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LABORATORY STUDY ON THE BIOLOGY OF AEDES MOSQUITO, *AEDES AEGYPTI* LINNAEUS

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ABSTRACT

The biology of aedes mosquito was studied in the central laboratory of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka from April to October 2015. Eggs were collected from Sher-e-Bangla Agricultural University campus which were emerged as aedes mosquitos both *Aedes aegypti* and *Aedes albopictus.* The gravid female laid eggs in a cluster. Each cluster having 105-129 eggs with mean of 117.71 ± 9.12. Each female laid 3-4 clusters. Initially the colour of the egg was white and gradually turned into black. The incubation period of eggs was ranged from 48h to 72h with mean of 60 ± 0.53. The development from the first instar larva to adult stage for aedes mosquitos was 8.37 ± 0.18 days for male and 9.5 ± 0.24 days for female, respectively. Female aedes mosquito fed on blood showed the highest mean survival which was 26.23 ± 2.17 days while the male aedes mosquito fed on 10% sucrose recorded 19.23 ± 2.21 days survival which was the shortest mean period. Depending on the gonotrophic cycle for aedes mosquito their number of eggs laid and longevity varied.

Keywords: Biology, aedes mosquito, mating, oviposition, incubation, instars, pupae, adults.

INTRODUCTION

The primary vector of dengue is *Aedes aegypti,* which is also called the 'yellow fever mosquito', first described by Linnaeus (1762). It is a member of the genus *Aedes* (Chistophers 1960). *A. aegypti* originated from Africa but is now found in tropical and subtropical regions thoughout the world. Apart from being responsible as dengue vector, both *A. aegypti* and *A. albopictus* have also become efficient

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vectors of other human diseases including Chikungunya and yellow fever (Hochedez *et al.* 2006, Phillips 2008) as well as some encephalitis viruses (Alto *et al*. 2013) and filariasis parasites (Cancrini *et al.* 2003). Adult *A. aegypti* is a small to medium-sized mosquito, approximately 3 to 6 mm in length, with two white stripes and a single curved line at each side forming a lyre shape on the dorsal thorax. The abdomen is generally dark brown to black, and is covered with white scales in the form of stripes and spots which create the unique distinguishing pattern. Each tarsal segment of the hind legs also possesses white stripes (Lee *et al.* 2003). On the other hand *Aedes albopictus* is characterized by its black-and-white-striped legs, and small black-and-white-striped body. (Chistophers 1960). The gravid female laid eggs in a cluster under favourable climatic and environmental conditions. The life cycle of this species can occur in less than 10 days. The lifespan for adult mosquitos typically ranges from 2 weeks to a month. (Lee *et al.* 2003b, Maricopa 2006). *A. aegypti* is a day-biting mosquito that prefers to feed on humans even if other hosts are available (Scott *et al.* 2000, Harrington *et al.* 2001). This species breeds and rests close to human habitation (Huber *et al.* 2008). From larva to emergence of adult ades mosquitos spent in aquatic environment (Alto *et al*. 2013).

Generally breeding sites of aedes mosquitos are likely to be in close proximity of their blood-feeding habitat (Alto *et al*. 2013, Ponlawat and Harrington 2005, Chaves *et al.* 2010, Valerio 2010). It is well recognised that both *A. aegypti* and *A. albopictus* can feed multiple times; therefore this behaviour may increase opportunities for the transfer of arboviruses to other vertebrate hosts (Delatte *et al.* 2010). Although some authors stated the life history of aedes mosquitos to some extent detailed study of the biology of this mosquito has been undertaken in this study.

MATERIALS AND METHODS

The biology of aedes mosquitos was studied in the Central Laboratory, Department of Entomology, Sher-e-Bangla Agricultural University (SAU), Dhaka during April to October 2015. An elaborate methodology for studying biology is presented herein.

