EFFECT OF NUTRIENT AND DENSITY AGAINST THE SUSCEPTIBILITY STATUS OF *Aedes aegypti* AND *Aedes albopictus* TOWARDS DIAGNOSTIC DOSE OF MALATHION AND PERMETHRIN

Salinah Abdul Farouk, Zairi Jaal & Siti Nasuha Hamzah*
School of Biological Sciences, Universiti Sains Malaysia,
11800 Minden, Penang, Malaysia.
*Corresponding author: *sitinasuha@usm.my*

ABSTRACT

The knockdown 50% values (KT_{50}) of *Aedes aegypti* and *Aedes albopictus* with variable nutrient and density conditions were determined based on World Health Organisation (WHO) Adult Bioassay standard protocol towards diagnostic dose of malathion (5%) and permethrin (0.75%) and the effect of nutrient and density on *Aedes* susceptibility status were investigated. Our results revealed that the susceptibility of these *Aedes* mosquitoes against malathion at $F(4, 116) = 42.103$, $p<0.05$ and permethrin at $F(4, 121) = 45.138$, $p<0.05$, gives the most delayed knockdown when fed with the optimum amount of nutrient which is 70mg compared with the lower amount of nutrient and the survival decreased once the nutrient amount was increased more than this amount. For density effect against *Aedes* susceptible status, at the highest density (600 larvae) examined, there was proportionally more larval mortality where the KT_{50} value was at the lowest value when compared with the optimal density which was between 150 to 250 larvae against malathion at $F(6, 159) = 62.203$, $p<0.05$ and permethrin at $F(6, 148) = 57.431$, $p<0.05$. However, the effect of nutrient and density factor of the different *Aedes* species significantly impacted their susceptibility to malathion and permethrin. The time required to knock down 50% (KT_{50}) of *Ae. albopictus* mosquitoes against the diagnostic dose of malathion as well as permethrin were relatively delayed compared to the values obtained by *Ae. aegypti* mosquitoes even though the environmental conditions were the same ($p<0.05$).

Keywords: *Aedes aegypti*, *Aedes albopictus*, diagnostic dose, environmental parameters, population parameters, malathion, permethrin

ABSTRAK

Nilai kepengsanan (KT_{50}) *Aedes aegypti* dan *Aedes albopictus* yang didedahkan kepada faktor nutrien dan kepadatan yang berbeza ditentukan berpandukan protokol World Health Organisation (WHO) Bioassay nyamuk dewasa terhadap dos diagnostik insektisid permethrin (0.75%) dan malathion (5%) dan kesan faktor tersebut terhadap kerentanan *Aedes aegypti* dan *Aedes albopictus* disiasat. Berdasarkan hasil yang diperolehi, didapati bahawa masa kepengsanan kedua spesies *Aedes* terhadap dos diagnostik insektisid malathion pada $F(4, 116) = 42.103$, $p<0.05$ dan permethrin pada $F(4, 121) = 45.138$, $p< 0.05$, memberikan nilai kepengsanan yang paling tinggi apabila 70mg nutrien diberi semasa tempoh tumbesarannya. Selain daripada itu, jumlah kematiannya juga meningkat apabila jumlah nutrien melebihi

jumlah optimum iaitu 70mg. Daripada segi kepadatan pula, masa kepengsanan *Ae. aegypti* dan *Ae. albopictus* didapati paling lambat untuk kepadatan antara 150 hingga 250 ekor larva per bekas malathion iaitu F (6, 159)= 62.203, $p<0.05$ dan permethrin iaitu F (6, 148) = 57.431, $p<0.05$ yang menunjukkan bahawa kepadatan ini merupakan kepadatan optimum. Kadar kematian larva paling tinggi manakala kerentanan kedua spesies *Aedes* juga paling rendah untuk kepadatan 600 larva per bekas. Kadar nutrien dan kepadatan memberikan impak yang signifikan terhadap tahap kerentanan *Ae. aegypti* dan *Ae. albopictus* apabila terdedah kepada dos diagnostik insektisid permethrin dan malathion. Berdasarkan masa kepengsanan *Ae. aegypti* dan *Ae. albopictus* terhadap dos diagnostik insektisid permethrin dan malathion, didapati bahawa *Ae. albopictus* mengambil masa yang lebih lama (KT_{50}) berbanding *Ae. aegypti* untuk pengsan dari segi faktor pemakanan dan juga faktor kepadatan ($p<0.05$) walaupun keadaan persekitaran kedua eksperimen adalah sama.

Kata kunci: *Aedes aegypti, Aedes albopictus,* dos diagnostik, kesan persekitaran, malathion, permethrin

INTRODUCTION

Dengue fever (DF) is the most common mosquito-borne viral disease which has spread rapidly in the past six decades with approximately 2.5 billion people of the world's population at risk of infection (Ferreira et al. 2012; Packierisamy et al. 2015). Dengue is transmitted by the bite of an *Aedes* mosquito infected with one of the four dengue viruses (Gubler 1998). *Aedes aegypti* has been identified as the major vector of dengue fever and dengue haemorrhagic fever while *Ae. albopictus* serves as the secondary vector in several South East Asian countries including Malaysia (Chua et al. 2005; Lumjuan et al. 2011; Farjana et al. 2012; Hamzah & Alias 2016a). *Aedes aegypti* is usually abundant in urbanized areas with highly crowded human population whereas *Ae. albopictus* is typically known as rural mosquito and breeds outdoors especially in natural habitats (Mackenzie et al. 2004; Mohiddin et al. 2015).

