Journal of Sustainability Science and Management
Volume 16 Number 8, December 2021: 240-247
eISSN: 2672-7226

HEIGHTENED BIOLOGICAL PROPENSITY OF *Aedes Albopictus* IN SELECTED DENGUE OUTBREAK PRONE AREA, SELANGOR

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Submitted final draft: 24 February 2021 *Accepted: 11 March 2021* http://doi.org/10.46754/jssm.2021.12.017

Abstract: Dengue fever is a complex disease caused by bites of infected female *Aedes* mosquitoes. The existence of hot spot (HS) areas increases in tandem with the escalating rise of dengue cases reported each week. This study was carried out to compare the differences between biological characteristics of *Aedes albopictus* in HS and non-hot spot (NHS) areas. Ovitraps were set up in HS and NHS areas in selected areas in Selangor. Throughout this study, biological characteristics; namely duration of immature stages, gonotrophic cycle, fecundity and longevity were observed from field collected populations under laboratory-controlled conditions. *Aedes albopictus* from HS areas demonstrated shorter duration (six days) than NHS (10 days) in immature stages. Longevity wise, longer periods were observed in HS as compared to NHS, with 60 and 36 days of survival, respectively. Higher fecundity was generated by *Ae. albopictus* from HS with a mean of 2605 eggs as compared to NHS with only 1140. In addition, a significant difference is observed on the gonotrophic cycle between both areas. The findings of this study indicated that repeated chemical control applications might have affected all biological characteristics between HS and NHS areas.

Keywords: Hot spot, immature stage, gonotrophic cycle, fecundity, longevity.

Introduction

Aedes albopictus, known as the Asian Tiger mosquito, is a maintenance vector that is occasionally involved with dengue transmission in Asia (Black IV *et al.,* 2002) . *Aedes albopictus* is known a human aggressive day-biting species, that has recently expanded to various countries outside its native Asian ranges (Benedict *et al.,* 2007). This mosquito breeds in natural and artificial containers, and its habitat selection is based on the accessibility of food and to complete its reproductive development (Hartman, 2011). *Aedes albopictus* is a container inhabiting mosquito that is strongly associated with human habitats, especially outside its native range and capable of ovipositing diapauses-destined eggs that could even survive harsh environments (Mogi *et al.,* 2012). In addition, the mosquito has been found thriving in backyard household containers that require treatment with larvicides and adulticides during outbreaks. Adulticiding is often required to control and prevent disease transmission (Marcombe *et al.,* 2014).

The success in completing the development from egg to adult and its lifespan are important factors in the viral transmission capability of the vector to its new host (Nur Aida *et al.,* 2011). Therefore, understanding the biological characteristics among mosquitoes in hotspot (HS) and non-hotspot (NHS) areas is essential in controlling dengue outbreaks. Furthermore, during outbreaks, shorter development duration of mosquito, from eggs to adult, will lead to increased population density, thus increasing the risk of dengue fever. The development of resistance against insecticide increases the agility of the mosquito, and consequently, its biological characteristics might be altered, such as shortening of the life cycle during immature stage, which would eventually increase the population size (Nur Aida *et al.,* 2011).

Dengue prevalence in Malaysia was reported to be 137 per 100,000 population in 2009 (Shepard *et al.,* 2013). Among all states in Malaysia, Selangor recorded the highest number of dengue cases with approximately 50% of

Malaysian dengue cases occurring in this state, with 62,867 cases and 51,652 cases in 2015 and 2016, respectively, and cases were reported to be increasing until 2020 by Selangor State Health Department, according to Ghani *et al.* (2019). A study in Subang Jaya Municipality by Dom *et al.* (2012) found that there were 4,651 cases of dengue fever reported between January 2006 and December 2009. Subang Jaya is classified into HS and NHS areas, where a HS is based on confirmed dengue cases occurring throughout the year. In controlling and containing the disease transmission in these areas during outbreaks, adulticides of organophosphates and pyrethroids have been repeatedly used. In the absence of an available vaccine at present, dengue control is limited to reduction of the vector population (Koedraadt *et al.,* 2006). This indicates that the sole practice of adopting adulticides might be an ineffective strategy. According to Dusfour. L *et al.* (2019), prolonged exposure to insecticides might lead to the development of physiological resistance to the type of insecticides used, thus the mosquito carries genes for resistance to the next generation. Needless to say, improper selection and excessive use of insecticides would lead to the development of a resistant population worldwide (Karunamoorthi & Sabesan, 2013).