Collection of aedes larvae: Larvae of aedes mosquitos were collected from 10 locations **(**in front of the Department of Agricultural Chemistry, behind the Department of Horticulture Seraj-ud-doula Hall, Sher-e-Bangla Hall, Kobi Kazi Nazrul Islam Hall, infront of the Kobi Kazi Nazrul Islam Hall, near the Vice Chancellor building and Northern part of central mosque, in front of Sheikh Hasina Hall and in front of Agriculture faculty) of Sher-e-Bangla Agricultural University (SAU) in order to study the biology (Figure 1). One ovitrap was set at each location to attract the gravid female for egg laying (Plate 1). For preparation of ovitrap an

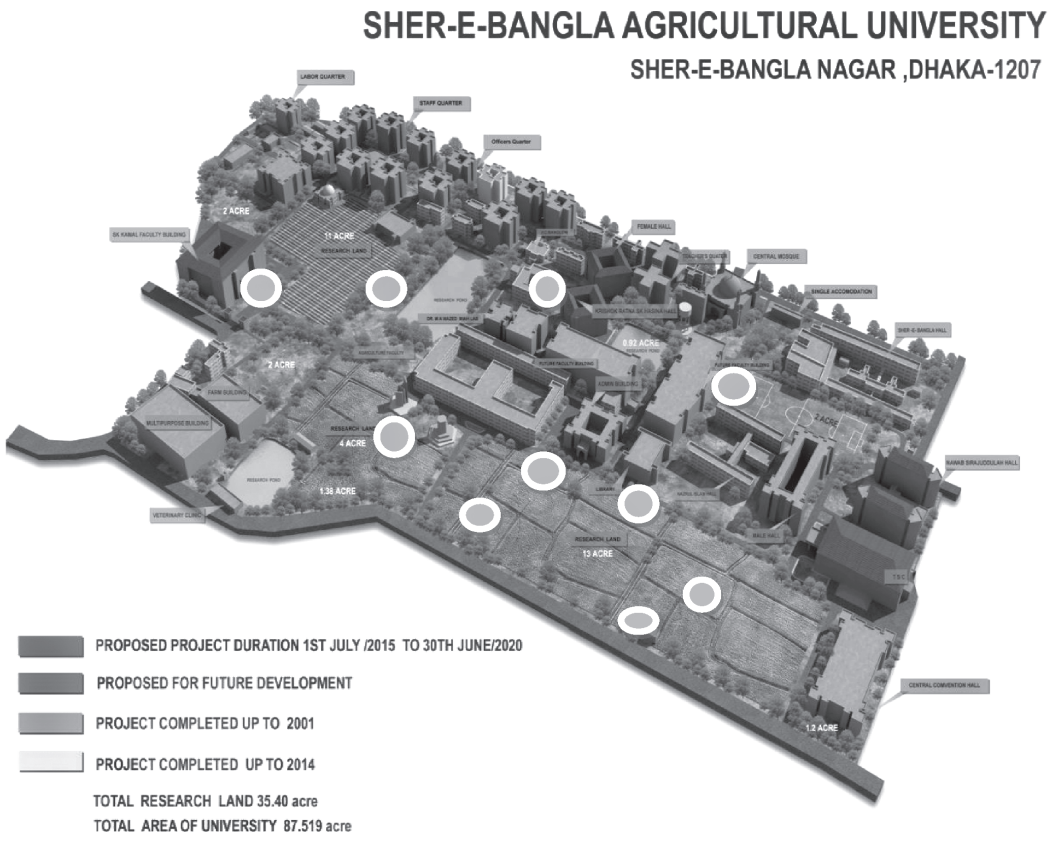

Fig. 1. Ten locations where earthen containers were placed in the Sher-e-Bangla Agricultural University

