ORIGINAL ARTICLE

A Comparison of Demographic Parameters of Aedes albopictus (Skuse) (Diptera: Culicidae) in Dengue Risk Area at Shah Alam, Selangor

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ABSTRACT

Introduction: *Aedes albopictus* is known for its aggressiveness towards human and recently expanded to more countries outside the native regions. Thus, the demographic parameters of *Aedes albopictus* are important to determine the characteristics of this species mosquitoes in terms of the reproduction rates and dispersal distance. Materials and **Methods:** This study, was performed using a Shah Alam strain of *Aedes albopictus* originally collected in twenty district areas of the central zone of Shah Alam. This research applies field work-study with a cross-sectional design to investigate the demographic parameters of Aedes albopictus. The demographic evaluation of *Aedes albopictus* was conducted under the control environment in insectarium. Results: Investigation on the demographic parameters of *Aedes albopictus* clearly showed that there is a significance different observed in the total number of mosquito eggs produced in both high and low incidence rate IR areas (p=0.03). In contrast, other parameters showed insignificant value between high and low IR areas. Conclusion: The key to control the mosquito vectors population is by tracking the vector's life cycle including its survival. Therefore, the outcome of this study may provide as a baseline to estimate the dengue outbreak in the current episystem.

Keywords: Demographic parameters, *Aedes albopictus*, Dengue, Shah Alam

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INTRODUCTION

Aedes albopictus (Asian Tiger mosquito) is an aggressive vector to human and has recently expanded to include more countries outside their home regions (1). This mosquito breeds in natural and artificial containers and the habitat selection are depends on access to food and the completion of reproductive development (2,3). Also, environmental factors are crucial in influence the mosquitoes' development and populations. The high population density of *Aedes albopictus* is translated to frequent biting activity creating public health concern (4,5). The ability to determine the demographic parameters of *Aedes albopictus* is of paramount ecological importance because longevity may affect the net reproduction rates and dispersal distance (6,7).

Surveillance of adult mosquitoes helps vector control technicians to understand the dynamic complexity in mosquito ecology and its relationship (8,9). Thus, the survival of a mosquito is the most important aspect that affects its ability in transmitting the disease (10,11). It is important to understand the demographic parameters of the mosquito in order to strengthen the vector control programmed (12). The studies of demographic parameters of *Aedes albopictus* are still limited compared to Aedes aegypti, especially in Malaysia (13). This parameter is remarkable for understanding the dynamics of the population. Completing the development from egg to adult and its lifespan successfully are important factors in the ability of the virus carrier to move to its preferred host (14,15,16). Therefore, it is necessary to understand the demographic parameters of mosquitoes in the areas of high incidence rate and low incidence rate (17,18). As well, during the outbreak period, if the mosquito development time from eggs to adult is fast, the population density and the risk of dengue fever will increase (19,20). Therefore, the demographic knowledge of mosquito parameters may set the basis for a successful vector control program (21).

Most of the entomologists have attempted utilizing the field data for the development of the life tables of

Aedes mosquitoes (14,16,22,23). Thus, this research applies field work-study with a cross-sectional design to investigate the demographic parameters of *Aedes albopictus* namely; (i) female fecundity, (ii) eggs development (iii) immature development period, (v) adult survivorships in the central zone of Shah Alam - Selangor, Malaysia. The main task of this research was to identify the demographic parameters of selected collected strains of Aedes in different dengue risk areas by using recent data of dengue surveillance.

MATERIALS AND METHODS

Study site

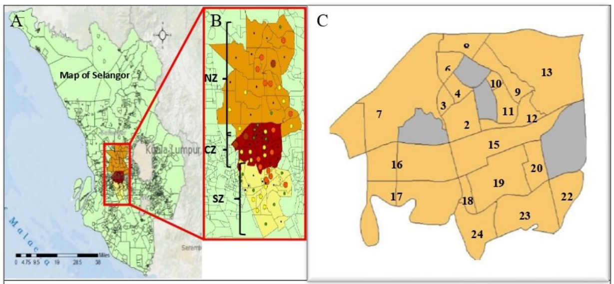
The entomological survey was conducted in the localities of the central zone of Shah Alam located approximately 25 km from Kuala Lumpur city at 3°05′48.74″ N 101°33′02.39″ E to 2°58′22.93″ N 101°44′ 39.69″ E altitude (Fig. 1). Based on the dengue surveillance data, this area experienced a large dengue outbreak every year. During this study, twenty residential localities had been explored for the surveillance activities and their surrounding environment was observed. The ecological condition of the study localities was summarized in Fig. 1D. From the general observation, this area was intense with the green landscape as most of the localities cultivated with vegetation.

