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EFFECTS OF LOW AND HIGH TEMPERATURE ON THE EGGS OF *Aedes aegypti* (L.)(Diptera : Culicidae) AND THE SUBSEQUENT STAGES DEVELOPED THEREFROM

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ABSTRACT

A study was undertaken to observe the effect of low and high temperatures on the eggs of *Ae. aegypti* and the different stages developed from there after hatching. The low and high temperatures for the specified periods affected the eggs of *Ae. aegypti* and the stages developed there from after hatching. The highest number of hatching of the eggs of *Ae.aegypti* was observed in control (90.67%) at 20°C. There was significant difference in the hatching efficiency of eggs of *Ae. aegypti* at control, 0.5 hour, 2 hour, 5 hour and 24 hour. There were insignificant differences in the duration of larval periods in treatments and control. Insignificant difference was also observed in the pupal duration in control and treatments. Although there were no significant differences among the treatments, length of the 2nd, 3rd and 4th instars larvae were higher in control than the larvae treated with different temperatures. The length of the pupae in control was higher than the different temperature treatments. Low and high temperatures showed no effect on the body length (mm) of adult male and female. Adults emerged from the eggs treated with different temperatures and exposed for different interval showed that egg laying decreased in low and high temperature than the control.

INTRODUCTION

Mosquitoes are the most important group of insect pests from the public health point of view throughout the world. Mosquito belonging to the family Culicidae, contains about 3450 species and subspecies. So far 113 species of mosquitoes have been identified from Bangladesh. Among them, *Aedesaegypti* (L.) (Diptera: Culicidae) is a serious pest and is considered to be a primary vector of viral diseases such as the dengue fever, chikungunya and yellow fever. The *Aedesaegypti* is a day biting mosquito. This species is most active for approximately two hours after sunrise and several hours before sunset. After the mosquito feeds on a virus-carrier, the virus starts to replicate in the mosquito. After eight to 12 days incubation period the mosquito can transmit the virus on subsequent feeding attempts (several times per day is not uncommon) depending on the availability of the host. Feeding generally occurs at one to two hour intervals. The *A. aegypti* is adapted to breed around human dwellings and prefers to lay its eggs in clean water free of other organisms. Artificial or natural water containers (water storage containers, flower pots, old tires, etc.) that are within or close to places where humans live are ideally larval habitats for the *A. aegypti*. It is estimated that up to 80 million people are infected annually.

*Aedesaegypti*is extremely common in the areas lacking piped water systems, and depend greatly on water storage containers to lay their eggs. Male and female adults feed on nectar of plants; however, female mosquitoes need blood in order to produce eggs, and are active in the daytime. Eggs have the ability to survive drying for long periods of time, allowing eggs to be easily spread to new locations. The artificial or natural water containers, water storage containers, flower pots, discarded tires, plates under potted plants, cemetery vases, flower pots, buckets, tin cans, clogged rain gutters, ornamental fountains, drums, water bowls for pets, birdbaths, etc that are within or close to the places where humans live, are ideally larval habitats for this mosquito. This species has also been found in underground collections of water such as open or unsealed septic tanks, storm drains, wells, and water meters.

Temperature is an important physical factor associated with blood feeding arthropodsand has a profound influence upon insects as well. The extreme high and low temperature limits insect activities both in space and time. Their rates of metabolism and consequently those of growth, reproduction and general behavior are largely controlled by temperature. The most important factors the environment which influence the physiology of insects are temperature and humidity (Wigglesworth 1965).

Extreme low temperatures often become critical for the continued active life of mosquitoes in the temperate zone but that is not the problem in subtropical countries like Bangladesh. Temperatures below 0°C appear to be fatal to the species if continued even for short time. Flu (1920) found that temperatures below freezing were certain to kill the adult insect in 24 hours. At such temperatures the insect is completely immobilized and it is only a question after exposure whether recovery takes place at a warmer temperature at 40°C insects exposed for one hour survived; longer exposures kill them (Otto and Neumann 1905). Khan and Rahman (2013), Ameen and Huda (1976), Ameen and Bhuiya (1979) and Khan and Hossain (2013) conducted several investigations on the effects of temperature on the various stages of mosquitoes.