Subang Jaya Municipality has been using insecticides of organophosphates and pyrethroids group during dengue outbreaks in the past five years (Table 1) as a prevention and control measures to combat dengue. Therefore, a study was carried out to explore the differences between biological characteristics of *Ae. albopictus* populations from dengue HS and NHS areas in Subang Jaya Municipality.

Materials and Methods

Site Selection

In this study, all of the HS and NHS were chosen according to Dom *et al.* 2012 for ovitrap set up. total of 12 dengue HS and NHS at different localities in the study area of Subang Jaya Municipality, namely Subang Jaya (N 3°04'52.37", E 101°35'03.88"), Seri Kembangan (N 3°01'19.19", E 101°42'19.95"), Seri Serdang (N 3°01'32.39", E 101°42'08.29") and Kinrara (N 3°04'12.33", E 101°41'27.62") (Table 2).

Ovitrap Setting and Collection

Standardized ovitraps were used in this study as described by Jaal. Z (2009). One thousand two hundred (1200) ovitraps, comprising a 300 ml black plastic container, were set up outdoors in dengue HS and NHS areas from March to September 2013. Each ovitrap was filled with seasoned tap water. After three days, the ovitraps were collected and the presence/absence of eggs or larvae were recorded in the laboratory. Percentage of ovitrap Index (OI) was calculated by dividing the number of positive ovitrap with the total number of recovered ovitrap and multiplying the result with 100.

Table 1: Commonly used insecticides by the local authority in the selected study area

Item	Trade Name	Active Ingredients	Concentration (w/w)
1	Malathion	Organophosphates	96.5%
2	Resigen	Permethrin	18.69%
		S-Bioallethrin	16.83%
		Piperonyl Butoxide (Synergist)	10.70%
3	Deltacide	S-Bioallethrin	0.85%
		Deltamethrin	0.60%
4	Pesguard	D-Tetramethrin	4.40%
		Cyphenothrin	13.2%
5	Sumithion	Fenitrithion	40.0%
		Tetramethrin	1.00%

Table 2: The hot-spot and non-hotspot area in selected study area

Study Area	Hot Spot	Non-hot Spot
Subang Jaya	USJ 6 PJS 7 Taman Subang Mas	USJ 20 USJ 13 USJ 8
Seri Kembangan	Taman Pinggiran Putra Taman Universiti Taman Sg. Besi Indah	Taman Bukit Belimbing Taman Sri Timah Taman Harmoni Indah
Seri Serdang	Taman Serdang Raya Taman Serdang Jaya Taman Sri Serdang	Taman Serdang Perdana Taman Muhibah Taman Bukit Pelangi
Kinrara	Puchong Perdana Kg. Bt. 13 Taman Kota Perdana	Taman Mutiara Indah Saujana Puchong Taman Bukit Kinrara

Egg Collection and Hatching

The water from each ovitrap was transferred into a white enamel basin with the diameter of 30 cm, together with the paddles, and left 1 - 2 days before the hatching process. The larvae were reared at 28°C ± 2, 80% RH. From day one to five, 2.5 mg of larval food was added to stimulate larval growth. Larvae were fed with food mixture in powdered form made of cat food (frieskies), dried beef liver, dried yeast and skimmed milk in the ratio of 2:1:1:1 as described by Jaal Z. (2009). The duration of immature stage was recorded daily until pupation.

Pupae Rearing

The pupae were transferred into a glass beaker and placed into a mosquito cage 30 cm x 30 cm x 30 cm for maturation into adults.

Adult Sexing and Rearing

All emerged mosquitoes were subjected to identification and sexing individually by observation using glass tube aspirator following a pictorial key for mosquito identification (Pratt *et al.,* 1963). Adults were fed with a 10% sucrose solution and vitamin B complex on cotton wool replaced every two days. The adult females (six days old) were then given a two-hour blood meal by placing restrained mice into the adult cage. Consequently, 20 females and ten males of *Ae. albopictus* adult mosquitoes were aspirated and transferred to another cage for gonotrophic cycle and fecundity experiments. After two to three days of mating process, mosquitoes were given access to a blood feeding source by placing one restrained mouse inside the cage.