Ovitrap surveillance

The study was performed using a Shah Alam strain of *Aedes albopictus* originally collected in March to December 2017. The eggs were collected using oviposition trap which is made up from plastic containers filled with a 150 ml distilled water with a paddle made of wooden hardboard (8cm x 2cm) as the site for mosquito oviposition. A total of 30 ovitraps were deployed in each locality (n=20) and placed in suitable habitat for the presence of mosquito with considering the factors of; (i) near adult resting sites (ii) in complete shade which is out of weather and human interference (iii) in direct line of sight (iv) near to other breeding containers (v) close to the ground (24). After four consecution days of export, the ovitrap were collected back.

The demographic parameters evaluation of Aedes albopictus

In the process of mosquito rearing to produce F1 generation, the F0 were (field strain) undergoes mass rearing. The process was conducted under controlled environment in insectarium at 29 ±3°C with 75 ±10% humidity and photoperiod 14:10 hours of dark : light cycle. The eggs collected for each locality are hosted separately by placing them in water for one hour. First instar larvae were transferred in plastic traps (29 x 23 x 6 cm) filled with dechlorinated tap water and were monitored until adult emergence. The larvae were fed daily with the pupal larval food. The observation of the immature mosquito development rate (days) on eggs to larval and larval to pupal were recorded accordingly



D

localities	Ecological condition
2	• Surrounded with various vegetation such as trees and shrubs
	• The environment is not properly managed and maintained
3	• There many scattered vegetation and tall trees around the residential areas
4	• Many households have ornamental plants on the balcony
	• Many rubbish and unused items in the residential areas
6	• Surrounded by with vegetation, trees, and vegetation
	• Not managed properly and unclean
7	• Have the vegetation in the surrounding area
	• A well-managed and clean area
8	• Surrounded with vegetation, trees, and shrubs
	• Properly managed and clean area
9	• Have the vegetation in the surrounding area
	• A well-managed and clean area
10	• There are many houses with vegetation
	• The environment is clean
11	• The backyard of the residential is poor sanitation
12	• Many houses have ornamental plants with proper maintenance
	• The area is properly managed
13	• Unclean environment
	• Rubbish ae scattered in abandoned area
15	• The commercial buildings is not well maintained
	• Poor sanitation
16	• The building is not well maintained and rubbish
	• Poor sanitation
17	• There are many scattered vegetation and tall trees around the residential area
	• The environmental is not well maintained
18	• Unclean environment
	• The residential backyard full of vegetation with poor maintenance
19	• The environment is clean
	• Houses with many vegetation are well maintained
20	• The environment is properly managed
22	• Unclean environment
	• Unused tyres were found at the backyard of some houses
23	• There many vegetation in the residential area
	• Rubbish can be found at the abandoned area
24	• The backyard of the houses is generally unclean and not well managed

Figure 1: (A) Map of Selangor highlighted Shah Alam in a different colour; orange: north zone (NZ), brown: central zone (CZ) and yellow: south zone (SZ). **(B) Distribution map in term of the number of DF cases in Shah Alam. (C) The central zone of Shah Alam,** *Seksyen* 1,5,14 and 21 (grey color) are excluded in this present study as they were subjected to the non-residential area. **(D) Ecological description of the central zone of Shah Alam**

(25).

