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EFFECT OF TEMPERATURE ON LIFE CYCLE AND BEHAVIOUR OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Abstract: In this study, effect of increasing temperature on life cycle, mortality, and behavior of the mosquito species, *Aedes aegypti* was evaluated. Heat shock was applied at 32°C, 37°C and 42°C for 20 minutes, 1 hour and 2 hours for each temperature to egg, 2nd instar, 3rd instar and pupal stage separately and changes in life cycle pattern was recorded on a routine basis. The control temperature was 27°C at which the mosquito was reared. When heat-shock was applied to eggs, the developmental period for each stage was found to be inversely proportional to temperature rise. The shortest embryonic developmental period was recorded at 32°C, and the shortest larval and pupal stages were recorded at 37°C (2h). The shortest full development period was also found to be at 37°C (2h). No hatching was recorded at 42°C. While heat shock was applied to 2nd instar larvae, 3rd instar larvae and pupae, similar decreased pattern was observed. In this study, 100% viability was observed upon heat shock to eggs, larvae (2nd and 3rd instar) and pupae at 27°C and 32°C. In case of 37°C, egg, 2nd instar and pupal viability decreased. No egg hatched at 42°C, while few 2nd instars survived. Lowest viability rate for 3rd instar larvae and pupae were counted at 42°C. Change in the rate of movement also decreased gradually with increasing temperatures in pupae. The change was irregular in case of 2nd and 3rd instar larvae. The highest movement was recorded for 2nd and 3rd instar larvae at 32°C and 27°C, respectively and lowest was recorded at 42°C.

Key words: *Aedes aegypti,* Heat-shock, Bioassay, Life cycle, Mortality

INTRODUCTION

Aedes aegypti is a vector of dengue fever and an extremely synanthropic insect (Lambrechts and Failloux 2012). About 3.9 billion people, in 128 countries, are at risk of infection with dengue viruses (Brady *et al.* 2012). Bangladesh recorded 81,832 cases in 2019, almost ten-time higher than 2018 (Hasan *et al.* 2019).

Ae. aegypti is abundant in neotropical regions, where environmental factors (e.g., rainfall, temperature, and relative humidity) favor its life cycle (Eisen *et al.*

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2014). Climatic factors extensively influence its global distribution. Optimal temperatures for its development, longevity, and fecundity are between 22°C and 32° C (Beserra *et al.* 2009). Research on mosquito adaptation with climate change has reported that global warming has shortened mosquitos' life cycle period and increased the disease transmission rates by mosquito vectors (Hoonbok *et al.* 2015). With higher temperatures in the favorable survival range of *Ae. aegypti*, egg-laying time decreases, causing an increase in egg number (Costa *et al.* 2010). Moreover, the extrinsic incubation period of the dengue virus is reduced, resulting in higher rates of viral transmission (Costa *et. al.* 2010). Vector-borne diseases like dengue, may be particularly sensitive to both periodic fluctuations and sustained changes in global and local climates because the vectors themselves are temperature and moisture dependent (Thai and Anders 2011). Recent studies focus on the investigation of the interaction between life cycle and global climate changes which may favor the transmission of dengue. Temperature can drastically alter the genetic structure and gene expressions and thus affect mosquito development (Gakhar and Shandilya 1999, Yadav *et al.* 2005, Monteiro et al. 2007, Zhao *et al.* 2009). This has implications on the larval development, survival ability in larvae as well as the resulting adult, size of the adult, gonotrophic cycle and competencies in transmission of pathogens (Reeves *et al.* 1994; Westbrook *et al.* 2010; Muturi and Alto 2011; Muturi *et al.* 2012).

In this study, the effect of temperature on the *Ae. aegypti* life cycle has been evaluated to observe whether increase in environmental temperature favors *Ae. aegypti* population growth.

MATERIAL AND METHODS

The study was conducted in Genetics and Molecular Biology laboratory (Department of Zoology, University of Dhaka) and Zoological Garden (Dhaka University). Larvae of *Ae. aegypti* were collected from different places of Curzon Hall (Dhaka University), from both their natural oviposition sites and by using artificial ovitraps.

Food Preparation: A suspension was made using chicken liver powder (5g) and dH_20 to a final volume of 500ml. It was stored at 4° C. Sucrose solution (5%) was used to feed the adults.