In the sub-tropical climatic areas like Bangladesh, temperature is not a critical factor. A mosquito development is never suspended due to unfavorable temperature. The mosquito population in Dhaka city is increasing day by day that it makes headlines in every national daily each year. There are several methods for the control of mosquito larvae in Bangladesh. Here mosquitoes are controlled mostly with the help of insecticides. Effects of temperature are strongly interacted to determine the mosquito's life history parameters. The present study was undertaken to observe the effect of low and high temperatures on the eggs of *Ae. aegypti* and the different stages developed from there after hatching, and the following aspects were observed after the eggs were

exposed to low and high temperatures: hatching efficiency of the eggs, larval and pupal duration, length of the different in star larvae and pupae, body length of the adult male and female and fecundity to study the relationship between temperature and *Ae. aegypti*

MATERIAL AND METHODS

The experiment was carried out in the Entomology laboratory, Department of Zoology, University of Dhaka. The room temperature and relative humidity were recorded (29±4)°C and 80%, respectively during the experimental period from 1st June, 2012 to 31st January 2013. The collected larvae were placed on slides with a few drops of water and were examined under a compound binocular microscope. The larvae were identified by following Service (1970).

Rearing of Ae.aegypti

The larvae of *Ae. aegypti* were collected from different breeding sources in the Curzon hall campus, University of Dhaka. The breeding sources included drains, aquaria in the zoological garden, earthen pots, coconut shells, tin cans, metal pots, tree holes. The larvae were collected with the help of a large dropper and were kept in a clean beaker and brought to the laboratory. They were then washed gently in tap water for several times to clean those from impurities. The collected larvae were reared in the ambient environment of the laboratory. They were kept in a plastic bowl containing tap water and provided with ground biscuits (commercially known as 'Energy plus' locally) as their food. The larvae were transferred from one bowl to another, whenever necessary, with the help of a large dropper. The water was changed and ground biscuits were provided daily. Similar procedure was followed to rear the subsequent 2nd, 3rd, 4th instars.

The fourth instar larvae were moulted into pupae and were separated daily from the larval bowl by using a dropper and placed them in a plastic bowl which was previously filled with tap water. Since the pupae don't take any food, no food was therefore, supplied to them. The plastic bowl with the pupae was kept in a mosquito rearing cage for the emergence of adult mosquitoes.

Adult emerged from the pupae in the rearing cage were provided with 10% glucose solution daily as their food. The glucose solution was soaked in a cotton wad on a petridish which was then placed in the cage. The male mosquitoes took only the glucose solution as food throughout their lifetime. The females were also fed on glucose solution during the first two or three days of emergence. Blood feeding is required for the nourishment and maturation of the egg of the mosquito. From the third day after emergence, the female mosquitoes were allowed to feed on blood from a pigeon. The feather was removed from the breast region of the pigeon and kept in a tight small iron cage which could easily be placed in the rearing cage. The blood feeding was initiated on the fourth day after emergence and continued as long as the females were alive. After taking the blood meal, the abdomen of the females become large and reddish colour.

The females mate with the males after taking blood meal. After mating the females laid eggs. Few filter paper strips were placed around the rim of a plastic bowl containing tap water. It was then placed inside the rearing cage for oviposition. The female mosquito laid eggs on the filter paper at the edge of the water in the plastic bowl. The number of eggs laid per female was recorded. After desiccation, the eggs were dipped in tap water contained in a plastic bowl. When flooded, the eggs were hatched within a few minutes.

Egg treatment with Temperature

Only the healthy eggs were selected for temperature treatment. Three strips of filter paper containing 50 freshly laid eggs of *Ae. aegypti* were placed in each filter paper contained petridish. Each of the petridish with eggs were kept in the incubator at temperature 10°C, 20°C, 30°C, 35°Cfor 0.5 , 2, 5 and 24 hours separately. There were three replications along with a control for each temperature and exposure time. After exposure of a stipulated period of each of the temperature, the petridishes were taken out of the incubator and kept it for five minutes for cooling. The strips of eggs were then dipped in separate plastic bowls containing tap water in an ambient laboratory condition. The eggs were immersed in the water for 24 hours. The number of 1st instar larvae hatched in 24 hours in the water was recorded with the help of a hand magnifying glass.

