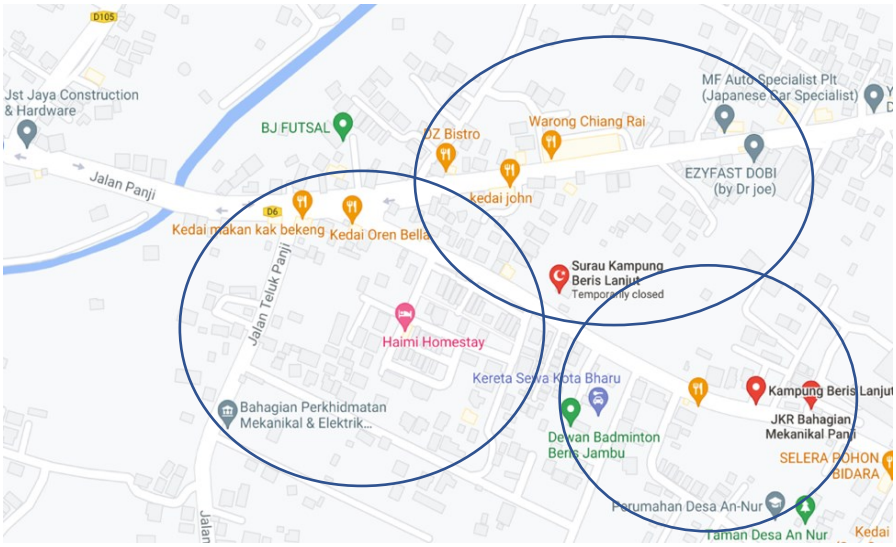

Figure 1. Circles area indicate the sampling locations spot in Panji, Kota Bharu.

Mosquito Preparation

F1 generation of the field caught *Ae. aegypti* were used in this study. The mosquitoes were obtained from a field sampling method using ovitrap as described by Lee (1992). The collection period was carried out from January 2017 to May 2019. Collected eggs were cultured and reared to the adult stage for species confirmation. The colonies were maintained in the insectarium at room temperature, 27.0 ± 2 °C. The adults were provided with a 10% sucrose contained B complex. All the survived mosquitoes from the bioassay test were preserved in the freezer (-80 °C) for the PCR analysis. A total of one hundred adult female *Ae. aegypti* mosquitoes, aged 3-5 days were used per tested insecticide in the adult bioassay study.

Bioassay

The bioassay procedure was conducted against the field and laboratory strain (F270 generation) of *Ae. aegypti* mosquitoes following the WHO guidelines (WHO, 2016) with some modification of test paper concentrations. The insecticide-impregnated papers and laboratory strain mosquitoes used in this study were purchased from the Vector Control Research Unit (VCRU) of Universiti Sains Malaysia, Pulau Pinang. The adult mosquitoes were tested against 4% DDT (Organochlorin), 0.75% Permethrin (pyrethroid I), and 0.05% Lambdacyhalothrin (pyrethroid II). OP Carbamate and PY were used as controls (DDT and permethrin/lambdacyhalothrin) respectively. Batches of 20 individual female *Ae. aegypti* were exposed to insecticide-impregnated papers for 60 minutes. Knockdown was observed and recorded for every five minutes within one hour of exposure. After the exposure periods, the mosquitoes were transferred into a clean paper cup, provided with a 10% sugar solution. The test mosquitoes and the controls were held for a 24-h recovery period and the mortality was recorded.

DNA Extraction and Amplification

In each PCR reaction, ten mosquitoes were used per pool. The mosquitoes used were from the preserved stock kept in -80C. The DNA of *Ae. aegypti* were extracted using a DNA extraction kit (Macherey-Nagel NucleoSpin ®). The mosquito was prepared by removing the legs and wings from the body. The thorax and bodies were cut into small pieces and kept at 4°C. The PCR reaction primers of F1534C and V1016G as shown in Table 1 were used to amplify the partial sequence following a standard PCR protocol. The reaction for primers F1534C was carried out in a final volume of 25µl, comprising 12.5 µl of 2X MyTaq™ Mix, 0.31 µl of 10 µM forward primer (C1534-f), 0.31 µl of 10 µM reverse primer (C1534-r), two internal allele-specific primers: 0.13 µl of 10 µM Ae1534F-r and 0.63 µl of 10 µM Ae1534C-f, 9.62 µl PCR water and 200ng of 1.5 µl DNA template.