Mosquito Rearing: Mosquito rearing was maintained under suitable condition of air temperature, 27-35°C and relative humidity, 55-77%. The collected larvae were transferred with a transfer pipette to large bowls containing 1.5L water in a quantity of 150 larvae per bowl to avoid overcrowding. Chicken liver powder suspension in an amount of 15ml was added to each bowl and covered with lid. Once larvae became pupae, they were transferred from the bowls into 500ml

plastic cups containing 250ml distilled water and these cups were placed into rearing cages to allow the pupae become adults. Three to four cotton balls were soaked in 5% sucrose solution, squeezed them together slightly to make one ball, and placed it on the top of a conical flask and then 2/3 of these conical flasks were placed in each mosquito rearing cage. For blood feeding, anesthetized pigeon was placed inside the netted cage for 15 min. When adult female mosquitoes became 3 days old, they were deprived of sucrose solution for 12-24 hr prior to blood feeding. A piece of brown paper (9 cm × 20 cm) was cut and labelled with the strain type, date, and time. Plastic cup (500ml) was filled with 250 ml distilled water and the paper was placed in direct contact with the inner wall of the cup along water/air interface. The cup was placed in the cage. The brown papers also called egg papers were collected after 3 days. The collected egg papers were allowed to be dried for 3 days in the insectary. Once dried, those were wrapped with a piece of folded paper towel and placed in a plastic container.

Bioassay: The second and third instar larvae and pupae of *Ae. aegypti* were exposed to heat shock at different temperatures (27°C, 32°C, 37°C and 42°C) for 20 minutes, 1 hour and 2 hours for each temperature. For each experiment around 25 second instar larvae, 15 third instar larvae and 8 pupae were taken.

Bio-assay Procedure: Larvae were collected from the rearing facility. The water bath was set into desired temperature. The larvae and pupae were carefully collected with a dropper from the container and replaced to falcon tubes which was filled with 3ml of dH_2O. All the falcon tubes were put into white cork-sheet and placed into the pre-set water bath. The time was monitored carefully with the help of a stopwatch. First, second and third batch of 2nd, 3rd instar larvae and pupae samples were taken out after 20 minutes, 1 hour and 2 hour respectively and replaced into 500ml plastic cups. The desired data were collected. The second batch of first, second instar larvae and pupae were collected after 1hour from the placement and again replaced in to 500ml plastic cups. Again, data were collected. The third batch of first, second instar larvae and pupae were collected after 2 hours, and the same process repeated. Regular monitoring of those were done to collect data.

Mortality rate: Mortality and survival rate was counted immediately after heat shock application was done.

Locomotory change: Larval and pupal average wriggling movement rate for control temperature was counted earlier. Then the average movement rate after the application of heat shock was counted. Average Changes in rate of movement for each sample was calculated in comparison to the control. To calculate the movement rate, each larva was monitored for 60 seconds.

Life cycle monitoring: Life cycle pattern for each heat shock sample was regularly monitored to find out if there is any deviation from the control. The period of emergence of first, second, third, fourth instar larvae and pupae were recorded and compared with those of control.

RESULT AND DISCUSSION

Heat shock applied to eggs: According to the study result, the length of developmental stages was inversely proportional to the temperature increase. The shortest embryonic developmental time was recorded at 32°C for 2 hours heat shock period and for larval and pupal stages it was recorded at 37°C for 2 hours heat shock period. The shortest full development period was also found at 37°C for 2 hours heat shock period. In 42°C heat shock, no hatching was recorded (Table 1, Fig. 1).

Table 1. Duration (days) of different developmental stages (egg, larva and pupa) of *Ae. Aegypti* when heat shock was applied to egg for a period of 20 minutes, 1 hour and 2 hours

Experimental Period	Stages	27°C	32°C	37°C	42°C
		Duration of different life cycle stages (days) ± SD			
20 minutes	Embryonic	3.18 ± 0.16	2.57 ± 0.12	3.63 ± 0.15	
1 hour	development	3.18 ± 0.16	2.48 ± 0.33	3.77 ± 0.03	No hatching
2 hours	(egg hatching)	3.18 ± 0.16	2.25 ± 0.25	3.82 ± 0.08	
20 minutes	Larval stage	7.35 ± 0.41	6.82 ± 0.08	5.97 ± 0.16	
1 hour	(larva to pupa)	7.35 ± 0.41	6.78 ± 0.03	5.77 ± 0.03	No hatching
2 hours		7.35 ± 0.41	6.65 ± 0.13	5.58 ± 0.14	
20 minutes	Pupal stage	2.5 ± 0.30	2.65 ± 0.13	1.63 ± 0.23	
1 hour	(pupa to adult)	2.5 ± 0.30	2.60 ± 0.17	1.48 ± 0.23	No hatching
2 hours		2.5 ± 0.30	2.30 ± 0.18	1.38 ± 0.06	
20 minutes	Full	13 ± 0.23	12.03 ± 0.08	11.23 ± 0.27	
1 hour	development	13 ± 0.23	11.87 ± 0.53	11.02 ± 0.28	No hatching
2 hours	(egg to adult)	13 ± 0.23	11.2 ± 0.56	10.38 ± 0.42	