Dengue has also been reported to be endemic in Malaysia, a tropical country of 27.5 million people located in Southeast Asia (Packierisamy et al. 2015; Hamzah & Alias 2016b). The best method to control dengue transmission due to unavailability of a stable vaccine is by a combination of long as well as short term measures to target adult as well as immature *Aedes* mosquitoes which include habitat reduction, Ultra Low Volume (ULV) space sprays as well as periodic fogging activities using insecticides and environmental management (Guzman et al. 2010; Packierisamy et al. 2015; Mohiddin et al. 2015). In Malaysia, the predominant choice of insecticides applied in the effort to control adult mosquitoes in the affected areas are permethrin (pyrethroids) and malathion (organophosphate) which have been proven to be significantly effective (Wan-Norafikah et al. 2013; Rosilawati et al. 2017; Hamzah et al. 2019). However, it should be noted that prolonged as well as extensive usage of insecticides have posed a great threat to the effectiveness of vector control programmes due to the emergence of insecticide resistance (Hemingway & Ranson 2000). Many studies reported that pyrethroid and organophosphate resistance were already widespread in *Aedes* mosquito especially in most of the Association of Southeast Asian Nations (Asean) countries including Malaysia (Koou et al. 2014; Rosilawati et al. 2017; Elia-Amira, et al. 2018).

The biotic and abiotic parameters that influence the development and transmission of mosquitoes such as nutrient, salinity, rainfall, temperature and humidity play a major role in the population dynamics of *Aedes* mosquitoes (Li et al. 1985; Smith et al. 2004; Legros et al. 2009; Couret et al. 2014; Rozilawati et al. 2017). Moreover, environmental conditions

encountered by larvae are capable of inflicting variable effects on the mosquito-arbovirus (Alto et al. 2005). Either on its own or in combination, environmental factors as well as population parameters such as nutrient availability and density of larvae affect the expression of adult characteristics as well as autogeny (Nayar 1969; Legros et al. 2009). The size of adult *Aedes* mosquitoes is reported to be highly influenced by the quantity of nutrient as well as population density during its larval development stage indicating that environmentally induced changes predominantly affect the size of the *Aedes* mosquitoes (Jirakanjanakit et al. 2007). Reduced nutrient availability results in increased larval mortality, delayed pupation, the emergence of smaller-sized adults starving to death in a shorter period of time and lower fecundity (Nayar 1969; Agnew et al. 2002; Zeller & Koella 2016). A study reported that high larval competition among *Ae. aegypti* results in prolonged development time and decreased wing length which negatively affected the longevity of its adults (Reiskind & Lounibos 2009). On the other hand, the large-sized *Ae. aegypti* adults emerging from high food availability or low-density environment exhibited a relatively larger as well as higher blood feeding success rates which not only affected their fecundity and longevity, but also consumed significantly more virus particles which elevated its vector competence status compared to smaller-sized adults (Nasci 1986; Nasci 1991; Nasci & Mitchell 1994).

It should be noted that an incomplete reduction in larval density due to vector control efforts results in higher food availability for the surviving immatures, leading to significant increase in the size of survivors and ultimately turns them into potentially superior vectors (Mori 1979; Arrivillaga & Barrera 2004; Jirakanjanakit et al. 2007; Yakob et al. 2008). Therefore, the most preferable control activities should aim to achieve a complete elimination of the vectors from the larval stages (Jirakanjanakit et al. 2007). By taking the influence of these parameters into consideration, the focus of this research is on the effect of nutrient as well as density against the susceptibility of *Ae. aegypti* and *Ae. albopictus* towards the adult mosquito's diagnostic dose of permethrin and malathion.

MATERIALS AND METHODS

Insecticides

Due to unavailability of established diagnostic doses for *Ae. albopictus* yet as well as lower rate of mortality using the WHO diagnostic dosages which has been set for *Ae. aegypti*, we decided to use the diagnostic doses for *Anopheles* spp. as a standard for this study (WHO 2016). Whatman standard filter paper was impregnated with diagnostic dose of malathion (5%) and permethrin (0.75%). To serve as the control treatments for permethrin as well as malathion, papers were impregnated with acetone mixed with silicone oil used for permethrin and olive oil for malathion respectively. All impregnated papers were prepared according to WHO protocol and were provided by Vector Control Research Unit (VCRU), Universiti Sains Malaysia.

Mosquitoes

In this study, a laboratory strain of *Ae. albopictus* eggs and the Bora Bora strain of *Ae. aegypti* eggs were obtained from the Vector Control Research Unit (VCRU) in USM. Both strains have been maintained in the insectarium for many years which is more than 190 generations without exposure to any insecticide as well as biological control agents.