A cone of round filter paper in a Petri dish filled with dechlorinated water was placed inside the cage for oviposition. The first gonotrophic cycle was recorded once the female completed the oviposition of eggs. The number of eggs per gonotrophic cycle was counted from the dried paper cone under a dissecting microscope. The female mosquito was then allowed to rest to get enough nutrition and strengthen its physical features before the next gonotrophic cycle. Only adult males were replaced should they die while the number of females remaining would be recorded until the last gonotrophic cycle (Ponlawat & Harrington, 2009). T-test analysis was used to compare means of positive ovitrap, ovitrap index, and *Aedes albopictus* biological characteristics between dengue hotspot and non-hotspot at $P<0.05$.

Results and Discussion

Aedes Abundance in Hotspot and Non-hotspot Area

The *Aedes* abundance in HS and NHS areas are shown in Figure 1 and 2. It was found that *Aedes*

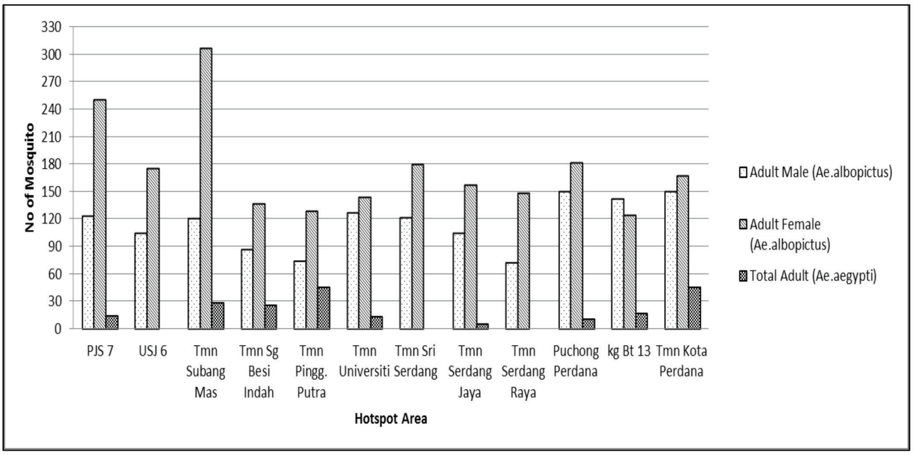

Figure 1: The abundance of *Aedes* spp. in dengue outbreak hotspot area

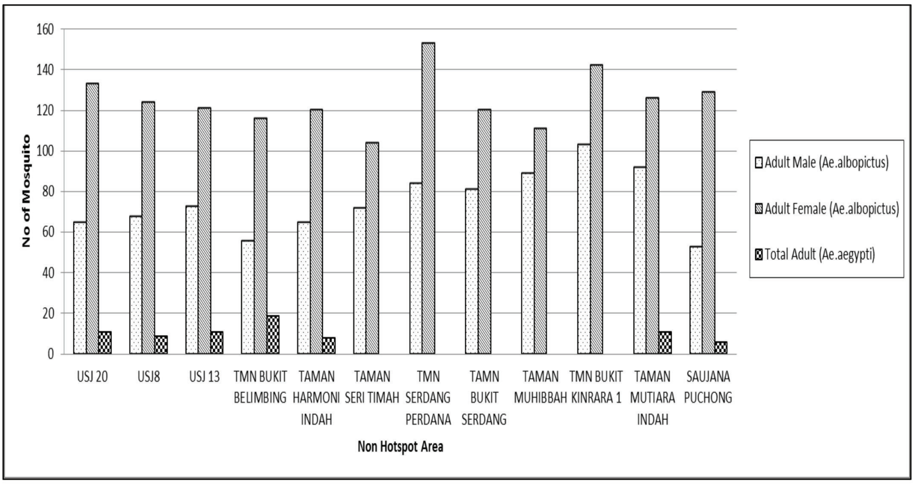

Figure 2: The abundance of *Aedes* spp. in dengue outbreak non-hotspot area

albopictus is a predominant species as compared to *Aedes aegypti,* and other species in both HS and NHS areas caught in the ovitraps. In addition, female *Aedes albopictus* was found to be higher in number than the male in all localities implying potential dengue viral transmission.