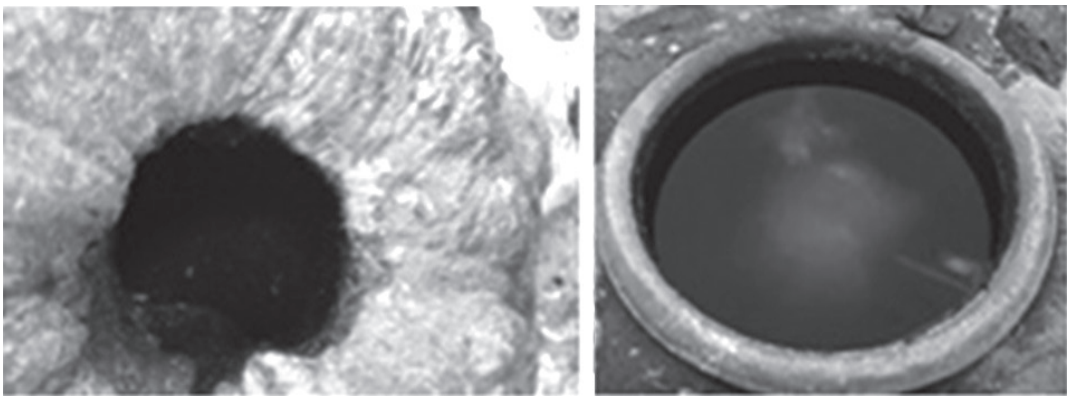

Plate 1. Tree hole and earthen pot in ten locations in SAU campus for collecting aedes mosquitos larvae

earthen pot was placed by making a hole in soil. Then five liter water was poured in each earthen pot. Fifty gram (50.0 g) Yeast powder and 20.0 g sugar were added with water and stirred with stick for mixing. Earthen pot with yeast solution was considered as ovitrap and sugar was added as larval food. A germination paper was placed in each ovitrap to support gravid female for egg laying. Ovitrap was monitored regularly to observe egg cluster and larvae of aedes mosquitos. Egg cluster of aedes mosquito was observed after seven days of placement of ovitrap. Larvae of aedes mosquito with water were collected from these ovitraps. Larvae were also collected from natural breeding sites such as old tyres in the yard of central laboratory, tree holes in front of soil science department (Plate 1), flower pots and containers those had water. Larvae were picked up with water by using pipettes. All the samples were brought to the laboratory for the purpose of mass rearing.

Rearing in the laboratory for biology study: Larvae of aedes mosquito collected from 10 ovitraps and natural sources were brought to the Entomology Laboratory of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. Water containing larvae from plastic bottles were poured into enamel trays and fed with larval food containing grounded biscuit, beef liver, powdered milk and yeast at a ratio of 2:1:1:1 (Toma *et al*. 2003) until they pupate. Then pupae were collected by pipettes and kept in separate plastic pots filled with water and placed within a rearing cage (Plate 3.3).

After emergence, adults were picked up one by one using a manually operated aspirator. Adult *Aedes aegyptii* were identified and separated by observing their morphological characteristics mainly the pattern of scutum with lyre shaped white marking and long median longitudinal white stripe on the thorax (Plate 2). Then ten adult mosquitos (5 males and 5 females) were kept together in each rearing cage. A chicken was kept at the bottom of the rearing case for feeding blood by adult female mosquitos. Legs and wings of the chicken were tied with rope for restriction its movement during feeding by female mosquitos. Moreover, 10% sucrose solution soaked cotton mass was placed at the bottom of each cage for feeding of adult male. They were allowed to mate freely inside the rearing cages at room condition having 27°C temperature and 75% RH. A wet filter paper folded into a shape of a cone in a Petri dish was placed into the cage for egg laying by female mosquitos after two days of blood feeding. The filter paper was taken out of the cage after six hours and the cone was inverted making sure that the eggs were on the inner surface of the filter paper. It was dried at room temperature to ensure development of the embryos

in the eggs and uniform hatching. Then 20 eggs were placed in each enamel tray with fresh water and monitored regularly to observe the incubation period. After hatching, water was changed and 10% sucrose solution was added for nourishing 1st instar larvae. This process was repeated upto the pupation. In the pupal period fresh water was added again to find clear emerging of adult. Just after the emergence adult took rest for 20-25 minutes. The adults were fed on chicken blood as their blood-meal source for female and cotton soaked with 10% sucrose solution for male (Clements 2000).