Table 2. Duration (days) of larval (2nd instar to pupa) and pupal stages in *Ae. aegypti when* heat shock applied to 2nd instar for a period of 20min, 1h and 2h

Experimental period	Stage	27°C	32°C	37°C	42°C
		Duration of different life cycle stages (days) ± SD			
20 minutes	Larval stage	8.45 ± 0.61	5.62 ± 0.16	5.23 ± 0.34	7.08 ± 0.59
1 hour	(2nd instar to	8.45 ± 0.61	5.4 ± 0.47	5.1 ± 0.33	All died
2 hours	pupa)	8.45 ± 0.61	5.03 ± 0.32	4.82 ± 0.26	All died
20 minutes	Pupal stage	2.95 ± 0.18	2.53 ± 0.06	1.55 ± 0.09	2.22 ± 0.26
1 hour	(pupa to	2.95 ± 0.18	2.42 ± 0.15	1.47 ± 0.06	All died
2 hours	adult)	2.95 ± 0.18	2.22 ± 0.06	1.3 ± 0.18	All died

Heat-Shock applied to 2nd instar larvae: The developmental period for each life cycle stage decreased with the increase in temperature and heat shock period,

except for 42°C (20min) while the larval and pupal stage duration were measured as 7.08 and 2.22 days. When, heat-shock applied for more than 20min at 42^{0}C, all larvae died. The shortest larval and pupal developmental period was measured at 37°C for 2h heat shock period(Table 2, Fig. 2).

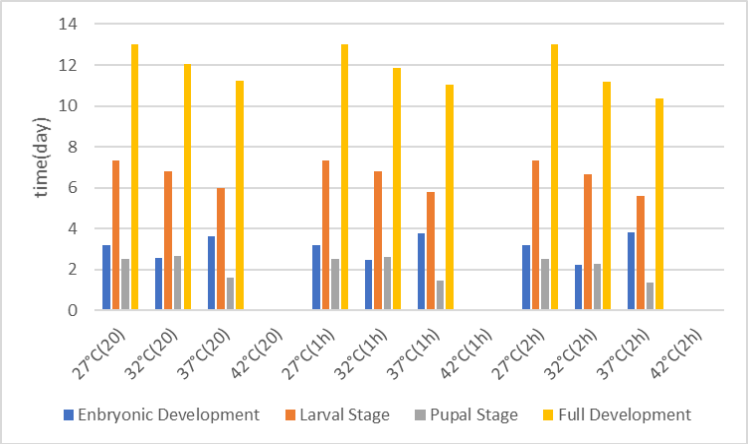

Fig. 1. Difference in duration (days) of embryonic development, larval and pupal stages, as well as full development (egg to adult emergence) when heat shock was applied at different temperature (27, 32 ,37 and 42°C) for same time and for different time (20min, 1h, 2h).

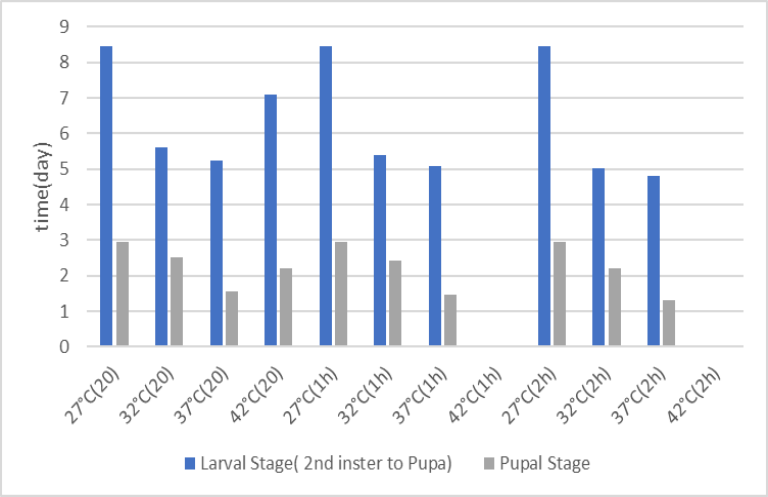

Fig. 2. Difference in duration (days) of larval (2nd instar to pupa) and pupal stage when heat shock was applied at different temperature (27, 32 ,37 and 42°C) for different time.