To Determine the Effect of Different Amount of Nutrients

Two hundred (200) newly hatched larvae were transferred by means of a pipette into each enamel tray filled with 2L of chlorine-free water (Jirakanjanakit et al. 2007). Mosquito larval food was prepared by mixing fine-ground dog biscuits, powdered milk, ground dried beef liver and yeast at the ratio of 2:1:1:1. Different amounts of larval food were added everyday into each tray; 20mg, 30mg, 50mg, 70mg and 100mg. The rearing medium was replaced with clean chlorine-free water every two to three days after the 2nd instar stage. Upon pupation, populations from each different tray were housed in separate cages with dimensions 30cm x 30cm x 30cm. Adults were provided with 10% sucrose solution using soaked cotton wicks. Prior to insecticide contact, 20 females non-blood fed *Aedes* mosquitoes aged between three to five days old were aspirated into holding tubes lined with clean paper and left for a period of 1 hour to precondition them. Female mosquitoes showing abnormalities were replaced with healthy ones. Later, the mosquitoes were gently transferred into 7 internally treated tubes (5 replicates with insecticides and 2 controls) lined with insecticide impregnated filter paper for 1 hours. For the control group, the females were exposed for 1 hour to papers impregnated with acetone mixed with silicone oil for permethrin and olive oil for malathion. The knockdown values of the test mosquitoes were recorded every minute up to 60 minutes. The test mosquitoes and the controls were held for a 24 hours recovery period in the holding tube and the mortality was recorded. The mosquitoes were fed on 10% sucrose solution by placing soaked cotton wicks on the top of the holding tubes. Treatment mortality was corrected if the mortality in the control group ranged between 5% and 20% by using Abbott's formula (Abbott 1925). All tests were conducted in triplicates, adhering to the same procedure. Data collected were analysed statistically by using probit analysis computer programme SPSS to determine the 50% knockdown values (KT_{50}).

To Determine the Effect of Density

Different amounts of larvae were placed in separate enamel trays filled with 2L of chlorine-free water starting from 150 larvae, 200 larvae, 250 larvae, 300 larvae, 350 larvae, 400 larvae and 600 larvae per tray. As described in section 2.3, 70mg larvae food was added to each tray every day and the culturing medium was replaced with clean chlorine-free water every two to three days after the 2nd instar stage. Populations from each different tray were housed in separate cages with the dimensions of 30cm x 30cm x 30cm upon pupation. Adults were provided with 10% sucrose solution using soaked cotton wicks. The bioassay procedure, according to WHO standard protocol (WHO, 2016), was repeated based on the same procedure stated above.

Statistical Analysis

Three replicates were performed for all samples and presented as mean ± standard deviation (S.D.). Significant ($p<0.05$) difference was tested accordingly with probit analysis, ANOVA between-groups as well as independent t-test through SPSS computer software version 24.

RESULTS AND DISCUSSION

The cumulative knockdown count was recorded for every one minute within the exposure period, or until 50% survival rate. From the analysis, the data shows that the optimum amount of nutrient required for developmental of *Ae. aegypti* and *Ae. albopictus.* The KT_{50} of these *Aedes* mosquitoes against the malathion and permethin gives the most delayed knockdown when fed with the higher amount of nutrient compared with the lower amount of nutrient (Fig. 1 and Fig. 2). The adults of these *Aedes* mosquitoes emerging from larvae which have been fed with 70mg of larvae food throughout its development stage showed the maximum number of

survival during its development stage with KT_{50} value 47.9±0.6 for *Ae. albopictus* and 32.3±0.6 for *Ae. aegypti*. From ANOVA, there were significant differences between the KT_{50} of the adult *Ae. albopictus* and *Ae. aegypti* which have been fed with 70mg nutrient and the adult *Aedes* mosquitoes which have been fed with 20mg, 30mg and 50mg larvae food throughout its immature developmental stages against malathion at $F(4, 116) = 42.103$, $p<0.05$ (Fig. 1). Similar results were obtained for *Ae. albopictus* and *Ae. aegypti* at $F(4, 121) = 45.138$, $p<0.05$, against permethrin with the same nutrient variable (Fig. 2). A consistent increase in its larval survival rate, faster pupation and subsequently its respective adult knockdown value were observed once the nutrient amount was increased because low nutrient larvae food is insufficient for the development of larvae due to deprivation of nutrient.