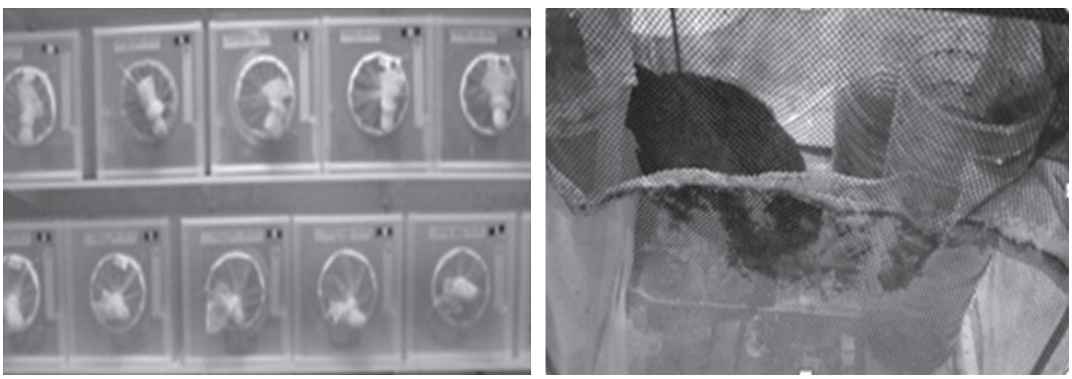

Plate 2. Larvae are rearing in trays and adult cages feeding with chicken blood

RESULTS AND DISCUSSION

The results on the biology of Aedes mosquitos in laboratory condition have been discussed under the following headings:

Mating of aedes mosquitos: Mating of aedes mosquitos occured 3-4 times during the fecundity period with mean value 3.43 ± 0.53 (Table 1). Black *et al*. (1989) concluded that *A. aegypti* males are more sexually aggressive and frequent than female.

Preoviposition and oviposition period and fecundity of aedes mosquitos: Adult females took 6-8 days for preoviposition with a mean period of 7.93 ± 0.19 days (Table 1). The duration of oviposition was as short as 6 days to a maximum of 7 days with a mean of 6.84 ± 0.35 days (Table 1). Females laid eggs in batch and produced cluster of eggs (Plate 3). Each female produced 3-4 clusters (Plate 3) with an average of 3.43 ± 0.53 clusters. Every cluster contained 105-129 eggs with mean of 117.71 ± 9.12 per cluster. A female laid a total of 327-516 eggs with a mean of 386.57± 64.63 (Table 1).

Table 1. Reproduction periods, fecundity and incubation period of aedes mosquitos in the laboratory

Character	Range	Mean ±Sd	No. of observation
Mating frequency	3 - 4	3.43 ± 0.53	7
Preoviposition period (Days)	6 - 8	7.93 ± 0.19	7
Oviposition period (Days)	6 - 7	6.84 ± 0.35	7
Postoviposition period (Hours)	24 - 42	36.00 ± 6.00	7
Incubation period (hours)	48-72	60 ± 0.53	7
No. of cluster per individual	3 - 4	3.43 ± 0.53	7
Number of eggs laid per cluster	105 - 129	117.71 ± 9.12	7
No. of eggs laid per female	327 - 516	386.57 ± 64.63	7
Egg Viability (%)	78 - 94	87.57 ± 5.19	7

