ORIGINAL ARTICLE

Progressive biological characteristics changes of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in hot spot and non-hot spot areas at Subang Jaya Municipality

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Abstract:

Dengue fever is a complex disease caused by bites of infected female *Aedes* mosquitoes. The existence of hot spot (HS) area increases as the number of dengue cases reported increase every week. Repeated chemical control application implemented in hot spot areas is expected to affect the normal life cycle of the mosquitoes. This study was carried out to compare the differences between biological characteristics of *Aedes albopictus* in HS and non-hot spot (NHS) areas in the context of chemical application**.** Ovitraps were set up at both HS and NHS areas in Subang Jaya Municipality. Throughout this study on biological characteristics, namely duration of immature stages, gonotrophic cycle, fecundity (number of eggs) and longevity were observed under laboratory controlled conditions. *Ae. albopictus* from HS areas demonstrated shorter duration, 6 days than NHS, 10 days in immature stages (p = 0.001). While, for longevity, longer days is observed in HS as compared to NHS with 60 and 36 days of survival (p = 0.001) respectively. Higher fecundity was recorded by *Ae. albopictus* from HS with a mean of 2605 eggs as compared to NHS with only 1140 eggs (p = 0.007). Similar significant differences were also observed for gonotrophic cycle as HS and NHS with 9 and 8 cycles (p = 0.043) respectively. It was found that repeated chemical control affected all biological characteristics except for gonotrophic cycle of *Ae. albopictus* found in both HS and NHS areas.

Keywords: Duration of immature stage, fecundity gonotrophic cycle, hot spot, longevity

1. INTRODUCTION

Aedes albopictus also known as the "Asian Tiger mosquito", is considered as a maintenance vector and occasionally, is involved with dengue transmission in Asia [1]. Black IV, Bennett [2] and other researchers suspected *Aedes albopictus* of being a vector or secondary vector of several viruses particularly of the viruses that cause dengue fever and dengue hemorrhagic fever and being implicated in the transmission of the dengue even though it is not classified as a major vector [3]. *Aedes albopictus* is known for its aggressiveness towards human and recently expanded to more countries outside its native regions [4]. This mosquito breeds in both natural and artificial containers. The habitat selection is based on the accessibility to obtain food and to complete its reproductive development [5]**.** Previous study also found that *Aedes albopictus* is a container inhabiting mosquito that strongly associated with human habitats especially outside its native range and capable of ovipositing diapauses-destined eggs that could even survive harsh environment [6]. In addition, the mosquito has been found thriving in backyard household containers that require treatment with larvicides and adulticides during episodes of outbreak. Adulticiding is often required to control and prevent the disease transmission [7]. 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 [8]. Therefore, understanding the biological characteristics among mosquitoes in the HS and NHS areas is essential. Furthermore, during outbreak, shorter development duration of mosquito from eggs to adult will lead to the increase in the population density thus increasing the risk of dengue fever. This is in accordance with findings by Nur [8] that development of immunity against insecticide increased the agility of the mosquito, at the same time its biological characteristics might be altered such as shortening of the life cycle during immature stage which would eventually increase the population size.

The transmission of the dengue virus (DENV) is vital in the development of dengue fever worldwide and Malaysia is no exception. The dengue prevalence in Malaysia was reported to be 137 per 100,000 population in 2009 [9]. In Selangor alone, dengue cases have spiked by 338%, with 29,972 cases recorded between January and July 27, 2014 as compared to only 6,842 cases for the same period in 2013 as reported in The Malay Online newspaper. Retrospectively, a study in Subang Jaya Municipality by Dom et al [10] discovered that there were 4,651 cases of dengue fever reported between January 2006 and December 2009. In the same study, he further classified Subang Jaya into HS and NHS based on confirmed dengue cases distribution. In controlling and containing the disease transmission in these areas during outbreak, adulticides from the family of organophosphates and pyrethroids has been repeatedly used. This practice is in agreement as reported by Koedraadt [11], that dengue control is limited to reduction of the vector population while waiting for an effective vaccine to be developed.

Nevertheless, despite the application of adulticide the cases continue to rise as revealed by studies conducted by Nazari. [12] and Nadirah [13]. From 980 reported cases nationwide, 442 were in Selangor, with 131 cases recorded in Subang Jaya [12]. In February 2014, the total dengue cases increased up to 4894 with 5 deaths as compared to 1154 cases with only 1 death in February 2013 in Selangor [13]. In June 2014, Subang Jaya Municipality alone recorded 184 dengue cases [14]. This situation indicated that the current adulticides applied might be ineffective. The study conducted by Lutz [15] reported that mosquito control and prevention practices by various localities may have a significant impact on its population. According to the World Health Organization [16] prolonged exposure to insecticides might develop physiological resistance to the type of insecticide used, thus, mosquito that carry resistance genes which had survived exposure would transfer these genes to the next generation.