Heat-Shock applied to 3rd instar: The lowest larval period (3rd instar to pupa) 3.63 ± 0.32 was recorded at 37°C for 2-hour heat shock period and the lowest pupal period (pupa to adult) 1.12 ± 0.10 was found at 42°C for 20 minutes heat shock period (Table 3, Fig. 3).

Heat-shock applied to pupa: The lowest pupal period (pupa to adult) 1.1 ± 0.1 was recorded at 42°C for 20 minutes heat shock period (Table 4, Fig. 4).

Effect of Heat-Shock on Survival Rate: In this study, 100% viability was observed upon heat shock to eggs, 2nd and 3rd instar larvae and pupae at 27°C and 32°C. In case of 37°C egg, 2nd instar and pupal viability decreased from 92% to 74.67%, 100% to 91.11% and 100% to 93.33%, respectively. No egg hatched at 42°C, while few 2nd instars survived (13.33%). Lowest viability rate for 3rd instar larva and pupa were counted at 42°C (2.22 and 3.33% respectively) (Table 5).

Effect of Heat-Shock on Locomotory Behavior: The rate of movement decreased gradually with increasing temperatures in pupa. The change was irregular in case of 2nd and 3rd instar larvae. Highest movement recorded for 2nd and 3rd instar larvae at 32°C and 27°C respectively and lowest were recorded at 42°C (Table 6).

Temperature is one of the important abiotic factors which influence the physiological processes of mosquitoes. This study aimed to evaluate the influence of temperature on the life cycle of *Ae. aegypti.* According to Beserra (2016) development time and temperature are inversely related, and the optimum growth range is between 22 and 32° C. At temperatures favorable to the life cycle, insects not only complete their development but do so more quickly, which may enhance vector competence for arboviruses (Muturi *et al.* 2012). Above the optimal temperature, development rates remain relatively stable and may decrease slightly until temperatures reach an upper limit, at which point development drops dramatically. This upper limit occurs at ~38 to 42° C (Eisen *et al.* 2014).

Table 3. Duration (days) of larval (3rd instar to pupa) and pupal stages in *Ae. aegypti* when heat shock applied to 3rd instar for a period of 20min, 1h and 2h

		27°C	32°C	37°C	42°C
		Duration of different life cycle stages (days) ± SD			
20 minutes	Larval stage	6.65 ± 0.35	4.19 ± 0.21	4.17 ± 0.19	5.28 ± 0.55
1 hour	(3rd instar to	6.65 ± 0.35	3.87 ± 0.38	3.9 ± 0	All died
2 hours	pupa)	6.65 ± 0.35	3.6 ± 0.20	3.63 ± 0.32	All died
20 minutes	Pupal stage	2.95 ± 0.18	2.27 ± 0.20	1.47 ± 0.13	1.12 ± 0.10
1 hour	(pupa to	2.95 ± 0.18	2.18 ± 0.16	1.25 ± 0.25	All died
2 hours	adult)	2.95 ± 0.18	2.03 ± 0.06	1.17 ±0.03	All died

In the study of Rafael *et. al.* (2016), temperatures of 16, 22, 28, 33, 36 and 39°C were used to evaluate the life cycle and thermal requirements for *Ae. aegypti* development. In temperature 28, 33 and 36°C the developmental period for egg, larvae and pupae were measured 3.35, 2.70 and 3.66 days; 6.16, 6.70 and 5.17

days; 2.08, 2.51 and 1.29 days respectively. Full development period for these temperatures were calculated 11.59, 11.92

