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RESEARCH ARTICLE

Effect of temperature on the embryronic development of *Aedes albopictus* (Diptera: Culicidae)

Mohd Fadzil Awang [a], Alicia Mindy Anak Rogie [a], Hazilia Hussain [a], Nazri Che Dom [a, b,*]

[a] *Centre of Environmental Health and Safety, Faculty of Health Science, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia*
[b] *Integrated Mosquito Research Group (I-MeRGe), Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia*

* *Corresponding author: nazricd@salam.uitm.edu.my*

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Abstract

Aedes albopictus (Skuse) is known to be an invasive species and it becomes the main concern on the changes of climate that will cause the expansion and spreading of the mosquito populations. Therefore, this study was designed to determine the development cycles of *Ae. albopictus* larval at different water temperatures and water holding containers in a controlled environment by using temperature regulated water bath to cover a range of temperature from 25ºC to 35ºC. The experiment was completely randomized in factorial 3 x 5 design (15 treatments), three populations (Selangor-strain, Malacca-strain and Sabah-strain) and three replications. The analysis was based on the observation of the immature stages development (days) in response to different water temperature regimes and types of water containers. The development between three different strains showed no significant difference (*p-value*: 0.594), however the effect of water temperature on local strains showed that the temperature might shorten the development period of *Ae. albopictus*. This study concluded that the information on the larval development could act as a preliminary warning in recognizing the pattern of outcome due to seasonal changes throughout the year in Malaysia.

Keywords: *Ae. albopictus,* development, water temperature, Malaysia

INTRODUCTION

Ae. albopictus is known to be poikilotherm organism which can adapt very well to various ranges of external temperatures (Hawley, 1988; Wu *et al*., 2013). Due to urbanization, the environmental changes are occurred and caused a significant impact on the ecology of the *Ae. albopictus* mosquito (Madzlan *et al*., 2016). In nature, it has a habit of exophagic and exophilic, which it prefers to feed and rest outdoor, making it preferably breeds in an outdoor environment (Petrić *et al*., 2014; Dom *et al*., 2013; Madzlan et al., 2018). The availability of different water containers due to improper sanitation facilities, the rapid growth of urbanization and presence of industries, affecting the intensity of transmission of dengue, as well as the abundance of *Aedes* mosquito (Nazri *et al*., 2013a; Rahman *et al*., 2017). A study conducted by Li *et al*., (2014) stated that the ecological characteristics of *Ae. albopictus* are identified to have more larval breeding sites, shorter development time at larval stage, higher emergence rate of adult mosquito, and longer lifespan in urban areas. This species also has a quick development in a combination of high oviposition and low immature loss of the species (Aida *et al*., 2011; Dom *et al*., 2016; Sairi *et al*., 2016). Temperature plays a crucial part by affecting all the important processess such as the larval development and survivorship (Dom *et al*., 2013). Water temperature is influenced by various parameters such as local climate, water depth and movement, habitat size, land cover types and canopy, presence of vegetation and soil properties (Paaijmans *et al*., 2008)

Study on mosquito ecology is important in order to gain information on population biology and dynamic, vectorial capacity and impact on the risk of disease outbreak. This study was aimed to determine the effect of different temperatures on the development of *Ae. albopictus*. The results obtained from this study were important to understand the life table and characteristics of *Ae. albopictus* including its life span, development rate, mortality rate, and survivorships in response to biotic factor and abiotic factor. In laboratory setting (with controlled temperature and relative humidity), the information gained is vital to determine its ability to grow in absence of environmental pressures such as predators. With the entire information gathered, it can be used to predict the development rate of Aedes mosquito in one locality especially in hotspot area for dengue cases. The outcome of this study might provide a base-line information that was essential for wider studies towards better understanding of population dynamics of *Ae. albopictus* under local conditions. This input was also critical for designing appropriate strategies to control the vector population and predicting the disease transmission in order to minimize the risk of outbreak among Malaysia population.

EXPERIMENTAL

Study design

The experimental study was designed in order to conduct the study on the *Aedes* mosquito under a controlled condition. This study involved three (3) phases. Phase 1 was carried out from the field setting. Phase 1 involved the process of collection of field strains and different types of water containers. The eggs samples were collected from four different strains; Selangor, Melaka, Sabah and lab strains. Lab strains were acted as the control strains. Different types of surface water containers; tires, plastic, coconut shell and glass bottles were also collected. The colonization of mosquito was conducted in Phase 2 in which the eggs were hatched in the laboratory for the mass rearing of *Aedes albopictus* to obtain the F_1 generation of eggs.