Most of the mosquito control programs is using the different types of insecticides to target the insects' central nervous system but, improper selection, repeated and overuse of insecticide would lead to the development of resistant population all over the world [17]. Since the application of the same group of insecticides over period of time was proven to develop resistance, the inventory of the insecticides used in Subang Jaya Municipality has shown the most commonly used insecticides are from organophosphates and pyrethroids group. Considering there was constant dengue outbreaks reported in Subang Jaya, Selangor, a study should be carried out to explore the differences between biological characteristics of *Aedes albopictus* population sampled from dengue HS and NHS areas in Subang Jaya Municipality as a result of repeated chemical application.

2. MATERIALS AND METHODS

2.1 Site selection

Twelve dengue HS and NHS were selected from 3 different localities in each study areas in Subang Jaya Municipality namely Subang Jaya, Seri Kembangan, Seri Serdang and Kinrara.

2.2 Ovitrap setting and collection

As described by Lee [18], standardized ovitraps were used in this study. A total of 1200 ovitraps comprised of 300ml black plastic container were placed outdoors in dengue HS and NHS area from March to September 2013. The opening and the base of the container is 9.1cm. An oviposition paddle made from hardboard (10cm x 2.5cm x 0.3cm) consisting of two different types of surfaces was placed diagonally into each ovitrap with the rough surface of the oviposition paddle upwards. Each ovitrap was filled with tap water to a level of 5.5cm. The ovitraps were collected after being left for three days, brought to the laboratory and the presence/absence of eggs or larvae was recorded. Any relocation of the trap position was also recorded. 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.

2.3 Egg collection and hatching

In the laboratory, the water from the each ovitraps was transferred into a white enamel basin together with the paddles and left for about one to two days before hatching process. The larvae were reared at 28°C ± 2, 80% RH. From day 1 to 5, 2.5mg of larval food was added to help stimulate the larval growth. All larvae were fed with food mixture made of cat food (frieskies), dried beef liver, dried yeast and skimmed milk in the ratio of 2:1:1:1 by weight prepared as a fine powder following Zairi. [19]. Daily monitoring was conducted to record the duration of immature stage until pupation.

2.4 Pupae rearing

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

2.5 Adult rearing

The adults were fed with 10 % (w/v) sucrose solution on cotton wool. The solution was prepared by dissolving 100g sugar, and 2 tablets of vitamin B complex in 1 liter of dechlorinated water. The cotton wool was replaced every 2 days. The adult females (6 days old) were then given a 24 hour blood meal by placing restrained mice into the adult cage. Consequently, 20 females and 10 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 blood feeding source by placing one restrained mice inside the cage for about two days. 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's mosquito was then allowed to rest to get enough nutrition and strengthen its physical features before observation of 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.

2.5 Statistical analysis

All of the data recorded were subjected to normality distribution test following Kolmogrov-Smirnov. Mean comparison testing was carried out using independent sample t-test for between dengue HS and NHS dengue area for its positive ovitrap, ovitrap index, and *Aedes albopictus* biological characteristics.

3. RESULTS AND DISCUSSION

3.1 Aedes abundance in hotspot and non hotspot area, its Positive Ovitrap and Ovitrap Index (%)

The *Aedes* abundance in hotspot and non hotspot area are shown in Fig.1 and Fig.2. It was found that *Aedes albopictus* is a predominant species as compared to *Aedes aegypti* and other species in both hotspots and non hotpots areas caught in the ovitraps. In addition, the female *Aedes albopictus* was found to be higher in number than the male in all localities implying potential dengue viral transmission. The results obtained from ovitrap survey clearly indicated there was a significant difference of *Ae.albopictus* breeding in both HS and NHS of Subang Jaya Municipality area ($p < 0.001$) (Table I).

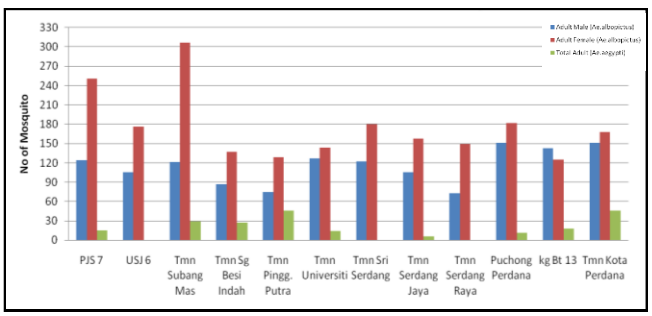

Figure 1:Aedes abundance in Hot Spot area

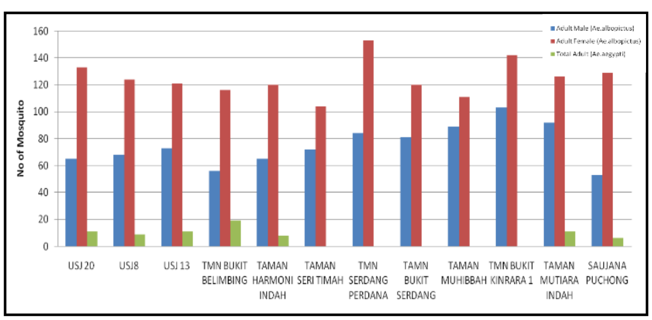

Figure 2:Aedes abundance in Non Hot Spot area

Table I: Mean Comparison of Positive and Ovitrap Index in 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
Positive Ovitraps	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 applied