Table 1. List of the primer used in this study (Saingamsook *et al.*, 2017).

Primer	Primer sequence (5′-3′)	Product size bp	Exon
1534 genotyping			
C1534-f	GCGTACCTGTGTCTGTTCCA	368	23
C1534-r	GGCTTCTTCGAGCCCATCTT		
Ae1534F-r	GCGTGAAGAACGACCCGA	232	24
Ae1534C-f	CCTCTACTTTGTGTTCTTCATCATCTG	180	24
1016 genotyping			
Gly1016-f	ACCGACAAATTGTTTCCC		15–16
Val1016-r	[short GC tail][a] AGCAAGGCTAAGAAAAGGTTAATTA	60	16
Gly1016-r	[long GC tail][b] AGCAAGGCTAAGAAAAGGTTAACTC	80	16

Notes: [a]short GC tail sequence: 5′-GCG GGC-3′

[b]Long GC tail sequence: 5′-GCG GGC AGG GCG GCG GGG GCG GGG CC-3′

On the other hand, reaction for the primer V1016G was carried out in a final volume of 25µl, comprising 12.5 µl of 2X MyTaq™ Mix, 0.31 µl of 10 µM forward primer (Gly1016-f), 0.16 µl of 10 µM reverse primer (Gly1016-r), two specific reverse primers 0.16 µl of 10 µM Val1016-r and 0.16 µl of 10 µM Gly1016-r, 10.37 µl PCR water and 1.5 µl DNA template. The amplification conditions for both primers, F1534C and V1016G consisted of 94 °C for a 15-sec initial denaturation, followed by 35 cycles each of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s and followed by a final extension at 72 °C for 10 min. The PCR products were then analyzed in agarose gel on gel electrophoresis.

Gel Electrophoresis

After PCR reactions, the amplified products of F1534C and V1016G primers were visualized on 1.5% and 3.0% agarose gel with a low molecular weight DNA ladder (DM1100 ExcelBand™ 50 bp DNA Ladder) to estimate the band size. A total of 8µL PCR product was mixed with 2µL loading dye and followed by 4µL of a ladder. The gel was then submerged in 0.5 X TBE buffer and was run for 60 minutes at 90V for genomic DNA and 60 min at 100 V for PCR products and visualized in a UV transilluminator.

Statistical Analysis

The bioassay was analyzed using SPSS software v.24. Control mortality between 5% and 20% is corrected using Abbott's (1925) formula. The KT50 value of all tested insecticides was analyzed with a probit formula (Raymond, 1985). The resistance ratio (RR) of all tested samples were calculated by dividing the KT50 of the field strain mosquitoes to the KT50 of the laboratory strain mosquitoes. When the RR value obtained > 10 indicates high resistance, values < 5 or equal are considered susceptible. Whereas RR between 5 and 10, mosquitoes are considered to have moderate resistance. In this study, the PCR analysis was conducted to confirm the susceptible/resistance genotyping of *Ae. aegypti*

Formula used:

$$\text{Abbott's formula: } \frac{\%\text{ test mortality - }\%\text{ control mortality}}{\%\text{ control mortality}} \times 100$$

$$\text{Resistance Ratio, RR} = \frac{\text{KT50 (field strain)}}{\text{KT50 (lab strain)}}$$

RESULT AND DISCUSSION

Population and Distribution

The results of the *Ae. aegypti* population distribution throughout the sampling collection years 2017 to early 2019 are presented in Table 2. The total numbers of *Ae. aegypti* collected were 3764 with male (1907) outnumbered the female (1857) population. The year 2019 recorded the highest number of *Ae. aegypti* collected were 2047. However, the total number of *Ae. aegypti* recorded in 2018 was the lowest among the three consecutive years, which was only 161. In general, throughout the sample collection duration male mosquitoes were produced higher numbers than female mosquitoes except in the year 2018. During the three years sampling collection, a fluctuating number of mosquitoes collected were observed.