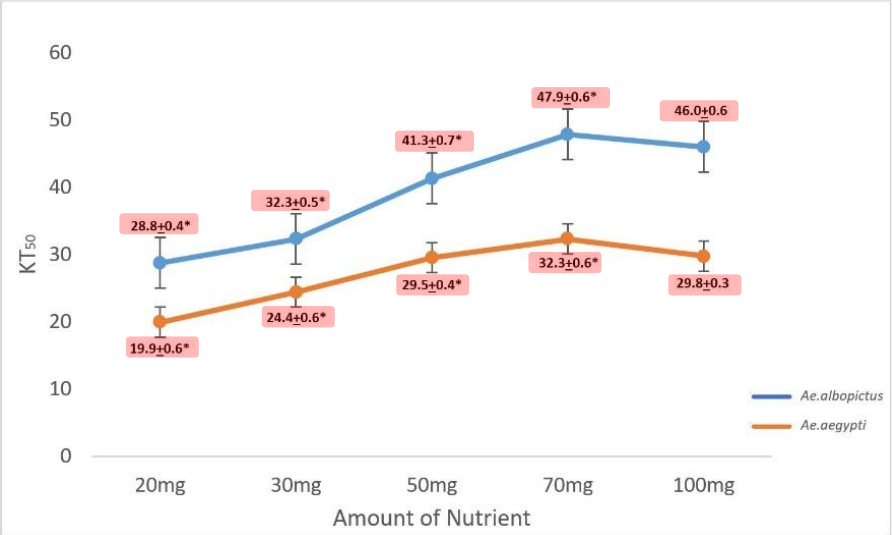

Figure 1. Knockdown time 50% (KT_{50}) of *Ae. aegypti* and *Ae. albopictus* mosquitoes fed with different amounts of food to 5% malathion, n=200. The (*) indicate significant difference ($p<0.05$) for each KT_{50} value between the different amounts of nutrient (mg) and red labelled data indicate significant difference ($p<0.05$) for each KT_{50} value between two different mosquito species.

Similarly, the survivorship of *Ae. albopictus* as well as *Ae. triseriatus* immatures was food dependent whereby their survival is lowest in the circumstances of low food availability (Teng & Apperson 2000). Adding the amount of food shortens the larval development rate, improved larval survival and results in a significant increase in the number of pupae produced (Lord 1998; Legros et al. 2009; Yoshioka et al. 2012). In this study, most of the adult *Aedes* mosquitoes which were fed with 100mg of larval food during its development period shows stagnant or a drop of survival level at the KT_{50} for both species compared to the adults fed with 70mg larvae ($p>0.05$). By increasing the nutrient amount excessively, it might reduce the survival rate and increase the mortality of the mosquitoes. According to Arrivillaga & Barrera (2004) and Gilles et al. (2011), increased diet results in increased survival while excessive diet diminished it.

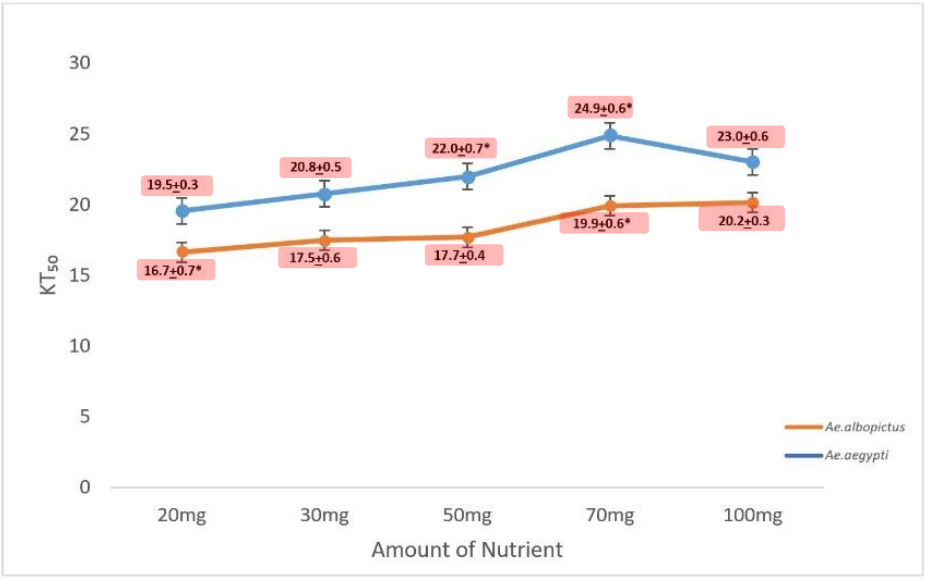

Figure 2. Knockdown time 50% (KT_{50}) of *Ae. aegypti* and *Ae. albopictus* mosquitoes fed with different amounts of food to 0.75% permethrin, n=200. The (*) indicate significant difference ($p<0.05$) for each KT_{50} value between the different amounts of nutrient (mg) and red labelled data indicate significant difference ($p<0.05$) for each KT_{50} value between two different mosquito species.