The F_1 eggs were used in Phase 3 for the experimental study on the development stages of immature stages of *Aedes albopictus*.

In Phase 3, analysis on the development stages of immature stages in five different temperatures was carried out by identifying the optimal temperature for pupal formation. The larval development in different water temperatures was observed by recording the numbers of larva and pupae emergences and the death of the larval daily. Next, the larval development in response to different water temperatures and different types of surface water containers would be monitored in another test. For both tests, 30 first instars with 3 replicates were placed in five different temperatures (25^0C, 27^0C, 30^0C, 33^0C and 35^0 C).

Collection of samples

In this study, (F1) eggs strains of *Aedes albopictus* were obtained from the (F0) strains that collected from 4 different locations to conduct this experimental study. The samples involved the four different strains that were obtained from Heath Districts Offices from three states, which were Selangor, Melaka and Sabah, and one lab strains was obtained from insectorium at UiTM Puncak Alam Campus. The strains were collected from different areas to represent some of the states in Malaysia. As for the lab strains, it was used as the control strains that not exposed to the outside environment. All field strains (Fo) eggs collected were hatched and reared under controlled condition to produce (F1) eggs for the experimental study.

Colonization of *Ae. albopictus*

In the process of mosquito rearing to produce F1 generation, the F0 were (field strains) undergone mass rearing under controlled conditions in the insectarium that maintained at 28 ± 20 C with 75% to 85% relative humidity. Each paddle containing eggs was left to be submerged in a large plastic container containing seasoned water tap. Paddles from each strains were placed in different basins. Each basin must be labeled according to their strains. The eggs were monitored daily. The eggs were left for few days until the first emergence of 1st instar larvae. The larvae were fed daily with the powdered chicken liver for their growth. As from the time of 1st instar emergence, the whole development cycles were observed during the entire of the monitoring period until the pupal stages. Once the larvae were reached pupae stage, the pupae were separated from the others. The pupae were then transferred into containers inside a cage with measurement of 23 cm x 23 cm x 23 cm, covered with white polyester tulle. After few days, the pupae would emerge into an adult mosquito.

The cage would be introduced with moist cotton that soaked in 10% sugar solution and a vitamin B complex solution to provide adult feeding. The solution would be provided inside an universal bottle with a cotton that wrapped around a wooden tongue dispenser. After mating process, the female mosquito would be laying eggs. For ensuring the egg development in the female mosquito, the female was fed with an artificial blood for four hours. The cage was covered with white tulle and contained a filter paper on a petri dish with a distilled water. The filter paper was observed daily to ensure the availability of eggs on the filter paper. After the female adult laying eggs, the eggs on the filter paper would be collected and dried. After drying process, the filter paper containing eggs was collected for the next experiment.

Experimental study on the development of *Ae. albopictus*

The effect of temperature on the development of embryonic stages in five different temperatures (25^0 C, 27^0 C, 30^0 C, 33^0 C and 35^0 C) was carried out. A total of thirty (n=30) first instars with three replicates were exposed with five different temperatures. In order to determine the impact of water temperature on the development of *Ae. albopictus* larvae at different strains, two aspects were monitored which were (i) commencement of pupation (CP_1) (first day of pupation) and (ii) cessation period (CP_2) (Final day pupation). The data on the developmental rate of embryonic in response to different water temperature regimes was analysed using the mean of larval development cycles, single factor ANOVA (One-Way ANOVA) and regression through MS - EXCEL 2010 and SPSS (Statistical Package of Social Science) Software.

RESULTS AND DISCUSSION

Developmental rate of larval to pupal stage in different water temperatures

The development rate of larval of *Aedes albopictus* from different strains was determined by observing the day of emergence of the pupae in response to five different water temperatures. Three field strains (Selangor-strain, Melaka-strain and Sabah-strain) were exposed to five different water regimes and the development days were recorded daily. In order to determine the impact of water temperatures on the development of *Aedes albopictus's* larvae at different strains, two aspects of larval development period were monitored which were the (i) commencement of pupation (CP_1) (first day of pupation) started and the (ii) cessation period (CP_2) (final day of pupation).

Table 1 Embryonic development (days ± SE) of *Ae. albopictus* in three different strains exposed with five different water temperatures.