Then the emerge pupae were separated from the rest of larvae into a new container and put inside the standard rearing cage (30 cm x 30 cm x 30 cm) until emergence of adult mosquito. At the adult stage, the mosquito sex separation was conducted. A container of 25 male and 25 female mosquitos (ratio 1:1) were transferred into a smaller cage (15 cm x 15 cm x 15 cm) by using an insect separator. The data of adult emergence was counted. Then, the mosquito was fed with 10% of sucrose solution filled in a universal bottle that has cotton wools for the mosquito to suck the solution. The universal bottle was placed in the cage and was refilled from time to time. After day 5 of the adult emergence, blood meal was given by placing a lab rat into a confined fit cage to the females for the development of the eggs within up to 2 hours (26). After that, a round black plastic container filled with dechlorinated water fitted with moist and placed the cone with folded filter paper on the top of the container into the cage served as the oviposition substrate. The moistened and cone folded filter paper was changed daily until no eggs were deposited on it. The filter paper that contained eggs were air-dried and the number of eggs was calculated. The observation on the survival, gonotrophic cycle and fecundity of the adult *Aedes albopictus* were conducted. The adult mosquitos were monitored according to localities that have been set. Therefore, the fecundity, gonotrophic cycle, and survival of the adults were monitored and recorded daily.

Data analysis and management

The present study was undertaken in twenty district areas of the central zone of Shah Alam. In order to summarize the demographic parameter of *Aedes albopictus*, the table analysis was conducted. A simple linear regression was used to estimate the general pattern of *Aedes albopictus*. Lastly, the incidence rate (IR) of DF cases in 2017 was used to compare the demographic parameters in high and low risk in the central zone of Shah Alam. The descriptive analysis on the IR based on the DF cases in year 2017 where, eleven section (n=11) were categorized into high IR (>20 cases in 1000 population) and nine sections (n=9) were categorized into low IR (<20 cases in 1000 population) areas. Since 20.42 is the mode number for the incidence rate, so above 20 cases considered as high incidence rate and less than 20 cases considered as low incidence rate. An independent t-test analysis was used to determine if there is a significant difference between the attribute of the *Aedes albopictus* demographic parameters in high and low IR areas.

RESULTS

Profile of population demographic parameters of Aedes albopictus in the central zone of Shah Alam, Selangor

The present study was undertaken in a twenty-district area of the central zone of Shah Alam in 2017. A total of 400 ovitrap were placed in the field during the study period, where two hundred ninety ovitraps were collected 29,066 eggs of mosquitos. In order to summarize the population parameter of *Aedes albopictus* in central zones of Shah Alam. Table I analysis comprising (i) female fecundity, (ii) egg development, (iii) immature development, and (iv) adult survivorships was developed. Table I summarized the measured life table features of *Aedes albopictus* for Shah Alam strain, under controlled laboratory conditions (28 ±2°C with 75% to 85% relative humidity). In general, adult females began taking a blood meal approximately two days post-emergence. The percentage of hatched eggs from the total oviposit (hatchability %) for each female ranged from 66.24% to 98.04% at and 82.89% overall hatchability and mortality ranged between 10.28% to 42.17% at and 28.17% overall mortality. The female mean fecundity was 58.13 eggs. The total developmental time was 8.8 days. The average survivorship was about 54.3 days from the first larva to the adult stage between the male

TABLE I: Life table attributes of Aedes albopictus mosquito from Shah Alam, Malaysia

Attribute	Mean ± SD	Range
i- Female fecundity (egg productivity)	58.13±19.11	26.68-136.08
ii- Egg development (%)		
Hatchability	82.89±18.57	66.24-98.04
Mortality	28.17±6.12	10.28-42.17
iii- Immature development (days)		
Eggs to Larvae	4.85±1.54	4-7
Larvae to Pupae	1.55±2.15	1-2
Total developmental time	8.8±1.33	7-11
v- Adult survivorships (days)		
Male ♂	48.55±13.28	29-70
Female ♀	60.05±16.57	41-79
Average	54.3±14.92	35-73.5

and female (48.55 and 60.05 days respectively).