For density effect against *Aedes* susceptible status, ANOVA demonstrated that crowding during the larval stages directly modulate adult susceptibility when exposed to malathion at $F(6,159) = 62.203$, $p < 0.05$ and permethrin at $F(6,148) = 57.431$, $p<0.05$ (Fig. 3 and Fig. 4). *Aedes* reared in 150-200 larvae densities gives the highest KT_{50} against diagnostic dose of malathion (with KT_{50} value 47.0±0.9 for *Ae. albopictus* and 38.7±0.3 for *Ae. aegypti)*, followed by 150-250 larvae per tray for mosquito against permethrin with KT_{50} value 26.9±0.6 for *Ae. albopictus* and 20.0±0.4 for *Ae. aegypti*) compared to others which implies the optimum density condition for the development of *Ae. aegypti* as well as *Ae. albopictus* mosquitoes. On the contrary, adult *Aedes* mosquitoes emerging from 600 larvae per tray showed the fastest KT_{50} value (malathion: 29.4±0.5 for *Ae. albopictus* and 29.7±0.3 for *Ae. aegypti*; permethrin: 21.5±0.5 for *Ae. albopictus* and 13.4±0.4 for *Ae. aegypti*) due to the overcrowded condition which ultimately affected its susceptibility status. Apart from that, larval competition due to overcrowding also results in prolonged life cycle of the larvae, retarded growth, increased larval mortality, reduced size of adults as well as decreased fecundity (Renshaw et al. 1993; Lord. 1998; Reiskind & Lounibos 2009; Yoshioka et al. 2012). In this current study, the degree of intraspecific competition had a significant effect on mosquito susceptibility and survival rate under controlled nutrient amount. It was noted that nutrition might be the most important factor and that the effect of density could be compensated by the availability of food. Corresponding results were observed whereby the survival of *Ae. albopictus* as well as *Ae. triseriatus* immatures were the lowest in the state of high density in controlled nutrient conditions (Teng & Apperson 2000). According Jirakanjanakit et al. (2007), as the density of larvae per tray increases, pupation is delayed, and the size of adults is reduced.

Predominantly, the size of adult mosquito is a factor of concern because it is proportional to its vector competence status and inversely proportional to its reaction to repellents as well as insecticides (Landry et al. 1988; Xue et al. 1995; Sumanochitrapon et al. 1991). Previous studies detected a relationship between mosquito size and its susceptibility

towards insecticides which bigger mosquito tend to be less susceptible and showed higher rates of oral infection with dengue virus (DENV) (Nasci 1986; Landry et al. 1988; Alto et al. 2008). According to Yeap et al. (2013) in his previous work, few field studies on the effects of *Ae. aegypti* size on field fitness have been performed where high nutrition produces large mosquitoes and are potentially fitter in terms fecundity, sperm quantity, survival and susceptibility. In contrast, food limitation due to overcrowded conditions produces smaller-sized adults (Jirakanjanakit et al. 2007). In relation to mosquito's susceptibility, the current data are consistent with previous findings in which *Anopheles* mosquitoes were exposed to DDT after varying larval nutrition and densities (Kulma et al. 2013; Oliver & Brooke 2013). From the data presented here, larvae that were bred under high density with sufficient nutrition had lower susceptibility than those at low density and low nutrition.

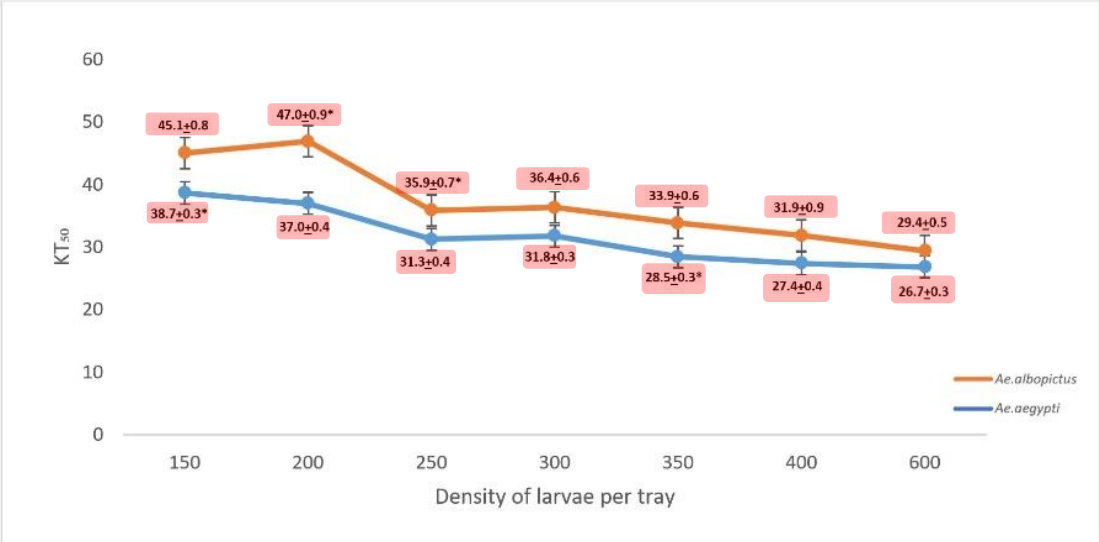

Figure 3. Knockdown time 50% (KT_{50}) of *Ae. aegypti* and *Ae. albopictus* mosquitoes in different density to 5% malathion, food=70mg. The (*) indicate significant difference ($p<0.05$) for each KT_{50} value between the different amounts of nutrient (mg) and red labelled data indicate significant difference ($p<0.05$) for each KT_{50} value between two different mosquito species.