A. Days of commencement of pupation (CP1)

Strain	Days of commencement of pupation (CP_1)					
	25	27	30	33	35	Mean
Mlk	7.0 ±0.00	5.0 ±0.00	5.0 ±0.00	4.0 ±0.00	3.3 ±0.33	4.86 ±1.39
Sgor	8.0 ±0.00	5.7 ±0.30	5.6 ±0.30	5.0 ±0.57	4.7 ±0.57	5.58 ±1.29
Sbh	8.0 ±0.00	6.0 ±0.00	5.0 ±0.00	4.3 ±0.33	4.0 ±0.00	5.46 ±1.61

B. Days of cessation of pupation (CP_2)

Strain	Days of cessation of pupation (CP_2)					
	25	27	30	33	35	Mean
Mlk	11.3 ±0.33	8.3 ±0.33	8.0 ±0.00	7.3 ±0.33	6.7 ±0.57	8.26 ±1.65
Sgor	11.0 ±0.00	9.0 ±0.00	8.0 ±0.00	7.7 ±0.30	7.0 ±0.00	8.54 ±1.55
Sbh	12.0 ±0.00	9.0 ±0.00	8.0 ±0.00	7.3 ±0.33	7.0 ±0.00	8.66 ±2.02

Based on the commencement period of larvae, the highest water temperature (35^0C) showed the shortest commencement period of larval development (Melaka-strain: 3.3±0.33 days; Sabah-strain: 4.0±0.00 days and Selangor-strain: 4.7±0.57 days). In contrary, the lowest water temperature (25^0C) showed the longest commencement period for larvae development (Mlk-strain: 7.0±0.00 days; Sgor-strain: 8.0±0.00 days; Sbh-strain: 8.0±0.00 days). In terms of the cessation period, the larval development of larvae was fastest during the exposures to the highest water temperature at 35^0C (Mlk-strain: 6.7±0.57 days; Sbh-strain: 6.0±0.00 days; Sgor-strain: 7.0±0.00 days). However, the lowest water temperature was recorded at temperature 25^0C which showed the longest cessation period of larvae

development (Sgor-strain: 11.0±0.00 days; Mlk-strain: 11.3±0.33 days; Sbh-strain: 12.0±0.00 days). Thus, as the temperature was rised, the development days were shortened and faster (Fig. 1A).

A One-Way between group analysis of variance (ANOVA) was used to investigate the impact of different water temperatures in response to the larval development days with different types of strains. Inspection of the skewness, kurtosis and Shapiro-Wilk statistics indicated that the assumption of normality was supported for the relationship between different larval strains and the development days of the immatures. Levene's statistic was shown not significant, F (2, 12) =0.23, p = 0.799, and thus the assumption of homogeneity of variance was not violated.

ANOVA was not statistically significant since it indicated that the type of strains did not influence the larvae development days in different water temperatures, F (2, 12) = 0.544, p = 0.594, n^2 = 0.083. Post hoc analysis with Tukey's HSD (using an α of .05) revealed that there was no significance between all different strains, Melaka-strain (M= 4.86, SD= 1.39), Selangor-strain (M=5.58, SD= 1.29), and Sabah-Strain (M = 5.46, SD = 1.61). Therefore, the largest effect sizes for these three comparison strains were d =0.23, 0.38 and 0.59, respectively.

In conclusion, the average of the embryonic development between three different strains during the commencement period showed that there was no significant difference (*p-value* = 0.594). The overall means showed that Melaka-strain has the fastest embryonic development duration (CP_1: 4.86 ± 1.39 days; CP_2: 8.26 ± 1.39 days) and followed by Sabah-strain (5.46 ± 1.61 days) and Selangor-strain (5.58 ± 1.29 days). Fig. 1 shows the average embryonic development of *Aedes albopictus* during the commencement period.