The temporal distribution of Aedes mosquito biology in the central zone of Shah Alam was further analysed in order to identify the dynamic pattern of the biology of *Aedes albopictus* for each zone. For a better view of the results, three histograms had been developed which coding with A, B, and C representing the density of eggs produced by mosquitoes, developmental time and survival of the mosquitoes respectively across twenty localities in central zones of Shah Alam. Then, a simple

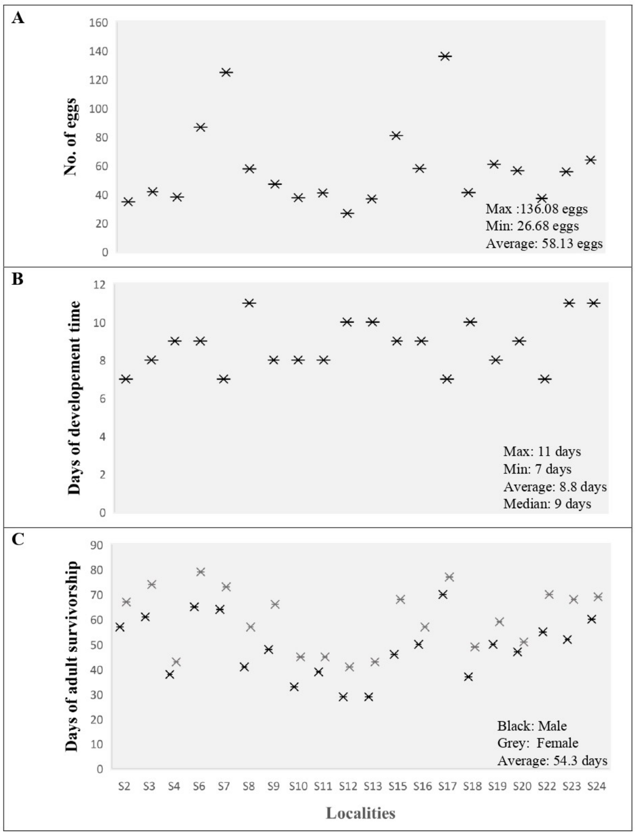


Figure 2: Summary of the demographic parameter of Aedes albopictus for twenty seksyen in the central zone of Shah Alam. **(A)** No of eggs produced per female; **(B)** Developmental time (days), and **(C)** Survivorship of an adult (days).

linear regression was used to estimate the general pattern of *Aedes albopictus* in response to a particular zone.

Fig. 2 shows the summary of the demographic parameter of *Aedes albopictus* for each zone during the study period for each parameter separately. Fig. 2A presents the schematic diagram of the egg produced by female mosquitoes. The maximum value for the density of eggs was recorded in section 17 (136.08 eggs), followed by section 7 (124.8 eggs), and then section 6 (86.64 eggs). Fig. 2B presenting the total development time (days), the shortest period was recorded in four sections (Section 2, 7, 17, and 22) for 7 days, followed by another four sections (section 3, 9, 11, and 19) in 8 days. In terms of the survivorship of adult *Aedes albopictus*, it shows the longest period was observed in section 17 (73.5 days), followed by section 6 (72 days), and then section 7 (68.5 days) (Fig. 2C).

There is a clearly defined threshold (black line) observations based upon the aggregate number of high thresholds in localities for all variables. Each black line represents fit to each leave-one-out cross validation sample. The observation on the trends of *Aedes albopictus* showed a positive relationship in egg productivity ($y = 0.3389x + 53.744$); development time ($y = 0.0643x + 7.9669$) and survivorship ($y = 0.0388x + 53.797$), which indicate that the biological character of *Aedes albopictus* for all localities are equally same in the central zone of Shah Alam.

Further information on the population parameter of *Aedes albopictus* for the adult stage was also been analyzed. Fig. 3 shows the spatial-temporal pattern of biology and demographic parameters of adult *Aedes albopictus* based on; (A) eggs density produced by female mosquitoes, (B) developmental time and (C) survivorship of an adult in each locality in the central zone of Shah Alam. Based on the outcome from Figure 3A, eighteen localities (78.2%) in the central zone show a developmental time between 6 to 9 days. In term of adult longevity, eleven localities (47.8%) was observed to have longer longevity which the adult can survive more than 54 days. The fecundity, gonotrophic cycle, and generation produced are also observed in order to determine the biology and demographic parameters of DF. Section 7 recorded has the highest fecundity with the total eggs of 3120 eggs and seven gonotrophic cycles until the adult mosquito died. The locality with the lowest fecundity is Section 12 with the total number of 667 eggs and only 6 gonotrophic cycles.