Comparatively, from the T-test analysis the effect of nutrient and density factors against *Aedes* mosquito susceptibility was significantly impacted by the different species ($p<0.05$). It takes longer for *Ae. albopictus* to be knocked down using diagnostic dose of malathion and permethrin compared to *Ae. aegypti* even though they were at the same nutrient and density conditions at $t(8) = 4.73$, $p< 0.05$ and $t(12) =5.92$, $p<0.05$ respectively. In this current study, *Ae. aegypti* was more susceptible to the diagnostic dose of malathion and permethrin with the range of KT_{50} from 28.8 ± 0.4 to 47.9 ± 0.6 compared to *Ae. albopictus.* The effect of nutrient on mosquito susceptibility or survival rate in different mosquito species has been the subject of multiple studies. Pridgeon et al. (2008) revealed in their study that different species of mosquitoes had different susceptibility to pesticides. In another study, reduced larval food resulted in decreased longevity of *Ae. triseriatus* (Say) exposed to harsh conditions compared with *Ae. aegypti* (L.) (Reiskind & Lounibos 2009). Therefore, susceptibility or survival of *Aedes* mosquitoes are dependent on environmental factors, population parameters as well as intraspecies interaction.

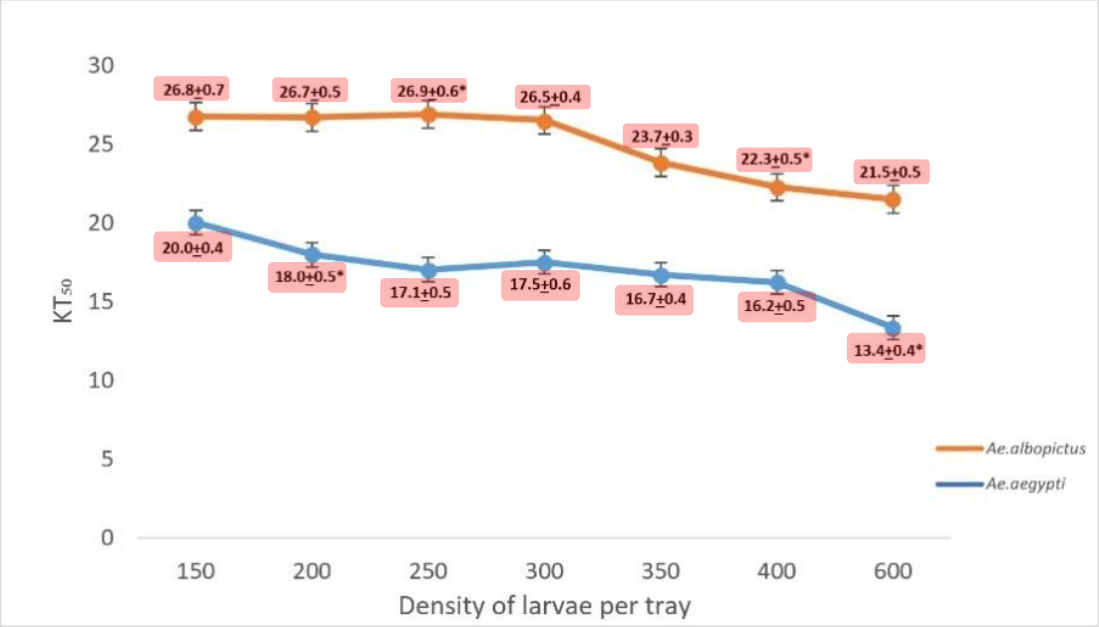

Figure 4. Knockdown time 50% (KT_{50}) of *Ae. aegypti* and *Ae. albopictus* mosquitoes in different density to 0.75% permethrin, food=70mg. The (*) indicate significant different ($p<0.05$) for each KT_{50} value between the different amounts of nutrient (mg) and red labelled data indicate significant difference ($p<0.05$) for each KT_{50} value between two different mosquito species.

CONCLUSION

This study provided information on optimal environmental and population parameters which are nutrient and larval densities against the susceptibility status of *Ae. aegypti* and *Ae. albopictus* using diagnostic dose of malathion and permethrin. The optimum amount of larvae food (70mg) gave the most delayed knockdown time compared to others for both species ($p<0.05$). The effects of density were more pronounced when the mosquitoes were reared in 150-250 larval densities implying the optimum density condition for their survival. *Ae. aegypti* and *Ae. albopictus* reared in 150-200 larvae densities gave the highest KT_{50} against diagnostic dose of malathion, followed by 150-250 larvae per tray against the diagnostic dose of permethrin when compared to others ($p<0.05$). However, our results revealed that different species of *Aedes* mosquitoes had significantly different susceptibility against both insecticides even under the same nutrient and density conditions ($p<0.05$). These findings show the need to select the most optimum conditions for mosquito species in order to achieve successful mosquito control strategies.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18(2): 265–267.

Agnew, P., Hide, M., Sidobre, C. & Michalakis, Y. 2002. A minimalist approach to the effects of density-dependent competition on insect life-history traits. *Ecological Entomology* 27(4): 396-402.

Alto, B.W., Lounibos, L.P., Higgs, S. & Juliano, S.A. 2005. Larval competition differentially affects arbovirus infection in *Aedes* mosquitoes. *Ecology* 86(12): 3279-3288.

Alto, B.W., Lounibos, L.P., Mores, C.N. & Reiskind, M.H. 2008. Larval competition alters susceptibility of adult *Aedes* mosquitoes to dengue infection. *Proceedings of the Royal Society of London B: Biological Sciences* 275(1633): 463-471.