The larval duration of each instar was recorded. In each treatment, 100 ml of tap water was taken in plastic bowl, 10 newly hatched larvae were released in the water. The time from the first larvae hatched up to the first pupation was recorded. Similarly, the larval period in 'control' was recorded. The food in the treatments and control was always provided in the same manner.

The first pupation of the matured 4th instar larvae was recorded. The period between first pupation and first adult emergence would be considered as the pupal period in this write up.

Measurement of the body length of the larvae, pupae and adult

The length of the 1st ,2nd,3rd and 4th instar larvae and pupae hatched from control and treated eggs were measured. Ten larvae were taken in a small bottle, containing 70% ethyl alcohol. Measurement was made on randomly selected larva. These inactive larvae were then placed longitudinally on a slide by using a forceps. The length of body was measured by Electronic bi-ocular microscope and also by oculometer.

To measure the body size of the male and female adults of *Aedesaegypti*,10 males and females were taken from a rearing cage to another cage. They were then exposed to aerosol spray to make them inactive/dead. Then, they were placed on the slide and their body lengths were measured in millimeter by an electronic bi-ocular microscope.

Fecundity

The number of adults emerged from the pupae was recorded. The females were given blood meal from a pigeon. Fifteen engorged female adults were taken in a rearing cage. The mosquito laid eggs on the filter paper at the edge of water in the plastic bowls. When the female completed their egg production, the total numbers of eggs were counted by using a simple microscope.

The data were reported as arithmetic mean ± standard deviation (SD).Linear regression was applied on the data to assess the treatment effect. All the statistical analyses were done on a computer using statistical software package SPSS (11.5).

RESULTS

Hatching efficiency of the eggs of the Ae.aegypti

At 10°C about 82, 86.67, 55.33, and 60.67 % of eggs were hatched in 0.5, 2, 5 & 24 h hours, respectively. The higher numbers of eggs were hatched in control condition. The highest hatching of the eggs of *Ae. aegypti*was observed in control (90.67%) at 20°C.There was significant difference in the hatching efficiency of eggs at control, 0.5 hour, 2 hour, 5 hour and 24 hour. But at 30°C the rate of hatchability of eggs were further decreased both in treated and untreated eggs (59.33, 40, 52.67, 44.67, 43.33% in control, 0.5, 2, 5 & 24 h respectively. With higher temperature of 35°C only 46% of eggs were hatched in control while 42.67, 54.67, 44, 46.67% of eggs were hatched in 0.5, 2, 5 & 24 h respectively (Table1).

Duration of the larval and pupa period

There were insignificant differences in the duration of larval periods in treatments and control. The duration of larval and pupal period was lowest at 20°C followed by 10°C. Both larval and pupal duration drastically increased when exposed to 30°C and 35°C. In all the temperatures larval duration was higher when exposed for 24 h, except at 35°C (Table 2).

Measurement of the length of different instar larvae, Pupae and Adult

Although there were no significant differences among the treatments, lengths of the 2nd, 3rd and 4th instars larvae were higher in control than the larvae treated with different temperature. Similarly, the length of the pupae emerged from the eggs treated with low and high temperatures in various exposure periods showed difference with control. The size of the pupae in control was higher than with the different temperature treatments. Low and high temperatures showed no effect on the body length (mm) of adult male and female. Female *Aeaegypti* emerged from the eggs treated with low and high temperatures (20°C and 35°C) in 0.5 and 2 hours had similarities.

Fecundity

Adults emerged from the eggs treated with different temperatures and exposed for different interval showed that egg laying decreased in low and high temperature than the control (Table 3).

Table 1. Hatching efficiency of the eggs of *Aedes aegypti* emerged from the eggs treated at 10°C,20°C, 30°C and 35°C in different exposure periods and control in an ambient condition of the laboratory (temperature range 8-35°C and relative humidity 60-88%).