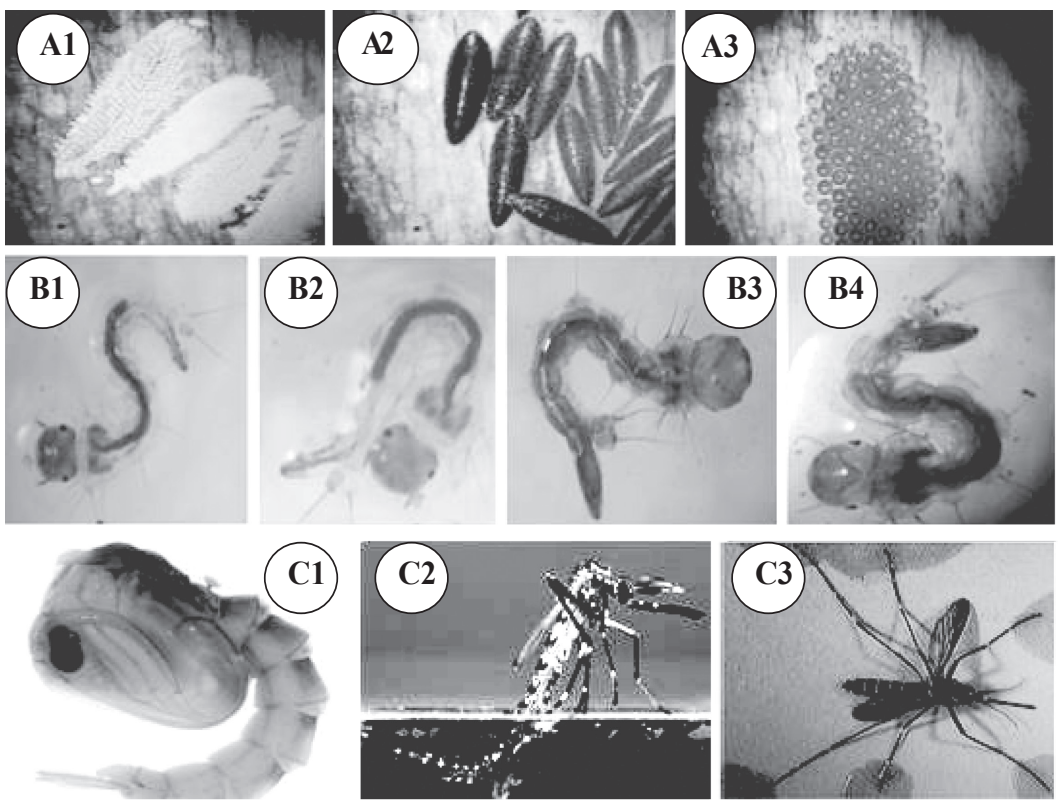

Plate 3. A1. Eggs cluster, A2. magnified eggs, A3. egg hatching, B1. 1st instar larva B2. 2nd instar larva, B3. 3rd instar larva, B4. 4th instar larva, C1. pupa, C2. emerging adult and C3. adults of *Aedes aegyti* mosquito

In this study a female aedes mosquito took 6-13 days to lay its full quota of eggs which was similar to that found by Toma *et al.* (2013) and Dieng *et al*. (2013). They found that a female of aedes mosquito took 6-14 days to lay its full quota of eggs. Almost similar finding was reported by other authors. Slight variation was recorded by Chaves (2011) and Nguyen (2012), who observed the preoviposition period of 39.32 ± 5.36. Difference could be due to environmental factors and impact of some chemicals in this study the incubation period was ranged from 48-72 h with mean of 60 ± 0.53. Farajollahi and Nelder (2009) reported that a single female laid up to 300-600 eggs. Depending on field condition an aedes female mosquito was found to lay 354-597 eggs as reported by Vitek and Livdahl (2006). Variation could be due to weather condition. Takeda *et al* (2003) found that variation of number of eggs laid depended on the availability of food sources of living habitat and species. Studies focusing the fecundity of this species which produced conflicting results. Hein (1976), Sucharit and Tumrasvin (1981), Black *et al*. (1989) and Galliard (1962) compared total lifetime fecundity of the this species, and they reported *A. aegypti* to be more fecund. Soekiman *et al*. (1984) observed that *A. aegypti* laid more eggs per batch than species with a Java strain of mosquito. In Hein's (1976) experiments, *A. albopictus* laid more eggs per milligram of blood ingested than did *A. aegypti*. Smith (1999) found egg production of *A. albopictus* dropped significantly at high temperatures, where as *A. aegypti* had only a slight decrease.

Egg viability rate of aedes mosquito was 78-94% with a mean of 87.57 ± 5.19. Chistophers (2010) observed and reported that the egg viability ranged 55-98% depending on the food sources and environmental conditions.

Larval biology: The first instar larva moulted to 2nd instar larvae and chronologically mounted to 3rd and 4th instars (Plate 3). The fourth instar larvae became pupae (Plate 3) and adult emerged from pupae (Table 2). Duration of four larval instars was 6-7.5 days. Almost similar observation was recorded by Hawley (1988). He reported that aedes mosquito larvae undergo four larval stages that required five to 10 days to complete the stages. The variation of duration depends on temperature (Alto *et al.* 2008) or larval diets (Hawley 1988).

Larvae were emerged and grown in transparent water with some debris having bacteria. Similar observations were noted by other research. Aedes mosquitos larvae were never found in turbulent waters because the larvae were unable to withstand wave action (Barrera *et al.* 2008).