A drastic drop-down in mosquito collection was recorded in 2018. The one possible reason could be the sweltering season in the year 2017 - 2018. According to Jabatan Metereologi Malaysia (2018), hot weather in 2017 and 2018 took place without the presence of El Nino unlike during the years 2015 and 2016 which has strongly contributed to the phenomenon. The world's average temperature estimation for 2018 was 0.66 ± 0.1°C above the average of 1961-1990. The current four years 2015 to 2018 were recorded as the warmest years. This condition is believed to affect the *Ae. aegypti* population in the area. A review is done by Reinhold *et al*. (2018) on the temperature of the environmental effects against *Aedes* sp has concluded that most abiotic factors play an important role in the life of insects, such as host-seeking behavior, breeding sites location, ecology, and dispersion. This condition has caused limited samples available for PCR analysis.

Table 2. Distribution of *Ae. aegypti* population during the sampling years 2017, 2018 and 2019.

Sampling years collection	Male	Female	Total
2017	779	777	1556
2018	74	87	161
2019	1054	993	2047
Total	**1907 (50.66%)**	**1857 (49.34%)**	**3764 (100%)**

Bioassay

The results of the WHO assay are presented in Table 3, which shows the KT50 and percentage of mortality rate values of *Ae. aegypti* adult laboratory and field strains exposed to tested insecticides. In the laboratory strain, lambda-cyhalothrin was recorded the lowest KT50 with the value of 5.02 min. This was followed by permethrin with the KT50 obtained 6.85 min. However, the KT50 of DDT was not calculated due to the zero knockdown observed in the 1hr exposure. The mortality obtained was 100% against all tested insecticides. On the other hand, results obtained for the KB population strain did not agree between KT50 values and mortality, while DDT has the highest mortality of 51.55 ± 2.63%, the KT50 was 5048.43 min.

Table 3. The bioassay of the F1 generation of field-caught *Ae. aegypti.*

Strains / Insecticides	KT50 (min) 24 hrs	95% Confidence Intervals	24h mortality Mean (%) ±SE	RR
Laboratory strain				
4% DDT	*	-	100.00±0.00	-
0.05% Lambda-cyhalothrin	5.02	4.76-5.29	100.00±0.00	-
0.75% Permethrin	6.85	6.55-7.17	100.00±0.00	-
KB strain				
4% DDT	5048.43	1103.673 – 500 000.0	51.55±2.63	-
0.05% Lambda-cyhalothrin	>500 000.0	> 500 000.0	14.58±9.45	> 10
0.75% Permethrin	793.77	196.664 - >500 000	18.41±1.74	> 10

Notes
1. * Cannot compute by probit due to no knockdown observed in 1 hr exposure
2. – Not available
3. S susceptible
4. RR <5 indicate susceptible
5. Susceptible criteria (WHO, 2016):
6. 98% - 100%: Susceptible to insecticide,
7. < 98%: Resistance suggested. Further tests are needed to verify
8.90% - 97% Presence of resistant genes in the vector population must be confirmed
9. < 90% - Confirmed of existence of resistant genes in the tested population

In contrast, permethrin gives the lowest KT50 of 793.77 min but produced low mortality in 24hr of 18.41 ± 1.74%. Nevertheless, KT50 of lambda-cyhalothrin was > 500 000 min with the lowest mortality of 14.58 ± 9.45%. Based on the susceptible criteria by WHO (2016), the field strain of *Ae. aegypti* was susceptible towards DDT > lambda-cyhalothrin > permethrin with mortality < 80% indicated resistance. Similarly, the calculated RR of the KB strain showed > 10 for lambda-cyhalothrin and permethrin. The inconsistency of the results obtained in KT50, mortality and resistance ratio are believed to be associated with the different reactions of the *Ae. aegypti* mosquito during the one-hour exposure. Some of them were observed to survive from the knockdown effects during the exposure but it caused mortality during the 24hr observation period. Because of this scenario, the RR value was not able to calculate. With the present data, evaluating the phenotype

insecticide resistance with the genotypic association of *kdr* mutations was limited because of the very small samples available. Thus, we suggested further investigation in the future for confirmation.