Temperature (°C)	Exposure periods (hours)				Control
	0.5	2	5	24	
	No. larvae hatched (mean ±SD)	No. larvae hatched (mean ±SD)	No. larvae hatched (mean ±SD)	No. larvae hatched (mean ±SD)	No. larvae hatched (mean ±SD)
10	41.00±1.00 (82%)	43.33±0.58 (82.67%)	27.60±2.52 (55.33%)	30.33±0.58 (60.67%)	43.67±1.53 (87.33%)
20	28.33±2.52 (56.67%)	26.33±1.15 (52.67%)	25.67±3.06 (51.33%)	25.67±3.79 (51.33%)	45.33±0.58 (90.67%)
30	20.00±2.00 (40%)	26.33±3.21 (52.67%)	22.33±3.78 (44.67%)	21.67±5.03 (43.33%)	29.66±4.51 (59.33%)
35	21.33±1.15 (42.67%)	27.33±2.08 (54.67%)	22.00±1.00 (44%)	23.33±4.16 (46.67%)	23.00±2.65 (46%)

Table 2. Mean duration of the larval period of *Ae. aegypti* emerged from the eggs treated at 10°C, 20°C, 30°C and 35°C in different exposure periods and control in an ambient condition of the laboratory (temperature range 8-35°C and relative humidity 60-88%)

Temperature (°C)	Exposure periods (hours)				Control
	0.5	2	5	24	
	Duration of larval/pupal stage in hrs (mean ±SD)	Duration of larval/pupal stage in hrs (mean ±SD)	Duration of larval/pupal stage in hrs (mean ±SD)	Duration of larval/pupal stage in hrs (mean ±SD)	Duration of larval/pupal stage in hrs (mean ±SD)
			Larvae		
10	115.6± 3.36	114.2± 0.84	117.0± 1.87	116.6± 3.21	116.0± 1.58
20	96.4± 2.61	95.8± 1.48	96.0± 2.0	96.0± 2.0	116.0± 1.30
30	234.4±16.33	217.8±14.94	216.4±20.51	254.4±57.17	230.6±27.46
35	316.8±35.59	278.4±40.16	312±37.95	244.8±31.29	292.8±31.29
			Pupae		
10	40.4 ± 1.14	39.8 ± 0.84	41.8 ± 4.66	39.0 ± 2.35	41.4 ± 0.55
20	40.0 ± 0.71	39.8 ± 1.30	36.0 ± 1.87	36.4 ± 3.36	42.0 ± 0.71
30	59.4 ± 5.22	54.4 ± 6.23	53.4 ± 6.58	53.2 ± 5.45	60 .0± 5.79
35	68.8 ± 2.59	68.4 ± 3.36	68.0 ± 4.30	67.2 ± 4.87	69 .0± 2.65

Table 3. No. of eggs laid per adult female of *Aeaegypti* emerged from the eggs treated at10°C, 20°C, 30°C and 35°C in different exposure periods and control in an ambient condition (temperature range 8-35°C and relative humidity 60-88%) of the laboratory.

Treatment by temperature(°C)	Exposure periods (in hours)	No. of eggs laid per female (mean±SD)	Control (mean±SD)
10	0.5	54.00±1.73	
	2	51.67±1.15	60.33±1.53
	5	51.67±0.58	
	24	50.67±2.08	
20	0.5	51.00±2.00	
	2	45.00±5.57	57.33±1.53
	5	46.00±6.24	
	24	48.00±2.65	
30	0.5	51.00±1.00	
	2	51.00±1.00	57.00±2.08
	5	50.67±2.08	
	24	48.67±1.53	
35	0.5	50.00±1.00	
	2	50.00±1.73	55.00±3.00
	5	52.00±2.65	
	24	48.67±1.53	

Table 4. Measurement of the length of 1st, 2nd, 3rd and 4th instar larvae of *Ae. aegypti* emerged from eggs treated at 10, 20, 30 and 35 in different exposure periods and control in an ambient condition (35 and RH 88%)