Pupal biology: After the fourth instar, Aedes mosquito entered into the pupal stage. Mosquito pupae were different from many other holometabolous insects in that the pupae were mobile and respond to stimuli. Pupae, also called "tumblers," did not

Table 2. Duration of different life stages of male and female aedes mosquito in the laboratory

Length of different stages	Female mosquito		Male mosquito	
	Range	Mean ±Sd	Range	Mean ±Sd
1st instar (Hours)	36 - 48	44.57 ± 4.72	36 - 42	44.57 ± 3.72
2nd instar (Hours)	30 - 36	34.29 ± 2.93	30 - 36	34.29 ± 2.93
3rd instar (Hours)	48 - 54	47.28 ± 2.93	42 - 48	46.28 ± 3.93
4th instar (Hours)	36 - 54	46.28 ± 4.72	36 - 42	46.28 ± 3.72
Pupa (Hours)	42 - 48	45.43 ± 2.27	42 - 48	45.43 ± 2.27
Adult female (Days)	14 - 19	16.43 ± 2.07	8 - 14	11.00 ± 1.91
Life span (Days)	22-29	26.23 ± 2.17	16-23	19.23 ± 2.21

feed and take approximately two days to develop. The pupal duration was ranging from 42 to 48 h with mean of 45.43 ± 2.27 h. Adults emerged by ingesting air to expand the abdomen thus splitting open the pupal case and emerging head first. Similar result was indicated by Dom *et al* (2013) and Tilak *et al* (2005). They found that the duration of aedes mosquito pupae was 2 days and pupae were moving but did not take food.

Adult biology: Female mosquitos lived longer than male. Longer developmental duration of female compared to male was due to higher longevity of 3rd instar of female as compared to male. The longevity of adult female was 14-19 days with mean of 16.43 ± 2.07 days. The life span of female ranged 22-29 days with a mean of 26.23 ± 2.17 (Table 2).

The longevity of adult male was 8-14 days with a mean of 11± 1.91 days. The life span of male ranged 16 to 23 days with a mean of 19.23 ± 2.21 days (Table 2). Almost similar result was revealed by Chen *et al* (2006). According to their observation the longevity of male and female of aedes mosquitos depending on the species and the conditions, a female mosquito's average lifespan was anywhere from two to four weeks, while a male mosquito lived for one to two weeks.

Adult *A. aegypti* is a small to medium-sized mosquito, approximately 3 to 6 mm in length, with two white stripes and a single curved line at each side forming a lyre shape on the dorsal thorax (Plate 3). The abdomen is generally dark brown to black, and is covered with white scales in the form of stripes and spots which create the unique distinguishing pattern. Each tarsal segment of the hind legs also possesses white stripes (Lee *et al.* 2003). On the other hand *Aedes albopictus* is characterized by its black-and-white-striped legs, and small black-and-white-striped body (Plate 3).

In terms of physical appearance, the mosquito's proboscis, a long, needle-like antenna that extends from the area of its mouth, is the best indication of the mosquitos gender (Plate 3). Male aedes mosquito have a feather-like proboscis, while the proboscis of the female aedes mosquito was relatively smooth, not bushy. The hair on the antenna is called antennal flagellum. It is a hearing organ. Male aedes mosquitos use their flagellum to help locate female aedes mosquito by the (very) quiet buzzing sounds.

Identifying characters of male and female larvae and pupae: In this study it was found that the larvae of *Aedes aegypti* (Linn.) the gonads and the imaginal discs of the antennae and the external genitalia show sufficient sexual dimorphism to permit separation of the sexes.

In case of pupae just before emergence of the adult, pupae show appreciable differences in size; however the female is normally larger than the male.

As in the male pupae the sexual dimorphism is easily seen in the central and lateral lobes. Also in the male, the central lobe of the female has a median cavity which appears to be connected, in some way with the anal opening of the adult.

CONCLUSION

Aedes mosquitos completed their life cycle through four stages like egg, larva, pupa and adult within 19-26 days. The adult stage are mosquitos are the active stage in the life cycle of aedes mosquito. All the stages can be recognized easily in the household containers which served as breeding place for them.

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