Arrivillaga, J. & Barrera, R. 2004. Food as a limiting factor for *Aedes aegypti* in water-storage containers. *Journal of Vector Ecology* 29: 11-20.

Chua, K.B., Chua, I.L., Chua, I.E. & Chua, K.H. 2005. Effect of chemical fogging on immature *Aedes* mosquitoes in natural field conditions. *Singapore Medical Journal 46*(11): 639-644.

Couret, J., Dotson, E. & Benedict, M. Q. 2014. Temperature, larval diet, and density effects on development rate and survival of *Aedes aegypti* (Diptera: Culicidae). *PLoS One 9*(2): e87468.

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

Farjana, T., Tuno, N. & Higa, Y. 2012. Effects of temperature and diet on development and interspecies competition in *Aedes aegypti* and *Aedes albopictus. Medical and Veterinary Entomology 26*(2): 210-217.

Ferreira, G.L. 2012. Global dengue epidemiology trends. *Revista do Instituto de Medicina Tropical de São Paulo 54*: 5-6.

Gilles, J.R.L., Lees, R.S., Soliban, S.M. & Benedict, M.Q. 2011. Density-dependent effects in experimental larval populations of *Anopheles arabiensis* (Diptera: Culicidae) can be negative, neutral, or over compensatory depending on density and diet levels. *Journal of Medical Entomology 48*(2): 296-304.

Gubler, D.J. 1998. Epidemic dengue and dengue hemorrhagic fever: a global public health problem in the 21st century. In W.M. Scheld, D. Armstrong, & J.M. Hughes, *Emerging infections 1.* ASM Press. Washington D.C pp. 1-14.

Guzman, M.G., Halstead, S.B., Artsob, H., Buchy, P., Farrar, J., Gubler, D.J., Hunsperger, E., Kroeger, A., Margolis, H.S., Martínez, E. & Nathan, M.B. 2010. Dengue: A continuing global threat. *Nature Reviews Microbiology* 8: S7-S16.

Hamzah, S.N. & Alias, Z. 2016a. Developmental expression and oxidative stress induction of proteome of glutathione S-transferases in *Aedes albopictus* (Diptera: Culicidae). *Journal of Asia-Pacific Entomology* 19(3): 869-875.

Hamzah, S.N. & Alias, Z. 2016b. Purification, expression and partial characterisation of glutathione s-transferases (GSTs) from three different strains of *Aedes albopictus* (Diptera: Culicidae). *Tropical Biomedicine* 33: 335-347.

Hamzah, S.N., Farouk, S.A. & Alias, Z. 2019. Isoenzymes of *Aedes albopictus* (Diptera: Culicidae) Glutathione S-transferases: Isolation and expression after acute insecticide treatment. *Pesticide Biochemistry and Physiology* 153: 116–121.

Hemingway, J. & Ranson, H. 2000. Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology 45*(1): 371-391.

Jirakanjanakit, N., Leemingsawat, S., Thongrungkiat, S., Apiwathnasorn, C., Singhaniyom, S., Bellec, C. & Dujardin, J.P. 2007. Influence of larval density or food variation on the geometry of the wing of *Aedes* (Stegomyia) *aegypti. Tropical Medicine & International Health* 12(11): 1354-1360.

Koou, S.Y., Chong, C.S., Vythilingam, I., Ng, L.C. & Lee, C.Y. 2014. Pyrethroid resistance in *Aedes aegypti* larvae (Diptera: Culicidae) from Singapore. *Journal of Medical Entomology* 51(1): 1-12.

Kulma, K., Saddler, A. & Koella, J.C. 2013. Effects of age and larval nutrition on phenotypic expression of insecticide-resistance in *Anopheles* mosquitoes. *PLoS ONE* 8(3): e58322.

Landry, S.V., DeFoliart, G.R. & Hogg, D.B. 1988. Adult body size and survivorship in a field population of *Aedes triseriatus*. *Journal of the American Mosquito Control Association* 4(2): 121-128.

Legros, M., Lloyd, A.L., Huang, Y. & Gould, F. 2009. Density-dependent intraspecific competition in the larval stage of *Aedes aegypti* (Diptera: Culicidae): Revisiting the current paradigm. *Journal of Medical Entomology* 46(3): 409-419.

Li, C.F., Lim, T.W., Han, L.L. & Fang, R.A. 1985. Rainfall, abundance of *Aedes aegypti* and dengue infection in Selangor, Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health* 16(4): 560-568.

Lord, C.C. 1998. Density dependence in larval *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology* 35(5): 825-829.

Lumjuan, N., Rajatileka, S., Changsom, D., Wicheer, J., Leelapat, P., Prapanthadara, L.A., Somboon, P., Lycett, G. & Ranson, H. 2011. The role of the *Aedes aegypti* Epsilon glutathione transferases in conferring resistance to DDT and pyrethroid insecticides. *Insect Biochemistry and Molecular Biology* 41: 203-209.

Mackenzie, J.S., Gubler, D.J. & Petersen, L.R. 2004. Emerging flaviviruses: The spread and resurgence of *Japanese encephalitis*, West Nile and dengue viruses. *Nature Medicine* 10(12s): S98.