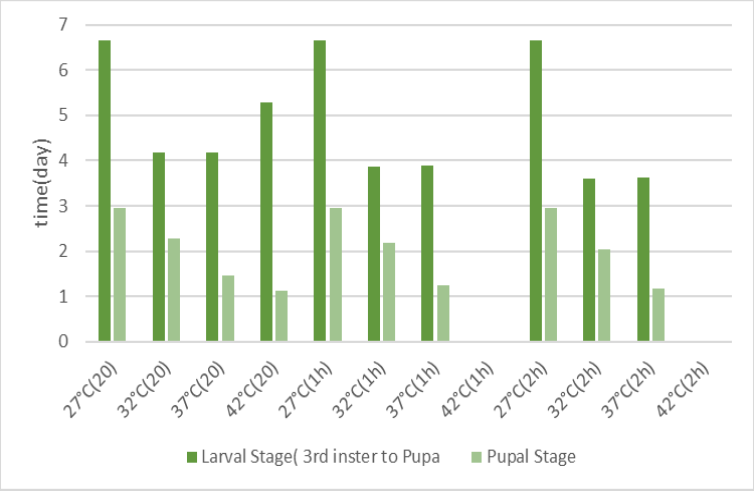

Fig. 3. Showing difference in duration (days) of larval (3rd instar to pupa) and pupal stage while heat shock was applied at different temperature (27, 32 ,37 and 42°C) for different times.

Table 4. Duration (days) of pupal stage in *A. aegypti* when pupa exposed to 27, 32, 37 and 42°C for 20min, 1h and 2h

Experimental period	Stage	27°C	32°C	37°C	42°C
		Duration of different life cycle stages (days) ± SD			
20 minutes	Pupal stage	2.95 ± 0.18	2.04 ± 0.07	1.28 ± 0.10	1.1 ± 0.1
1 hour	(pupa to	2.95 ± 0.18	2.02 ± 0.13	1.17 ± 0.14	0.83 ± 0.08
2 hours	adult)	2.95 ± 0.18	1.8 ± 0.33	1.02 ± 0.18	All died

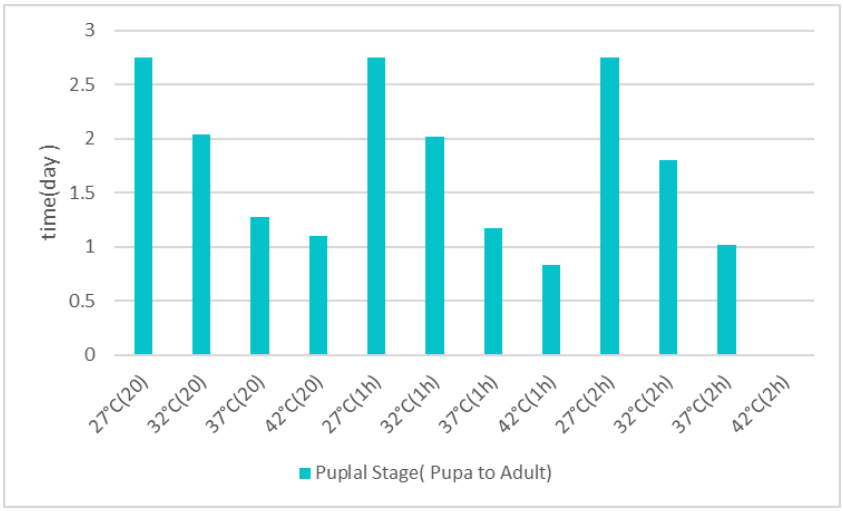

Fig. 4. Difference in duration (days) of pupal stage when heat shock was applied at different temperature (27, 32 ,37 and 42°C) for different times.

Table 5. After heat-shock viability rate of egg, larval stages and pupa at different temperature for different period of times

Experimental period	Stage	27°C	32°C	37°C	42°C
		Duration of different life cycle stages (days) ± SD			
20 minutes		100 ± 0	100 ± 0	92 ± 5.29	All died
1 hour	Egg	100 ± 0	100 ± 0	81.33 ± 4.16	
2 hours		100 ± 0	100 ± 0	74.67 ± 11.02	
20 minutes	2nd instar	100 ± 0	100 ± 0	100 ± 0	18.67 ± 4.62
1 hour	larva	100 ± 0	100 ± 0	97.78 ± 3.85	13.33 ± 6.11
2 hours		100 ± 0	100 ± 0	91.11 ± 3.85	All died
20 minutes	3rd instar	100 ± 0	100 ± 0	100 ± 0	73.33 ± 6.67
1 hour	larva	100 ± 0	100 ± 0	100 ± 0	37.78 ± 10.18
2 hours		100 ± 0	100 ± 0	100 ± 0	2.85 ± 3.85
20 minutes	Pupa	100 ± 0	100 ± 0	100 ± 0	66.67 ± 15.28
1 hour		100 ± 0	100 ± 0	100 ± 0	33.33 ± 5.77
2 hours		100 ± 0	100 ± 0	93.33 ± 11.55	3.33 ± 5.77

