

The Adaptation of Field Collected *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Laboratory Condition

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Abstract-*Aedes aegypti* and *Aedes albopictus* has been incriminated in dengue transmission in Malaysia, and all available measures has been taken to reduce the dengue cases and mortality. However, statistical data showed increasing trend in dengue incident. The aim of this study is to evaluate the adaptability F1 offspring of field collected *Aedes* mosquitoes in a new environment (laboratory). Biological parameter of *Aedes* mosquito, such as the larval growth, survival and fecundity were scrutinized under laboratory condition. Data obtained from this study showed that both *Aedes* species were highly adaptable to laboratory condition. The finding of this study is essential for vector control.

Keywords- *Aedes Albopictus*; *Aedes Aegypti*; Larval Growth; Survival; Fecundity

I. INTRODUCTION

The transmission of dengue fever in Malaysia is caused by two *Aedes* mosquito: *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) [1]. *Aedes albopictus* is a semidomestic mosquito, while *Aedes aegypti* is a domestic mosquito in urban area [2]. These species are effective vectors of dengue because of their ability to breed in artificial containers in and around the house, close to human being [3].

Aedes aegypti is one of the most efficient mosquito vectors for arboviruses, because it is highly antropophilic and thrives in close proximity to humans [4] and prefer to live indoors [4, 5]. It is commonly in urban areas especially in the most densely populated districts [6].

Aedes albopictus occurs throughout the geographical region consisting of the countries of South East Asia, and it has been found in all types of country, urban, suburban, rural [7], farmland or deep forest [8].

The purpose of this study is to determine the adaptability F1 offspring of field collected *Aedes* mosquitoes by studying their bionomics. It is hoped that biology of field strain *Aedes* could shed some information which is vital for vector control. This study will investigate the biological parameters, such as egg hatchability, growth development, fecundity and survival F1 offspring of field collected *Aedes aegypti* and *Aedes albopictus* reared under laboratory condition.

II. MATERIAL AND METHOD

A. Breeding of *Aedes* Mosquito in the Laboratory

The method used was adapted from Adanan *et al.* [9] with modification. Field strain of *Aedes* species were collected using the ovitrap method (*Aedes aegypti*'s eggs collected from Bagan Dalam, *Aedes albopictus*'s eggs collected from Paya Terubong) and hatched under laboratory condition (temperature: 26 ± 4 °C and relative humidity: $60 \pm 10\%$). Emerging larvae were fed with larval food (dog biscuit, sun-dried beef liver, yeast, milk powder in a ratio of 2:1:1:1, ground into a powder). Pupae were separated from the larvae using a pipette into paper cups (size: diameter = 5 cm, height = 12 cm) and transferred to into separate cage (size: 30 cm x 30 cm) according to their species.

Emerging adult mosquitoes were fed with sucrose (10% sugar solution and vitamin B complex). On the fifth day after emerging, female *Aedes* mosquitoes were given blood meal by placing mice confined in a small screen cage (for approximately 3 hours).

Twenty-four hours after blood feeding an oviposition substrate for egg collection were placed in the middle of the cage. Oviposition substrate was made of filter paper (Whatman No. 1 filter paper; 24 cm diameter), which were folded into a cone shape and placed in a petri dish. One third of petri dish was filled with dechlorinated water to allow the filter paper to remain moist.

Eggs collected from these mosquitoes were identified as F1 generation and were air-dried before placed in air-tight container at room temperature until further study.

B. The Hatching Rate of Field Strain *Aedes Aegypti* and *Aedes Albopictus*

Gravid *Ae. aegypti* and *Ae. albopictus* female mosquitoes (n=150 females from each *Aedes* species) were individually transferred to paper cups containing filter paper for oviposition. Filter papers were removed from the paper cups and air-dried for 24 hours, only filter papers with more than 70 eggs were used in this study. Dried filter papers were divided into 9 groups and labelled according to designated time for hatching.

Filter papers from each group were submerged into dechlorinated water according to the designated time as described below:

Group 1: One week after egg collection;

Group 2: Two weeks after egg collection;

Group 3: Three weeks after egg collection;

Group 4: One month after egg collection;

Group 5: Two months after egg collection;

Group 6: Three months after egg collection;

Group 7: Four months after egg collection;

Group 8: Five months after egg collection;

Group 9: Six months after egg collection.

At the designated time, the filter papers were placed in the plastic trays (size: height = 5 cm, length =17 cm, width=12 cm) and flooded with dechlorinated water (200 ml). No food was added during the experiments. All larvae emerged was recorded every day until no hatching observed.