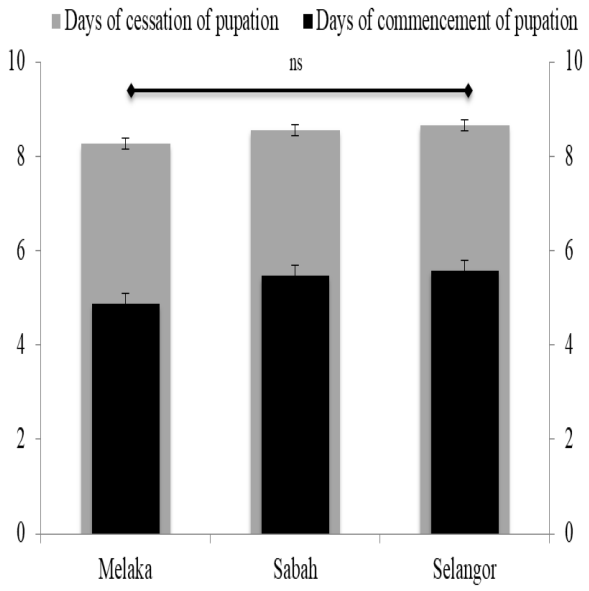

Fig. 1 Profile of embryonic development of local *Ae. albopictus*. Data showed an average of embryonic development (days ± SE) of *Ae. albopictus* in three different strains exposed with five different water temperatures. (ns Not significant (ANOVA; p ≤ 0.05).

The effect of water temperature on the development of local strain of *Ae. albopictus* at immature stages was shown in Fig. 2. Melaka-strain's larval were developed slightly faster in both commencement and cessation period of pupation, followed by the Selangor-strain and Sabah-strain. As the water temperatures were reached the optimum temperature of 27ºC to 30ºC, the development was started to stabilize. However, as the water temperatures were continued to rise, the larval developmental was accelerated constantly for all strains. Generally, the trend showed a stable accelerating trend for both aspects: Commencement and cessation of the pupation indicated that an increasing temperature might shorten the development period of *Ae. albopictus*.

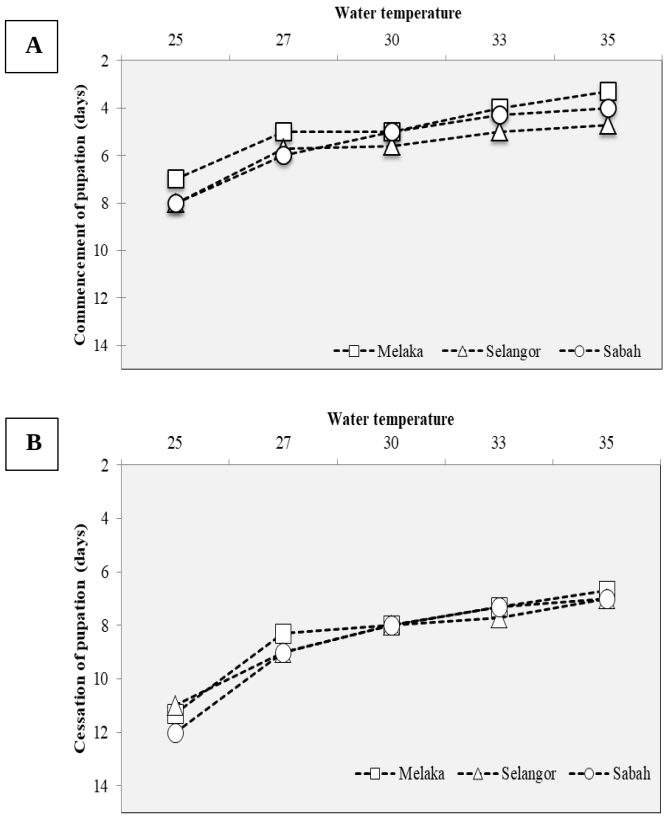

Fig. 2 Effect of water temperature on the development (days) of local *Ae. albopictus* strain (Melaka-strain, Selangor-strain, Sabah-strain) based on (A) commencement of pupation and (B) cessation of pupation.

Development cycles of immature stages in different water temperatures and water containers

A One-Way between group analysis of variance (ANOVA) was used to investigate the impact of water temperatures on the larvae development days by different types of containers based on the effective cumulative temperatures (days degree). Inspection of the skewness, kurtosis and Shapiro-Wilk statistics indicated that the assumption of normality was supported for the relationship between types of strains and larvae development days and normally distributed. Levene's statistic was not significant, F (3, 8) =1.36, p = .322 and thus the assumption of homogeneity of variance was not violated.

The ANOVA was statistically significant differ, indicating that the types of containers would influence the larvae development days in different water temperatures, F (3, 8) = 2.502, p = 0.013, n2 = 0.484. Post hoc analysis with Tukey's HSD (using an α of 0.5) revealed that coconut shell (M = 47.67, SD = 26.16) has significantly shortest effective cumulative temperatures followed by tire (M = 54, SD =29.46), glass (M = 102.00, SD = 42.15) and plastic (M =126.33, SD = 59.91). Therefore, the largest effect sizes for these four comparison containers were d = 1.64, 1.51, 0.99, 0.51 respectively.