Positive Ovitrap and Ovitrap Index (%)

The results obtained from the ovitrap survey indicate there is significant difference of *Ae. albopictus* abundance in both HS and NHS of Subang Jaya Municipality area ($p < 0.001$) (Table 3).

Duration of Immature Stages

There were significant differences between HS and NHS in the duration of first instars to pupal stage. Shorter development time was observed among HS mosquito population with a mean value of 6.16 ± 1.88 days as compared to NHS with a mean value of 9.77 ± 1.98 days before it emerged into pupae (Table 4). Shorter duration of immature stages in HS might have contributed to the increase in population size of *Ae. albopictus.* The shorter immature stage of mosquitoes will eventually allow rapid emergence of adult mosquitoes into the environment, thus contributing to a higher density of adult mosquitoes and increasing

Table 3: The comparative analysis of *Aedes albopictus* abundance in both hotspot and non-hotspot area using positive ovitrap and its index (POI%)

Variable (s)	HS Mean (SD)	NHS Mean (SD)	Mean Score (95% CI)	t-Stats (df)	p-Value
Positive Ovitrap	40.05 (5.26)	33.11 (1.93)	6.944 (4.25 – 9.63)	5.25 (34)	0.001*
Ovitrap Index (%)	86.86 (8.10)	70.1 (5.02)	16.70 (12.13 – 21.26)	7.408 (28.38)	0.001*

*There is a significant difference as p-value < 0.05, t-test

the chances of adult *Aedes* to bite humana in the area. A similar finding on this important epidemiological implication favoring high population size of pupae due to the rapid development of larvae (Wan-Norafikah *et al.,* 2009). Moreover, rapid development time in the immature stage also influences the size, wing size and longevity of the adult (Reiskind & Lounibos, 2009).

Gonotrophic Cycle

There is a significant difference ($p < 0.05$) observed in the gonotrophic cycles of both HS and NHS with mean values of 9.11 and 8.08 cycles respectively (Table 4). The gonotrophic cycles obtained by *Ae. albopictus* was able to produce up to ten reproductive cycles between 17˚C – 32˚C which is in accordance with a study by Briegel and Timmermann (2001). In addition, Dieng *et al.* (2010) found that wild strain *Ae. albopictus* mosquitoes collected in Northern Peninsular of Malaysia had up to seven gonotrophic cycles. The differences of gonotrophic cycles between HS and NHS areas in the study area suggests that there are heightened biological propensity of the mosquito itself and this might be due to continuous environmental stressor available in the HS area by means of frequent mass insecticide spraying to control dengue outbreaks. However, the fitness cost study over ramification of gonotrophic cycle between HS and NHS areas have yet to be focused by looking into genetic mutation which may result in greater cycle of mosquito population.

Fecundity (Number of Eggs)

In terms of fecundity, HS mosquitoes produced higher number of eggs than NHS mosquitoes. There were 221.75 and 157.4 eggs representing the mean number of eggs produced by 20 females *Ae. albopictus* from HS and NHS respectively (Table 4). Hence, one female *Ae. albopictus* oviposited between 200 to 300 eggs per gonotrophic cycle. These findings were similar to mean number of eggs laid per female 221 (220.65) conducted by Nur Aida *et al.* (2011). A similar study by Nur Aida *et al.* (2011) revealed the production of eggs was influenced by the mosquito body size, quality and quantity of mating process, blood feeding and preferred location of laying eggs. Larger size mosquitoes would be able to produce more eggs in comparison to smaller mosquitoes since larger female mosquitoes consume more blood (Nur Aida *et al.,* 2011). In addition, body size of mosquitoes could be related to the duration of immature stage taken during first instars until its emergence into pupae, where the faster the duration, the lesser the exposure to survival risk during the aquatic life hence producing larger mosquitoes. Additionally, it was found that protein is an essential component for egg development and energy for the survival for adult female mosquitoes (Harrington *et al.,* 2001). It is noteworthy that in this study, both samples were fed with the same blood source so as to be in line with findings established by Xue *et al.* (2009), where they found that the source of blood meal influenced the fecundity or number of eggs produced.