C. The Development Rate of Field Strain *Aedes Aegypti* and *Aedes Albopictus*

Aedes eggs were vacuum-hatched to ensure the uniform age of the larvae. One hundred first instar larvae were randomly transferred into 100 individual vials (diameter = 2.5 cm and height = 7.5 cm) filled with dechlorinated water (water volume: 5 ml water on day 1 and 10 ml of water from day 2 onwards). About 50 mg of larval food (dog biscuit, sun-dried beef liver, yeast, milk powder in a ration of 2:1:1:1, ground into a powder) was diluted with 5 ml of dechlorinated water. On day one, only one drop of larval food was dropped into each vial and the drops were increased to two or three drops a day after day two. Water was changed everyday to remove scum. The development rate of the larvae and pupae were monitored at a fixed time and recorded.

D. Survival of Field Strain *Aedes Aegypti* and *Aedes Albopictus* Fed on Different Diets

Field strain of *Aedes* mosquitoes was separated into three groups of adult mosquitoes. The first group consists of male *Aedes* mosquitoes fed with 10% sucrose, the second group consists of female *Aedes* mosquitoes fed with 10% sucrose, and the third group consists of female *Aedes* mosquitoes fed with blood meal and 10% sucrose. Each group consists of 50 adults kept in individual paper cup. Each cup was covered with a piece of muslin cloth with a slit in the middle. Each cup was monitored daily and mortality was recorded.

E. The Length of Gonotrophic Cycle and the Number of Eggs Laid by Field Strain *Aedes Aegypti* and *Aedes Albopictus*

Two sets of 30 paper cups were prepared with moist cotton wool and filter paper for oviposition. The top of the cups were covered with muslin cloth. A day after blood meal was given, 30 gravid female *Aedes sp.* were transferred individually into the first 30 cups. Cotton balls soaked with sucrose were left on the top of the cups as food source. The next day (day 2), the females from the first set were transferred to the second set of cups. Filter paper from the first set of cups were removed and replaced with new set of filter paper. Each set of filter paper removed daily were air-dried before the number of eggs recorded. This experiment was continued until there were no eggs deposited.

F. Data Analysis

Data was expressed as mean $\pm$ S.E (Standard Error). Data obtained were analysed for normality test. As the data was found not normally distributed, a non-parametric test (Mann Whitney U test) was used to analyse the data (SPSS analysis version 11.0, all statistical test was considered significant at $p = 0.05$).

III. RESULTS AND DISCUSSION

A. The Hatching Rate of Field Strain *Aedes Aegypti* and *Aedes Albopictus*

Under laboratory condition, eggs produced by field strain *Ae. aegypti* and *Ae. albopictus* (F1 generation) showed a very low hatching rate. Highest hatching rate produced by *Ae. aegypti* eggs was 49.1% for one month old eggs, while *Ae. albopictus* eggs achieved highest hatching rate for 3 weeks old eggs at 42.4% (in Table 1, Mann Whitney U test at $p = 0.05$). The hatching activity starts to decrease after the peak hatching rate for both *Aedes* eggs. The hatching activities has stopped for 5 or 6 months old *Ae. albopictus*'s eggs. Similarly no hatching was observed for 6 months old *Ae. aegypti*'s eggs.

TABLE 1 THE HATCHING RATE OF AEDES AEGYPTI AND AEDES ALBOPICTUS

Egg submerged	N	Hatching rate (mean $\pm$ S.E) (%)	
		<i>Aedes aegypti</i>	<i>Aedes albopictus</i>
after kept			
<i>albopictus</i>			
Week 1	3	33.4 $\pm$ 3.51a (107.7 $\pm$ 5.55)	25.0 $\pm$ 3.14a (101.7 $\pm$ 10.9)
Week 2	3	39.5 $\pm$ 5.25a (101.3 $\pm$ 13.7)	33.4 $\pm$ 7.26a (95.0 $\pm$ 6.00)
Week 3	3	31.1 $\pm$ 1.34b (98.0 $\pm$ 11.2)	42.4 $\pm$ 3.45a (76.7 $\pm$ 1.67)
Month 1	3	49.1 $\pm$ 5.56a (96.7 $\pm$ 6.67)	38.0 $\pm$ 1.25b (84.0 $\pm$ 1.15)
Month 2	3	43.9 $\pm$ 5.35a (120.7 $\pm$ 8.11)	31.1 $\pm$ 1.63b (106.0 $\pm$ 4.16)
Month 3	3	26.7 $\pm$ 2.25a (108.0 $\pm$ 5.03)	19.1 $\pm$ 2.13b (88.3 $\pm$ 0.67)
Month 4	3	18.1 $\pm$ 4.95a (124.0 $\pm$ 16.5)	11.3 $\pm$ 1.66a (94.0 $\pm$ 12.1)
Month 5	3	9.74 $\pm$ 1.52a (120.7 $\pm$ 12.8)	0b (87.0 $\pm$ 3.51)
Month 6	3	0 (107.3 $\pm$ 10.8)	0 (86.3 $\pm$ 2.84)