Larval instar/ pupae/adult (in mm)	Temperature (◦ C)	Control length of larvae/ pupae and adult.(in mm)				
		Exposure periods (in hour)				
		0.5	2	5	24	Control
		Measurement of larvae, Adult(mm)				
1st Instar	10	1.10	1.14	1.12	1.20	1.20
	20	1.18	1.14	1.18	1.04	1.08
	30	1.12	1.16	1.08	1.16	1.08
	35	1.18	1.18	1.22	1.10	1.14
2nd Instar	10	4.02	4.02	3.32	3.78	4.52
	20	4.02	4.10	3.88	3.79	4.54
	30	4.00	3.94	3.66	3.78	4.52
	35	3.84	3.90	3.78	3.80	4.46
3rd Instar	10	5.86	5.60	5.76	5.60	6.56
	20	6.18	5.90	5.72	5.44	6.46
	30	5.82	6.00	6.06	5.94	6.90
	35	5.76	6.12	5.66	5.68	6.44
4th instar	10	7.24	7.50	7.56	6.64	8.00
	20	7.54	7.46	7.14	6.80	8.02
	30	7.62	7.40	7.42	7.46	7.96
	35	7.46	7.52	7.54	7.30	7.92
Pupae	10	4.64	4.44	4.52	4.28	4.80
	20	4.34	4.34	4.34	4.22	4.80
	30	4.54	4.36	4.30	4.32	4.82
	35	4.56	4.54	4.48	4.50	4.76
		Male/Female				
Adult	10	2.80/3.36	2.98/3.26	2.70/3.42	3.06/3.46	3.14/3.44
	20	3.06/3.50	3.00/3.50	2.98/3.36	3.04/3.46	3.36/3.50
	30	3.02/3.52	3.06/3.40	3.06/3.36	3.00/3.40	3.20/3.32
	35	2.88/3.46	3.00/3.46	3.22/3.62	3.04/3.36	3.10/3.54

DISCUSSION

There is less information about the effect of low temperature on the hatching efficiency of the eggs. In this experiment there was significant difference on the hatching efficiency of the eggs treated with temperature between 10, 20, 30 and 35°C temperatures on different exposure periods. Begon *et al* (1990) reported that in low temperatures eggs of mosquitoes hatched, larvae pupated and adult emerged, that is immature stages continued their development throughout the cold months, but to a slower speed than they did under warmer conditions.

The highest larval duration observed in control and gradually decreased as the temperature treatment periods increased. Briegel and Timmermann (2001) stated that median developmental time from hatching to pupation of *Ae. alpopictus* was seven days at 32^0C. Alto and Juliana (2001) found that the development time of *Ae. albopictus* decreased with increased temperatures that is similar to the present study.

The highest pupal duration observed in control and gradually decreased as the temperature treatment periods increased. In present study, the results show similarity with the result obtained by Briegel and Timmermann (2001).

The results showed that the length of larvae in temperatures 10°C, 20°C, 30°C and 35^0C for different exposure periods was relatively the same among them, but they differed with control. In the present work, the length of 2^{nd}, 3^{rd} and 4^{th} instars were similar with the results obtained by Christophers (1960). The length of pupae for different exposure periods was relatively the same among them, but they differed with control. This result indicated that the temperature has effect on the length of pupae. The lengths of *Ae.aegypti* at 10°C, 20°C, 30°C and 35^0C in different exposure periods were around 3 mm for the male and 3.5 mm for the female.

The highest fecundity (60.33±1.53) was obtained in control, at 10°C. In 10°C, 20°C, 30°C and 35°C in different exposure periods the number of eggs laid per female decreased with the increase of the length of exposure period. In the present work it was observed that the size of adult female (3.5) of *Ae. aegypti* decreased with the increase of the length of exposure periods and fecundity decreased gradually with the increase of the length of exposure periods. The present findings are agreeable with the results obtained by Service (1977).

CONCLUSION

The eggs of *Ae. aegypti* were exposed to 10,20,30,35 C for half an hour, two, three, four, five and 24 hours each treatment level. There was a significant hatching efficiency of eggs of *Ae. aegypti* at control,, half an hour, 2 hour, 5 hour and 24 hour. There were insignificant differences observed in the larval and pupal period. There were variations in the sizes of larval instar, pupal and adult lengths in different exposure periods in different temperatures.

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