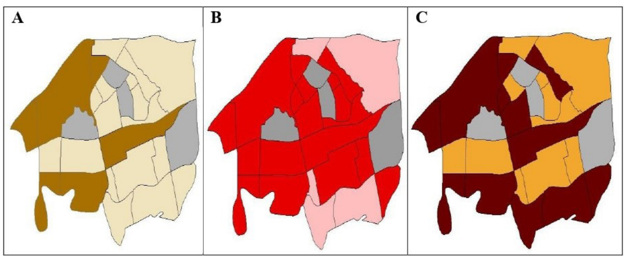


Figure 3: Population parameter of Aedes albopictus (adult stages); (A) Eggs density produced by Aedes albopictus, **(B)** Developmental time and **(C)** Survivorship of an adult. Note: The developmental time: Dark red (6 to 9 days); light red (10 to 12 days). Adult survivorship: Dark orange (>54 days); light orange (<54 days). Eggs density produces by Aedes albopictus: Dark brown (<77.4 eggs per ♀) and light brown (>77.4 eggs per ♀).

The biological data of Aedes of central zones of Shah Alam was further investigated by assessing the gonotrophic cycle in terms of total eggs produced for each twenty spot areas. Gonotrophic cycle is the period of the cycles from the blood supply to the oviposition over time. Fig. 4 shows the schematic representation of the gonotrophic cycle of in different localities in the central zone of Shah Alam. It showed that section 3, 6 and 15 were recorded nine gonotrophic cycles with total no of eggs produced are 1043, 2166 and 2022 respectively. The highest no of eggs produced in one gonotrophic cycle was observed in section 17 (G2: 1250 eggs) followed by section 7 (G2: 1050 eggs). Other localities recorded less than thousand eggs for each cycle (< 500 eggs per GC).

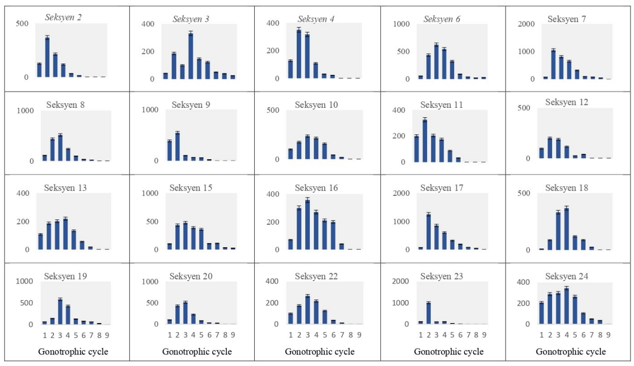


Figure 4: Schematic representation of the gonotrophic cycle (total eggs produced) of Aedes albopictus in twenty localities in central zones of Shah Alam.

The analysis of the gonotrophic parameter were analysed according to the total numbers of eggs produced. The high no of eggs produced was reported in Seksyen 17 (3402 eggs). In summary, the total eggs per gonotrophic cycle was significantly higher in all localities and the densities of total number of eggs was in order from greatest to least were Seksyen 7 (3120 eggs) > Seksyen 6 (2166 eggs) > Seksyen 15 (2022eggs) > Seksyen 24 (1595 eggs) > Seksyen 19 (1522 eggs) > Seksyen 16 (1446 eggs) > Seksyen 8 (1441 eggs) > Seksyen 20 (1407 eggs) > Seksyen 23 (1390 eggs) > Seksyen 9 (1178 eggs) > Seksyen 3 (1043 eggs) > Seksyen 18 (1030 eggs) > Seksyen 11 (1023 eggs) > Seksyen 4 (953 eggs) > Seksyen 10 (940 eggs) > Seksyen 22 (930 eggs) > Seksyen 13 (921 eggs) > Seksyen 2 (870 eggs) > Seksyen 12 (667 eggs).