Mean number of hatching followed by the same letter within the same rows are not significantly difference ($P = 0.05$, Mann Whitney U- test).
(mean number of eggs used for each experiments $\pm$ S.E)

Laboratory studies by Macgregor [10], Shanon and Putnam [11] and Hien [12] have proved that the age of the *Aedes* eggs at the time of drying influence the eggs to withstand desiccation but the eggs will die if they become too dry when they were first laid. Eggs with fully developed embryo can withstand dry conditions for long periods of time [13]. The production of eggs that are resistant to desiccation makes *Aedes* mosquitoes and ideal colonisers of temporary collections of water [14].

B. The Development Rate of Field Strain *Aedes Aegypti* and *Aedes Albopictus*

The development rates from larval to adult stages of *Ae. aegypti* were shorter than that of *Ae. albopictus*. *Ae. aegypti* took about 6 days to reach the adult stage while *Ae. albopictus* took about 7 days respectively to reach the adult stage. The pupal stage for both *Aedes* species lasted 1 to 2 days (in Table 2, Mann Whitney U test).

TABLE 2 THE DEVELOPMENT RATE OF. FIELD STRAINS AND LABORATORY STRAINS AEDES AEGYPTI AND AEDES ALBOPICTUS

Stages	N	No. of days (mean $\pm$ S.E)	
		<i>Ae.aegypti</i>	<i>Ae. albopictus</i>
Larva	100		
First instar		1.70 $\pm$ 0.08a	1.20 $\pm$ 0.06b
Second instar		0.84 $\pm$ 0.04b	1.02 $\pm$ 0.04a
Third instar		0.86 $\pm$ 0.04b	1.12 $\pm$ 0.05a
Four instar		1.24 $\pm$ 0.07b	1.58 $\pm$ 0.06a
Pupa	100	1.37 $\pm$ 0.07b	1.90 $\pm$ 0.06a
Total development from first instar to adult stage		6.84 $\pm$ 0.30b	7.73 $\pm$ 0.24a

Mean number of days followed by the same letter within the same rows are not significantly difference ($P = 0.05$, Mann Whitney U-test).

Numerous experiments have been conducted to determine the development rate of *Ae. aegypti* and *Ae. albopictus*. In a tropical environment, immature stages of *Aedes* require about 7 days before adult emergence [15]. Vythilingam *et al.* [16] and Lee [17] found that the larval period of *Aedes sp.* was 6-8 days while pupae took about 1-2 days. Abu Hassan and Yap [18] also found that the life cycle of *Aedes* from the egg to adult stage is 6-8 days.

The duration of larval development are influenced by temperature, food supply [19-22], sex [22, 23], crowding [22], depth of water, and salinity [24].

C. Survival and Longevity of Field Strain *Aedes Aegypti* and *Aedes Albopictus* Fed on Different Diets

During the study, females of both *Aedes* mosquitoes fed with blood and sucrose averaged about 45 to 49 days, while females of both *Aedes sp.* fed with sucrose only averaged about 49 to 51 days. Male *Ae. aegypti* fed with sucrose survived 51 days while *Ae. albopictus* lived only 37 days (in Table 3). Based on the findings, female and male *Ae. aegypti* fed with sucrose live longer compared to other test groups under controlled laboratory condition.

TABLE 3 SURVIVAL AND LONGEVITY OF FIELD STRAIN AND LABORATORY STRAIN AEDES AEGYPTI AND AEDES ALBOPICTUS FED ON DIFFERENT DIETS

Treatment	N	No. of days alive (mean $\pm$ S.E)	
		<i>Ae. Aegypti</i>	<i>Ae. Albopictus</i>
Male + sucrose	50	51.7 $\pm$ 1.22a	37.7 $\pm$ 0.74b
Female + sucrose	50	51.7 $\pm$ 1.31a	49.2 $\pm$ 1.47a
Female + sucrose + blood meal	50	45.1 $\pm$ 1.25b	49.9 $\pm$ 1.12a

Mean number of days followed by the same letter within the same columns are not significantly difference ($P = 0.05$, Mann Whitney U-test).