3.2. Duration of immature stage

Based on the laboratory observation on *Ae. albopictus* larvae, there were significant shorter duration of immature stage periods of *Ae. albopictus* between HS and NHS. Shorter development time was observed among HS mosquito population with a mean value of 6 days as compared to NHS with a mean value of 10 days ($p = 0.001$) before it emerged into pupae (Table 2). Shorter duration of immature stages in HS might have contributed to the increase in population size of *Ae. albopictus*. This finding has an important epidemiological implication favoring high population size of *Ae. albopictus* in HS area due to the rapid development of larvae [21]. Moreover, there was a study had reported that shorter development time during the immature stage had influenced the body size, wing size, and longevity of adult mosquito [22]

Table 2: Mean Comparison of Positive and Ovitrap Index in 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*

3.3. Gonotrophic Cycle

There is a significant difference ($p = 0.043$) observed in the gonotrophic cycles of both HS and NHS with mean values of 9.11cycles and 8.08 cycles respectively (Table 2). The gonotrophic cycles obtained were similar to a study conducted by Briegel and Timmermann [23], where *Ae.albopictus* was able to produce up to 10 reproductive cycles between 17˚C – 32˚C. In addition, Dieng et.al [24] found that wild strain *Ae. albopictus* mosquito collected in Northern Peninsular of Malaysia had gonotrophic cycles up to seven. The slight variation in the cycles probably is due to differences in *Aedes* mosquito strains originated from different areas.

3.4. Fecundity (number of eggs)

HS mosquitoes produced higher number of eggs than NHS. There were 221.75 and 157.4 eggs representing the mean number of eggs produced by 20 female *Ae.albopictus* from HS and NHS respectively (Table II). Hence, one female *Ae.albopictus* oviposited between 200 to 300 eggs per gonotrophic cycle. Similar pattern of egg production was also reported by Nur [8]. In addition, the study has revealed that the production of eggs was also influenced by the mosquito body size, quality and quantity of mating process, blood feeding and preferred location of laying eggs. Larger size mosquito would be able to produce more eggs in comparison to smaller mosquito since larger female mosquito consumes more blood [8]. Similar study indicated, body size of mosquito could be related with the duration of immature stage taken during first instars until emerge into pupae where the faster the duration, the lesser the exposure to survival risk during aquatic life hence producing larger mosquito. Additionally, it was found that protein is an essential component for egg development and energy for the next generations survival for adult female mosquito [25]. It is noteworthy that in this study, the female mosquitoes were fed with the same blood source for 2 consecutive days so as to be in line with findings established by Xue, Barnard [26], where they found that the source of blood meal was proven to have influenced the fecundity or number of eggs produced.

3.5. Longevity

The lifespan of adult mosquito emerging from pupae until it dies is known as longevity. The longer the lifespan of adult mosquito, the higher the number of population size thus increasing the probability of dengue transmission. Results in Table 2 showed that HS mosquito lived longer, with mean = 65.33 days , than NHS mosquito mean = 35.5 days, $p < 0.001$ ensuring sustainability of a new generation as stated in the study conducted by Nur Aida and Abu Hassan [27]. In this study, both HS and NHS mosquitoes were reared under laboratory control condition mimicking outside environment with standard laboratory protocols in order to get similar condition for development to prevent any bias [28].

4 CONCLUSION

In conclusion, it was found that repeated chemical control application affected all biological characteristics except for gonotrophic cycle of *Ae. albopictus* found in both HS and NHS. The competency of HS *Ae. albopictus* to survive from its aquatic life which is from eggs, larval and pupal stages, until completion of its lifecycle allow the *Ae. albopictus* to rapidly transmit the virus into the new generation and new host. The ability of HS *Ae. albopictus* to live longer would enable the production of more eggs as compared to NHS. Thus, the reinforcement of surveillance and implementation of control measures against this invading species are required in Subang Jaya Municipality in both HS and NHS. As repeated application of insecticides has been practiced in Subang Jaya Municipality due to frequent dengue outbreaks, it is advisable to practice insecticides rotation so as to avoid prolong exposure to the same active ingredient thus preventing further changes in biological characteristics and possibly resistance in both HS and NHS. The awareness of the community members towards the seriousness of dengue cases and the importance of the source reduction activity should be emphasized for instance ‘The search and destroy’ program and also through health education campaigns at both HS and NHS areas. This is due to the fact that the transmission of dengue could not be minimized or totally interrupted without the participation and cooperation of the whole communities.

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