The comparison of the population demographic parameters of *Aedes albopictus* in high and low incidence rate areas

The incidence rate (IR) was used to compare the biological parameters of *Aedes albopictus* in high and low risk localities in the central zone of Shah Alam. This IR data for residential area was categorized into two groups; namely (i) high IR (>20 cases in 1000 population) and (ii) low IR (<20 cases in 1000 population). Table II shows the descriptive analysis on the IR based on the DF cases in year 2017 where, eleven section (n=11) were categorized into high IR and nine sections (n=9) were categorized into low IR areas.

TABLE II: Summary of the profile of the incidence rate in the central zone of Shah Alam

Section	No. dengue cases	Population	IR	Categorized of IR
2	51	3,000	17.00	<20
3	13	1,525	8.52	<20
4	29	2,510	11.15	<20
6	85	3,490	24.35	>20
7	950	37,415	25.39	>20
8	145	9,528	15.22	<20
9	110	10,455	10.52	<20
10	40	1,929	20.73	>20
11	69	3,768	18.31	<20
12	4	484	8.26	<20
13	382	9,789	39.02	>20
15	237	5,436	43.60	>20
16	120	6,479	18.52	<20
17	271	8403	32.25	>20
18	132	10,320	12.79	<20
19	201	10,900	18.44	<20
20	187	11,165	16.75	<20
22	64	2,764	23.15	>20
23	49	2,101	23.32	>20
24	336	15,910	21.12	>20

Note: IR >20 is high and IR <20 is low (*) The dengue cases data are retrieved from Ministry of Health in 2017

An independent t-test analysis was used to determine if there is a significant difference between the biological parameters of *Aedes albopictus* in high and low IR areas. Generally, most of the data that was recorded greater in the high IR areas compare to the low IR areas except for the immature development time (larvae to pupae and the total development time) (Table III). There was a significant difference (p=0.03) terms of eggs produced by the female in high IR (M=73.27 ±15.69 eggs) and in IR rate (M=45.75 ±11.43 eggs). However, there was not a significant difference for the hatchability (p=0.80) and mortality (p=0.14) in the high IR (M=83.49 ±17.00%; M=31.42 ±7.40%) and in the low IR (M=82.39 ±17.94 days; M=25.51 ±7.63%) respectively. The total immature development (days) period was absorbed shorter as compared the high IR (M=8.78 ±1.35 days) to low IR (M=8.82 ±1.42 days). Finally, the average of the adult survivorships in high IR in general (M=59.22 ±8.45 days) was observed longer as compared to low IR (M=50.27 ±10.43 days. Specifically, the same trends were also observed for male (high IR; M=52.67 ±7.44 days; and low IR; M=45.18 ±8.69 days) and female (high IR; M=65.78 ±9.64 days and low IR M=45.18 ±8.69 days). The result from the analysis indicated that there is no different on the development time (days) and adult survivorship of *Aedes albopictus* in high and low IR areas (p= 0.95 , p=0.10) respectively.

DISCUSSION

The ecology of Aedes mosquito and the mechanism of dengue transmission is important to recognize the vulnerable time in the life cycle of mosquitos. The demographic parameters study of *Aedes albopictus* is still limited compared to Aedes aegypti especially in Malaysia. Therefore, the establishment of the demographic parameters profile of the *Aedes albopictus* and its geographical distribution may provide a foundation for developing effective control strategy. In order to contribute to the knowledge, this study was

TABLE III: Life table attributes of Aedes albopictus mosquito from Shah Alam based on the incidence rate (IR) of dengue from 2013 to 2017

Attribute	High IR		Low IR		*p-value
	Mean ± SD	Range	Mean ± SD	Range	
i- Female fecundity (egg productivity)	73.27±15.69	36.84-136.08	45.75±11.43	26.68-60.88	0.03
ii- Egg development (%)					
Hatchability	83.49±17.00	68.52-94.01	82.39±17.94	66.24-98.04	0.80
Mortality	31.42±7.40	18.71-42.17	25.51±7.63	10.28-39	0.14
iii- Immature development (days)					
Eggs to Larvae	5.11±1.42	4-7	4.64±1.53	4-6	0.20
Larvae to Pupae	1.33±2.15	1-2	1.73±1.98	1-2	0.09
Total developmental time	8.78±1.35	7-11	8.82±1.42	7-11	0.95
v- Adult survivorships (days)					
Male ♂	52.67±7.44	29-70	45.18±8.69	29-61	0.18
Female ♀	65.78±9.64	43-79	55.36±12.23	41-74	0.06
Average	59.22±8.45	36-73.5	50.27±10.43	35-67.5	0.10