Longevity

Longevity is defined as the lifespan of an adult mosquito. The longer the lifespan of adult mosquitoes, the larger the population size, thus increasing the probability of dengue transmission (Padmanabha *et al.,* 2012). Results in Table 4 showed that HS mosquitoes lived longer than NHS mosquitoes, ensuring sustainability of a new generation as described by Nur Aida *et al.* (2008). Despite both HS and NHS mosquitoes were reared under laboratory control conditions using standard protocol to mimic natural environment to prevent any bias (Lambrechts & Failloux, 2012), a study conducted by Oliver and Broke (2016) has suggested that insecticide-resistant mosquito population is capable of withstanding oxidative stress and had proven to sequester more oxidative enzyme, therefore increasing lifespan. In this study, it was noteworthy that *Ae. albopictus* in HS could survive longer as compared to *Ae. albopictus* in NHS area.

Conclusion

All biological characteristics except for gonotrophic cycle of *Ae. albopictus* found in both HS and NHS were different. The ability of HS *Ae. albopictus* to live longer would enable the production of more eggs, as compared to NHS. This implies that repeated use of insecticides to control dengue outbreaks in HS areas is expected to affect the normal life cycle of the mosquitoes (Ranson *et al.,* 2010). On top of that, prolonged exposure to insecticide due to continuous outbreak in the study area had increase oxidative burden to the exposed mosquito population, hence possibly inducing oxidative stress resistance, to less exposed mosquito population, therefore, inducing heightened biological propensity namely duration of immature stage, longevity, fecundity and gonotrophic cycle of the *Ae. albopictus* in the respective area (Oliver & Broke., 2016). Therefore, there is an urgent need for the reinforcement of surveillance and implementation of control measures against this invading species in both HS and NHS. In relation to this, it is recommended and advisable to rotate between insecticides to avoid prolonged exposure, thus preventing further changes in biological characteristics and possibly resistance in both HS and NHS.

Furthermore, the awareness of the community towards the seriousness of dengue and the importance of source reduction activity should be emphasized through health education campaigns in both HS and NHS areas. This is because the transmission of dengue cannot be minimized or totally interrupted without the participation and cooperation of whole communities. Future consideration on the study on the development of resistance towards

Table 4: The comparative analysis of biological characteristics of *Ae.albopictus* between HS and NHS dengue areas in Subang Jaya

Variable (s)	HS Mean (SD)	NHS Mean (SD)	Mean Score (95% CI)	t-Stats (df)	p-Value
Duration of immature stage (Days)	6.16 (1.88)	9.77 (1.98)	-3.61 (-4.95-(-2.29))	-5.59 (33.9)	0.001*
Longevity (Days)	65.33 (11.14)	35.5 (8.77)	29.37 (23.02 – 36.64)	8.923 (32.22)	0.001*
Fecundity (No. of eggs)	221.75 (86.76)	157.4 (38.98)	64.30 (18.74-109.87)	2.868 (34)	0.007*
Gonotrophic cycle	9.11 (1.67)	8.08 (1.304)	1.055 (0.035 -2.075)	2.108 (32.06)	0.043*

*There is a significant difference as p-value < 0.05, t-test

Note: Hotspot (HS); Non-Hotspot (NHS)

repeated insecticide exposure should be carried out in order to improve existing vector control programs. It is recommended to rotate between insecticides from different insecticide groups with different active ingredients to prevent changes in biological characteristics as a strategy to manage insecticide resistance.

Acknowledgements

This research was funded by the Research-Intensive Fund Scheme, Research Management Institute, UiTM and Long-Term Research Grant (LRGS) for Infectious Diseases, 2011 - 2014, Ministry of Higher Education, Malaysia, and USM (304/Pbiology/650575/U112). We would also like to thank staff at the Environmental Health and Safety Department, Faculty of Health Sciences, UiTM Puncak Alam Campus, for their assistance.

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