According to WHO (2016), as resistance takes a variety of forms the impact will have on the effectiveness of the vector control program management. Thus, a scheduled evaluation of the resistance/susceptibility at the targeting hotspot area should be enforced. Some of these insecticides are categorized into pyrethroids, organochlorines, and organophosphates that acted and share a similar specific mode of action mechanism, the VGSC. For instance, dichlorodiphenyltrichloroethane (DDT) is being reported to have closely resembled effects mode of action with pyrethrin and pyrethroid (Davies *et al.,* 2007). In the previous year, DDT was being used widely around the world including in Malaysia. It was later banned due to the mounting evidence on the adverse effects to wildlife animals, risks to human health**,** and the environment.

Today, it is classified as a probable human carcinogen by the US Environmental Protection Agency (EPA, 2021). However, in Malaysia, the usage of DDT has been banned since 1998 (Ramachandran, 2006). After nearly 23 years when it was banned we continue to experience its long-lasting effects. From the bioassay testing, we confirmed the DDT has its continuous resistant effect in the current *Ae. aegypti* population of Kota Bharu, Kelantan. These findings were similar to previous studies that reported the presence of the DDT-resistant phenotype in *Ae. aegypti* (Nazni *et al.,* 2009), *Culex quinquefasicatus* (Low *et al.,* 2013), and *Anopheles darlingi* (Fonseca *et al.,* 2009). Therefore, the observed resistance in *Ae. aegypti* population may have been due to the extensive usage of pyrethroids in public health activities and pest control. These conditions agreed with many researchers who have reported the similar DDT and pyrethroid mode of action on the peripheral nervous system of the insects in which VGSC were targeted (Davies *et al.,* 2007; van den Bercken, 1973). In this present study, no knockdown/mortality observed during the first-hour exposure against DDT has explained the slow effects of DDT compared to other insecticides in its mode of action (Davies *et al.,* 2007).

Mosquito Genotyping and Kdr Alleles

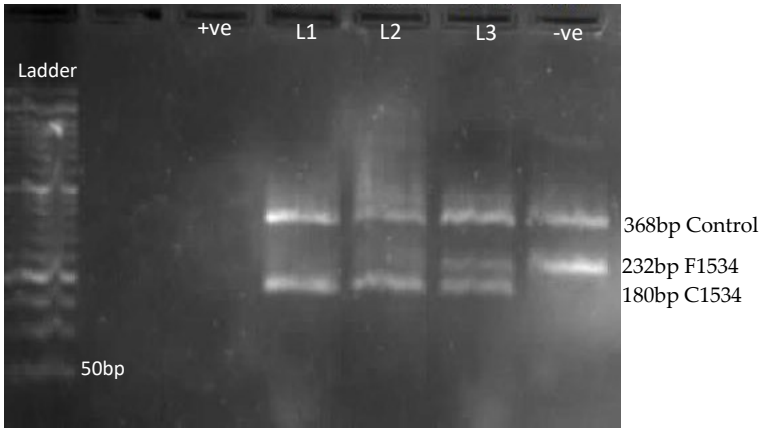

Figure 2. Gel photograph showing AS-PCR assay for genotyping of F1534C alleles *Ae. aegypti* in KB. DNA Ladder: 50 bp, Lanes 1-2: homozygote resistance (C/C), Lane 3: heterozygote resistance (F/C), Lane +ve: positive control, Lane -ve: negative control.