Survival is prolonged by feeding [25], but the type of nourishment provided to the females affects their longevity [26]. Lewis [27] assumed that mosquitoes depend on blood and nectar for survival as they do not possess an efficient mechanism to prevent water loss.

Temperature and relative humidity appears to be the primary factors for adult survival [22, 28]. Bates [29] and Hylton [30] observed that longevity of female *Ae. albopictus* increase under constant humidities and low temperature. At higher temperature, [30] found that the life span of female *Ae. albopictus* was reduced regardless of humidities. At low humidities, insects are unable to survive probably because of their inability to control water loss [31].

D. The Length of Gonotrophic Cycle and the Number of Eggs Laid by Field Strain *Aedes Aegypti* and *Aedes Albopictus*

About 80% of the total eggs were laid during the first four days of experiments. Both females *Ae. aegypti* and *Ae. albopictus* took their first blood meal 5 days after emergence. Female *Ae. aegypti* and *Ae. albopictus* took about 14 days and 16 days respectively to deposit all their eggs. Furthermore, female *Ae. aegypti* laid more eggs than female *Ae. albopictus*. Under laboratory conditions, gonotrophic cycle of both *Ae. aegypti* and *Ae. albopictus* are 3.00 and 2.73 days respectively. Mann Whitney U test showed that mean number of eggs deposited by female *Ae. aegypti* and *Ae. albopictus* are significantly different (at $p=0.05$) (in Table 4).

TABLE 4 THE LENGTH OF GONOTROPHIC CYCLE AND THE NUMBER OF EGGS LAID

<i>Aedes sp.</i>	Gonotrophic cycle	No. of eggs laid* (days)							Total no. of eggs laid	N	Average per female
		2-3	4-5	6-7	8-9	10-11	12-13	14-15			
<i>Ae. Aegypti</i>	3.00 $\pm$ 0.83a	1484 (53.0)	1013 (36.2)	164 (5.86)	72 (2.57)	0 (0)	68 (2.43)	0 (0)	2801 (100)	30	93.4 $\pm$ 3.88a
<i>Ae. albopictus</i>	2.73 $\pm$ 0.18a	1033 (46.2)	769 (34.4)	286 (12.8)	123 (5.51)	20 (1.0)	2 (0.09)	1 (0.045)	2234 (100)	27	74.5 $\pm$ 5.88b

Mean number of eggs followed by the same letter within the same column are not significant different ($P=0.05$, Mann Whitney U-test).

(percent number of eggs laid by N females)

*First batch of eggs

A similar observation as the present study was made by Gillett, [32] and Curtin and Jones [33]. They found that *Ae. aegypti* do not always lays all their eggs at once but deposit their eggs in batches over several days. In an earlier study, Macfie [34] found that *Ae. aegypti* oviposition begins on the 3rd or 4th day after a blood meal.

According to Clements [35] and Pant *et al.* [36], temperature affects the length of gonotrophic cycle of *Ae. aegypti*. In tropical conditions, gonotrophic cycle lasts two days for most species [14, 35]. At a temperature of 25 °C to 26 °C and relative humidity of 50% to 60%, Gubler and Bhattacharya [37] found that gonotrophic cycle of *Ae. albopictus* lasted three to five days while Hien [26] found that the gonotrophic cycle lasted three to three and a half days under the same temperature but relative humidity of 60% to 70%. In another experiment, Mori and Wada [38] demonstrated that under natural condition (average field temperature: 25 °C), the duration of gonotrophic cycles for *Ae. albopictus* was five days. A number of studies conducted in the field and laboratory showed that *Ae. albopictus* took an average of five days for the first and second gonotrophic cycles [22].

IV. CONCLUSION

Based on the findings, F1 offsprings of field collected *Ae. aegypti* and *Ae. albopictus* were able to adapt to laboratory condition. *Ae. aegypti* is a domestic breeder, while *Ae. albopictus* is an outdoor breeder. Therefore, it explains that *Ae. aegypti* has better adaptability than *Ae. albopictus*. The ability to thrive in any given conditioned of *Aedes* causes difficulty to vector control agency in controlling *Aedes* population. Hence, the vector control approach must be revised in order to effectively reduce the dengue vector population.

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