Mohiddin, A., Jaal, Z., Lasim, A. M., Dieng, H. & Zuharah, W.F. 2015. Assessing dengue outbreak areas using vector surveillance in north east district, Penang Island, Malaysia. *Asian Pacific Journal of Tropical Disease* 5(11): 869-876.

Mori, A. 1979. Effects of larval density and nutrition on some attributes of immature and adult *Aedes albopictus*. *Tropical Medicine* 21(2): 85-103.

Nasci, R.S. 1986. The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field. *Journal of American Mosquito Control Association* 2(1): 61-62.

Nasci, R.S. 1991. Influence of larval and adult nutrition on biting persistence in *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* 28(4): 522-526.

Nasci, R.S. & Mitchell, C.J. 1994. Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. *Journal of Medical Entomology* 31(1): 123-126.

Nayar, J.K. 1969. Effects of larval and pupal environmental factors on biological status of adults at emergence in *Aedes taeniorhynchus* (Wied.). *Bulletin of Entomological Research* 58(4): 811-827.

Oliver, S.V. & Brooke, B.D. 2013. The effect of larval nutritional deprivation on the life history and DDT resistance phenotype in laboratory strains of the malaria vector *Anopheles arabiensis*. *Malaria Journal* 12:44.

Packierisamy, P.R., Ng, C.W., Dahlui, M., Inbaraj, J., Balan, V.K., Halasa, Y.A. & Shepard, D.S. 2015. Cost of dengue vector control activities in Malaysia. *The American Journal of Tropical Medicine and Hygiene* 93(5): 1020-1027.

Pridgeon, J.W., Pereira, R.M., Becnel, J.J., Allan, S.A., Clark, G.G. & Linthicum, K.J. 2008. Susceptibility of *Aedes aegypti, Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 pesticides with different modes of action. *Journal of Medical Entomology* 45(1): 82-87.

Reiskind, M.H & Lounibos, L.P. 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Medical and Veterinary Entomology* 23(1): 62-68.

Renshaw, M., Service, M.W. & Birley, M.H. 1993. Density-dependent regulation of *Aedes cantans* (Diptera: Culicidae) in natural and artificial populations. *Ecological Entomology* 18(3): 223-233.

Rosilawati, R, Lee, H.L., Nazni, W.A., Nurulhusna, A.H., Roziah, A., Khairul Asuad, M., Siti Futri Farahininajua, F., Mohd Farihan, M.Y. & Ropiah, J. 2017. Pyrethroid resistance status of *Aedes (Stegomyia) Aegypti (Linneaus)* from dengue endemic areas in peninsular Malaysia. *International Medical Journal Malaysia* 16(2):73-78.

Rozilawati, H., Mohd Masri, S., Tanaselvi, K., Mohd Zahari, T.H., Zairi, J., Nazni, W.A. & Lee, H.L. 2017. Life table characteristics of Malaysian strain *Aedes albopictus* (Skuse). *Serangga* 22(1): 85-121.

Smith, D.L., Dushoff, J. & McKenzie, F.E. 2004. The risk of a mosquito-borne infection in a heterogeneous environment. *PLoS Biology* 2(11): e368.

Sumanochitrapon, W., Strickman, D., Sithiprasasna, R., Kittayapong, P. & Innis, B.L. 1998. Effect of size and geographic origin of *Aedes aegypti* on oral infection with dengue-2 virus. *The American Journal of Tropical Medicine and Hygiene* 58(3): 283-286.

Teng, H.J. & Apperson, C.S. 2000. Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: effects of density, food and competition on response to temperature. *Journal of Medical Entomology* 37(1): 40-52.

World Health Organization. 2016. *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. World Health Organization, Geneva 2.

Wan-Norafikah, O., Nazni, W.A., Lee, H.L., Zainol-Ariffin, P. & Sofian-Azirun, M. 2013. Susceptibility of *Aedes albopictus* Skuse (Diptera: Culicidae) to permethrin in Kuala Lumpur, Malaysia. *Asian Biomedicine* 7(1): 51-62.

Xue, R.D., Barnard, D.R. & Schreck, C.E. 1995. Influence of body size and age of *Aedes albopictus* on human host attack rates and the repellency of deet. *Journal of the American Mosquito Control Association* 11(1): 50-53.

Yakob, L., Alphey, L. & Bonsall, M.B. 2008. *Aedes aegypti* control: the concomitant role of competition, space and transgenic technologies. *Journal of Applied Ecology* 45(4): 1258-1265.

Yeap, H.L., Endersby, N.M., Johnson, P. H., Ritchie, S.A. & Hoffmann, A.A. 2013. Body size and wing shape measurements as quality indicators of *Aedes aegypti* mosquitoes destined for field release. *The American Journal of Tropical Medicine and Hygiene* 89(1): 78–92.

Yoshioka, M., Couret, J., Kim, F., McMillan, J., Burkot, T. R., Dotson, E. M., Kitron, U. & Vazquez-Prokopec, G. M. 2012. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). *Parasites & Vectors* 5: 225.

Zeller, M. & Koella, J.C. 2016. Effects of food variability on growth and reproduction of *Aedes aegypti*. *Ecology and Evolution* 6(2): 552-559.