Ae. aegypti field strain from KB was successfully amplified on the gel electrophoresis for the genotyping alleles of F1534C and V1016G by the Allele-Specific PCR (AS-PCR) (Figure 2 and 3). AS-PCR of the F1534C showed distributions of F and C in the gene domain III. In the resistance phenotypes, the homozygotes frequencies of C/C were 66.7% and the heterozygotes frequencies of F/C were 33.3%. AS-PCR of the V1016G showed the distribution of the V and G in the gene domain

II. The results obtained (Figure 3 and Table 4) showed the homozygotes frequencies, V/V were 100% susceptible. While on the other hand, no other *kdr* mutants allele of domain II were detected in the PCR analysis. With the present data, evaluating the association of phenotype insecticide resistance with the genotypic of *kdr* mutations was very limited. Nevertheless, concerning the small sample analysis process, the population of *Ae. aegypti* in KB was highly resistant against the lambda-cyhalothrin, permethrin and DDT. However, further investigation would be suggested for confirmation.

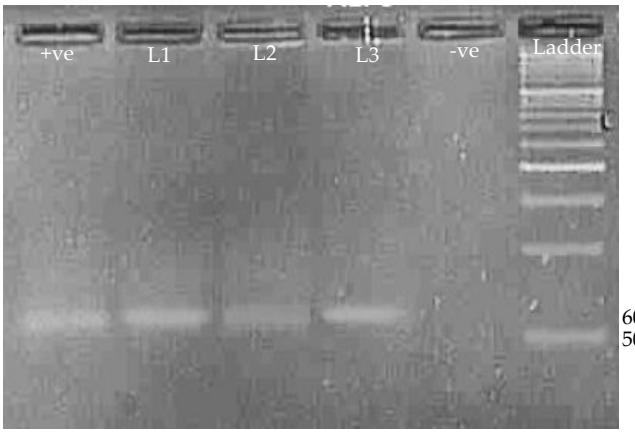

Figure 3. Gel photograph showing AS-PCR assay for genotyping of V1016G alleles *Ae. aegypti* in KB. DNA Ladder: 50 bp, Lanes 1-3: homozygote susceptible (V/V), Lane +ve: positive control, Lane -ve: negative control.

Table 4. Distribution frequency of *kdr* alleles in field strain *Ae. aegypti.*

Alleles	Primers	Allelic frequency	
F1534C	F/F1534	0/3	100%
	F/C1534	1/3	33.3%
	C/C1534	2/3	66.7%
V1016G	V/V1016	3/3	100%
	V/G1016	0/3	0
	G/G1016	0/3	0

AS-PCR was conducted to investigate the association of resistant genotyping *kdr* alleles F1534C/V1016G with the bioassay phenotyping. In general bioassay, findings were in agreement with the detection of *kdr* mutation alleles. In KB strain, the majority of the 2/3 *Ae. aegypti* populations were homozygote (C/C 1534) resistant against the pyrethroid type I. The rest of 1/3 populations were heterozygotes resistance (F/C 1534). These conditions were expected as the area was a dengue hotspot that received frequent insecticide treatments during the outbreak seasons. For V1016G allele which associates with the pyrethroid type II, lambda-cyhalothrin, gives 100% susceptibility (V/V 1016). According to Chen *et al.* (2019), mosquitoes carrying both F1534C and V1016G showed a greater level of pyrethroid resistance than those carrying F1534C alone, which was exhibited by the *Ae. aegypti* population in Kota Bharu. In relation to the present studies, small sample analysis and PCR reaction of the mosquito that was not conducted individually could result in inaccuracy in the detection of the V1016G allele in the *Ae. aegypti* population.

CONCLUSION

In conclusion, our randomized investigation to evaluate the susceptible/resistant status of field-collected *Ae. aegypti* against DDT-pyrethroid insecticides concerning the *kdr* resistant alleles F1534C

and V1016G managed to show the long-lasted resistance effect of DDT in an existing *Ae. aegypti* mosquito population. The *Ae. aegypti* population investigated showed resistance phenotypically against type I (permethrin) and type II (lambda-cyhalothrin) insecticide. It also showed resistance genotypically against the pyrethroid type I (permethrin). Further investigation is however required to confirm the present status of the V1016G allele in the population. Large sample size and individual PCR reactions against mosquitoes are recommended in the future for better and reliable findings.