Note: *p*-value generated from *t-test* analysis designed to investigate the demographic parameters of *Aedes albopictus* in selected high risk of DF cases in Selangor. The assessment was attempted by examine the fecundity, hatchability and mortality rate of the eggs immature development period and survival of *Aedes albopictus* which conducted under the controlled environment which mimicking outside environment (29 ±3°C with 75 ±10% humidity) in order to get similar condition for development to prevent any bias.

In general, the mean number of eggs produced by the *Aedes albopictus* female (Shah Alam strain) is in the range (58 eggs/female) recorded for other local strain from different locality (Kuala Lumpur strain: 77 eggs/ female; Penang strain : 77.4 eggs /female) (27,28). Investigation on the number of eggs produced by *Aedes albopictus* in high and low IR found that female *Aedes albopictus* in high IR areas produced higher number of eggs than low IR areas. There were 73.27 and 45.75 eggs representing the mean number of eggs produced by the female *Aedes albopictus* from high and low IR areas respectively. Similar pattern of eggs production was also reported by (29) based on the date captured, the study revealed that the production of eggs was influenced by many factors including mosquito body size, quantity and quality of mating process, blood feeding and preferred location of laying eggs (30). There is a significance different observed in the total no of eggs produced of both high and low IR areas (p=0.03). This finding has an important epidemiological implication favouring high population size of *Aedes albopictus* in high IR areas. Apart from that, the duration of immature stage is another factor affecting the life pattern of mosquitoes. Shorter development that was observed among *Aedes albopictus* is high IR areas (8.78 days) as compared to low IR areas (8.82 days) before it emerged as adult. Even though they are no significant (p=0.95). it has a potential in dengue control program as they provide information on the number of gravid females. Shorter duration of immature stage into adult might contribute to the increase in population size. The investigate is done by Awang (25) reported that shorter development time day immature stage had influenced the wing size, and longevity of adult mosquito.

The adult survivorship is central to demographic parameters of any population. The need to estimate the adult survival can give the preliminary and basic indication of the status of the population. In this study, both high and low IR areas mosquitos were recorded under laboratory condition mimicking outside environment in order to get similar condition for development of *Aedes albopictus*. Overall, the mean survival values for the *Aedes albopictus* (Shah Alam strain) showed that males lived (48 days) shorter than females (60 days) mosquitoes. The present study also identify that survival of *Aedes albopictus* in high IR areas (59 days) is longer than low IR areas (50 days). Study done by Nur Aida et al. (28) supported that a critical analysis of mosquito population is crucial in epidemiological study. Thus, the input from this study can help in assessing the impact of local vector control measure.

Studies have done to study the Aedes parameter swith various factor influencing their survival, fecundity, and mortality (4, 6, 27, 28, 31, 32, 33, 34). The demographic parameters study of *Aedes albopictus* is still limited compared to Aedes aegypti, especially in Malaysia. Aedes life table model is important in providing the based information on its different development aspects under specific understanding life strategies of each species including life span, reproductive potential and stage specific survivorship to proliferate and its vectorial capacity for disease transmission. Most of the studies have used the laboratory data collection under controlled conditions, known to provide the maximal growth potential that may not occur in nature. To address this issue, the entomologists have attempted utilizing the field data for the development of the life table of Aedes mosquitoes. In order to highlight the biology of *Aedes albopictus* such as development, survival, mortality, and fecundity, the experiment had been conducted in order to determine some of the demographic parameters of selected strains of the *Aedes albopictus* under laboratory condition.

CONCLUSION

In conclusion, the key of transmission chain breakage is about to control the mosquito vectors population. The abundance of the Aedes mosquito is a challenge in tracking the dengue cases as the increasing population is affected by the multiple factors. Vector's life cycle including its survival has been suggested as to influence dengue transmission in recent years. Therefore, the understanding on Aedes characteristic might provide knowledge about the dengue fever epidemiology thus; allow to forecast the outbreak in current episystem.

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