Among all containers, the coconut husk was found to be the container with the shortest development period (6.06 days) and followed by tires (6.74 days), glass containers (7.48 days) and plastic containers (8.48 days) such as illustrated in Fig.3. Thus, water temperatures and type of containers would influence the development of *Aedes albopictus*'s larva.

Water temperatures are one of the crucial determinants for the mosquitoes to complete their developmental stages from the immature to adult (Couret *et al*., 2014). The findings showed that the selected water temperatures in this study were able in completing the development cycles of the *Ae. albopictus*. However, the most optimum water temperature was recorded at temperature 30ºC. As the water temperature was increased, the developmental periods of the embryonic were shorter and faster. The lower temperature showed that the developmental period was much longer and slower. Delatte *et al*. (2009) highlighted that the minimum threshold of embryonic

stages for their development was found at temperature of 10.4ºC and achieved an optimum growth at temperatures of 29.7ºC. The shortest period of embryonic development was also found at temperature 30ºC and the optimum intrinsic rate of growth was between 25ºC and 30ºC which was concurrent with the finding by Farjana *et al*. (2012) which found that among four water temperatures; 20°C, 25°C, 30°C and 35°C,the highest temperature showed the increase in the mortality rate among both species; *Ae. aegypti* and *Ae. albopictu*s. This result was also in accordance with the study conducted by Briegel *et al*. (2001) that found that the larval developmental period from the hatching to pupation stages was correlated inversely with temperature. Laura *et al*. (2007) also found that the eclosion rate would increase within the temperature ranging 20ºC to 35ºC. The maximum survival rates of immatures stages were also found in the range of temperature between 20ºC to 30ºC (Tun-Lin *et al*., 2000). Higher temperature might allow larvae to develop and grow faster to the pupae stages until become an adult (Neto *et al*., 2004).

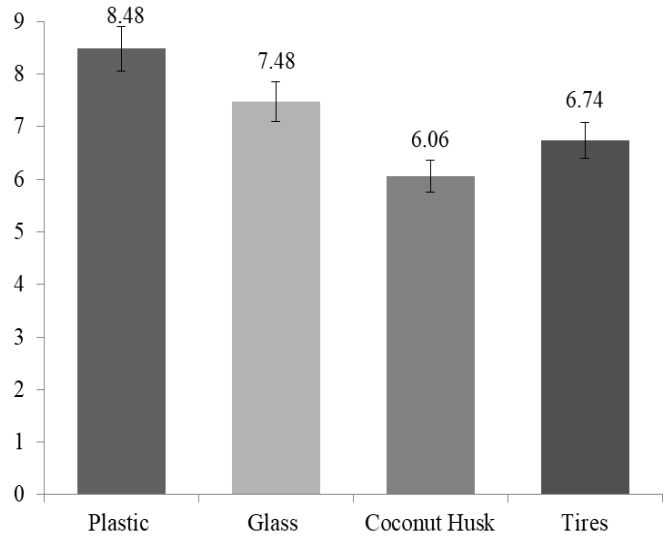

Fig 3 Average day of larval development based on different types of containers in response with different water temperatures.

Further study on the development rate of the Aedes larval within different types of container should be carried out. Since the larval stages of dengue vectors were usually inhabited containers in residential landscape especially *Ae. albopictus*. As explained by few researchers, a darker type containers were the most prefered containers for the Aedes species to breed due to the character of this container which able to hold warmer water temperature that indirectly could increase the development rate of the immature stages.

CONCLUSION

The information on the larval development days of *Ae. albopictus* in different water temperatures regimes was important in planning an effective dengue control in Malaysia. The ability of *Ae. albopictus* to survive in vary ranges of water temperatures showed that the vector could adapt very well in the changing and transforming environment throughout times.This study also found the relationship between water temperatures and the development of the *Ae. albopictus* where as the temperature was increased, the developmental period was also accelerated. Greater temperatures were influenced the adult mosquitos' production. It could also help in yielding higher and lower eclosion rates, as well as higher mortality among the species. The *Aedes* life table could also act as a preliminary warning in recognizing the pattern of outcome due to seasonal changes throughout the year in Malaysia.

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