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REFERENCES

[1] Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Entomology,* 18(2), 265-266.

[2] Amelia-Yap, Z.H., Chen, C.D., Sofian-Azirun, M. & Van, L.L. 2018. Pyrethroid resistance in the dengue vector *Aedes aegypti* in Southeast Asia: present situation and prospects for management. *Parasites Vectors,* 11(1), 332.

[3] Chen, M., Du, Y., Wu, S., Nomura, Y., Zhu, G., Zhorov, B.S. & Dong, K. 2019. Molecular evidence of sequential evolution of DDT-and pyrethroid-resistant sodium channel in *Aedes aegypti*. *PLOS Neglected Tropical Diseases*, 13(6), e0007432.

[4] Davies, T.G., Field, L.M., Usherwood, P.N. & Williamson, M.S. 2007. DDT, pyrethrins, pyrethroid and insect sodium channels. *IUBMB Life*, 59(3),151–162.

[5] EPA (Environmental Protection Agency). 2021. *DDT-A brief history and status*. (https://www.epa.gov/ingredients-used-pesticide-products/ddt-brief-history-and-status). Last accessed on July 2021.

[6] Fonseca - González, I., Quiñones, M.L., McAllister, J. & Brogdon, W.G. 2009. Mixed-function oxidases and esterases associated with cross-resistance between DDT and lambda-cyhalothrin in *Anopheles darling* Root 1926 populations from Colombia. *Memorias Do Instituto Oswaldo Cruz,* 104(1),18–26.

[7] Grossman, M.K., Uc-Puc, V., Rodriguez, J., Cutler, D. J., Morran, L. T., Manrique-Saide, P. & Vazquez-Prokopec, G.M. 2018. Restoration of pyrethroid susceptibility in a highly resistant *Aedes aegypti* population. *Biology Letters*, 14(6), 20180022.

[8] Hemingway, J., Hawkes, N.J., McCarroll, L. & Ranson, H. 2004. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology,* 34(7), 653–665.

[9] Jabatan Metereologi Malaysia. 2018. *Laporan tahunan.* Kementerian Tenaga Sains, Teknologi, Alam Sekitar dan Perubahan Iklim. Jabatan Metereologi Malaysia.

[10] KKM (Kementerian Kesihatan Malaysia). 2014. *Kejadian denggi di Kelantan 2014.* (https://jkt.kpkt.gov.my/jkt/resources/PDF/Persidangan_2015/persidangan%20kesihatan/DENGGI_DI_KELANTAN_2014.pdf). Last accessed 10 August 2021

[11] KKM (Kementerian Kesihatan Malaysia) 2017. *Prosedur semburan residu dan semburan perimeter untuk kawalan denggi.* (https://www.moh.gov.my/moh/resources/Penerbitan/Garis%20Panduan/Garis%20Panduan%Umum(KKM)/Prosedur_Semburan_Residu_dan_Semburan_Perimeter_Untuk_Kawalan_Denggi.pdf). Last accessed on 10 August 2021.

[12] Lee, H.L. 1992. *Aedes* ovitrap and larval survey in several suburban communities in Selangor, Malaysia. *Mosquito Borne Disease Bulletin*, 9(1), 29-33

[13] Leong, C., Vythilingam, I., Liew, J.W., Wong, M.L., Wan Yusoff, W.S. & Lau, Y.L. 2019. Enzymatic and molecular *characterization* of insecticide resistance mechanisms in field populations of *Aedes aegypti* from Selangor, Malaysia. *Parasites Vectors,* 12, 236.

[14] Low, V.L., Chen, C.D., Lee, H.L., Lim, P.E., Leong, C.S. & Sofian-Azirun, M. 2013. Current susceptibility status of Malaysian *Culex quinquefasciatus* (Diptera: Culicidae) against DDT, propoxur, malathion and permethrin. *Journal of Medical Entomology*, 50(1), 103–111

[15] Nazni, W., Selvi, S., Lee, H., Sadiyah, I., Azahari, H., Derric, N. & Vasan, S.S. 2009. Susceptibility status of transgenic *Aedes aegypti* (L.) against insecticides. WHO Regional Office for South-East Asia. *Dengue Bulletin,* 33, 124–129.

[16] Ramachandran, R. & Mourin, J. 2006. Overview of the POPs Pesticide Situation in Malaysia *International POPs,* 1-15

[17] Raymond, R. 1985. Log-Probit analysis basic programme of microcomputer. *Cahiers ORSTOM Serie Entomologie et Parasitologie,* 23, *117–121.*

[18] Reinhold, J. M., Lazzari, C.R. & Lahondère, C. 2018. Effects of the Environmental Temperature on *Aedes aegypti* and *Aedes albopictus* Mosquitoes: A Review. *Insects*, 9(4), 158.

[19] Rohani, A., Aidil Azahary, A.R., Yu, K.X., Zurainee, M.N., Wan Najdah, W.M. & Lee, H.L. 2019. Insecticide susceptibility status and resistance mechanism of *Anopheles cracens* Sallum and Peyton and *Anopheles maculatus* Theobald (Family: Culicidae) from knowlesi malaria-endemic areas in Peninsular Malaysia. *Asian Pacific Journal of Tropical Medicine,* 12(2), 79-86.

[20] Rozendaal, J.A. & World Health Organization. 1997. *Vector control: methods for use by individuals and communities.* World Health Organization.

[21] Saingamsook, J., Saeung, A., Yanola, J., Lumjuan, N., Walton, C. & Somboon, P. 2017. A multiplex PCR for detection of knockdown resistance mutations, V1016G and F1534C, pyrethroid-resistant Aedes aegypti. *Parasites & Vectors*, 10(1), 465.

[22] Sayono, S., Hidayati, A.P., Fahri, S., Sumanto, D., Dharmana, E., Hadisaputro, S., Asih, P.B.S. & Syafruddin, D. 2016. Distribution of voltage-gated sodium channel (Nav) alleles among the *Aedes aegypti* populations in central Java Province and its association with resistance to pyrethroid insecticides. *PLoS One,* 11, e0150577.

[23] Skae, F. M. T. 1902. Dengue Fever in Penang. *British Medical Journal*, 2(2185), 1581–1582.

[24] Stenhouse, S.A., Plernsub, S., Yanola, J., Lumjuan, N., Dantrakool, A., Choochote, W. & Somboon, P. 2013. Detection of the V1016G mutation in the voltage-gated sodium channel gene of *Aedes aegypti* (Diptera: Culicidae) by allele-specific PCR assay, and its distribution and effect on deltamethrin resistance in Thailand. *Parasit Vectors,* 6(1), 253.

[25] van den Bercken, J., Akkermans, L.M.A. & van der Zalm, J.M. 1973. DDT-like action of allethrin the sensory nervous system of *Xenopus laevis. European Journal of Pharmacology,* 21(1), 95-106.

[26] Wuliandari, J., Lee, S., White, V., Tantowijoyo, W., Hoffmann, A. & Endersby-Harshman, N. 2015. Association between three mutations, F1565C, V1023G and S996P, in the voltage-sensitive sodium channel gene and knockdown resistance in *Aedes aegypti* from Yogyakarta, Indonesia. *Insects*, 6(3), 658–685.

[27] WHO (World Health Organization). 2016. *Monitoring and managing insecticide resistance in Aedes mosquito populations: interim guidance for entomologists.* (https://apps.who.int/iris/handle/10665/204588). Last accessed on 20 July 2021.

[28] Yanola, J., Somboon, P., Walton, C., Nachaiwieng, W. & Prapanthadara, L. 2010. A novel F1552/C1552 point mutation in the *Aedes aegypti* voltage-gated sodium channel gene associated with permethrin resistance. *Pesticide Biochemistry and Physiology*, 96(3), 127–131.