Table 6. Rate of wriggling movement (number of strokes per 60s) of 2nd and 3rd larval stages and pupa in control temperature (27°C) and upon heat shock (32, 37 and 42°C) at different temperature for different period of times

Heat-Shock Period	Stage	27°C	32°C	37°C	42°C
		Rate of wriggling movement per 60s ± SD			
20 minutes		94.33 ± 4.04	106.67 ± 4.73	84 ± 7.21	42.33 ± 6.66
1 hour	2nd instar	94.33 ± 4.04	91.67 ± 17.93	93.67 ± 5.13	38.67 ± 9.87
2 hours		94.33 ± 4.04	96.33 ± 6.03	65 ± 7.81	All died
20 minutes		93 ± 2.65	77.67 ± 4.04	86.33 ± 6.03	68.33 ± 8.50
1 hour	3rd instar	93 ± 2.65	68.67 ± 6.51	38.67 ± 3.21	35.67 ± 6.66
2 hours		93 ± 2.65	74.33 ± 6.66	62.67 ± 4.16	25.33 ± 4.51
20 minutes		75.33 ± 7.64	61 ± 9.64	55 ± 7	32 ± 8.19
1 hour	Pupa	75.33 ± 7.64	55.33 ± 10.97	28.33 ± 9.07	24.33 ± 5.69
2 hours		75.33 ± 7.64	48.67 ± 3.51	24.67 ± 6.35	16.33 ± 4.73

and 10.13 days respectively. The sole exception was 39° C, which suppressed embryonic development and led to larval death within hours of hatching. The negative effect of the 39° C condition is consistent with the influence of temperature on culicid vector development (Mohammed and Chadee 2011; Zequi and Lopes 2012) and suggests that 39° C approaches the lethal temperature (Mourya *et al.* 2004, Aghdam *et al.* 2009, Couret *et al.* 2014). Despite the lack of full life cycle data at 39° C, the fact that some larvae still hatched indicates a possible physiological adaptive response to high water temperatures.

In this study, when heat shock was applied to eggs, for each temperature along with the raise in heat shock period developmental stages became shorter,

except for 37°C when the egg development period got longer from 3.63 to 3.82 days upon the raise of heat shock period from 20 min to 2h. Even, while the heat shock temperatures were increased keeping heat shock period constant the result was same, except for the pupal stage which lasted for 2.65 and 2.6 for 20min and 1h respectively for 32°C. The lowest larval and pupal stage were found at 37°C upon 2h heat shock and the lowest egg development period was at 32°C for 2h heat shock (Table 1, Fig. 1), which is consistent with the previous work (Rafael *et al.* 2015). At 42°C, no hatching was recorded. So, it has been considered lethal for their growth, which appreciate the previous work (Eisen *et al.* 2014; Mourya *et al.* 2004, Aghdam *et al.* 2009, Couret *et al.* 2014).

In 2nd instar larvae, the developmental period for each life cycle stages decreased with the increase of temperature and heat shock period, except in 42°C when the larval and pupal stages measured 7.08 and 2.22 days for 20min heat shock and upon further heat shock all larvae died. The shortest larval and pupal developmental period was measured at 37°C for 2h heat shock period (Table 2, Fig. 2).

In case of 3rd instar larvae (Table 3, Fig. 4) and pupae (Table 4, Fig. 4) same decrease in developmental period for each life cycle stages with the increase of temperature and heat shock period were observed.

A previous study showed that longevity in mosquito become reduced with increasing temperatures (Beserra *et al.* 2009). However, mean viability of egg, larval, and pupal stages for all population samples was high (above 80%) for optimal growth temperature but reduced gradually above optimal temperature (Beserra *et al.* 2006, Tejerina *et al.* 2009). In this study, 100% viability have been observed while heat shock was applied to eggs, 2nd and 3rd instar larvae and pupae at 27 and 32°C for 20min, 1h and 2h. But when heat shock was applied to 37°C for different heat shock period (20min, 1h and 2h) egg, 2nd instar and pupal viability decreased from 92 to 74.67%, 100 to 91.11% and 100 to 93.33% respectively. No egg hatched after heat shock at 42°C, while few 2nd instars survived with a viability rate of 13.33%. The lowest viability rate for 3rd instar larva and pupa were counted in 42°C for 2h heat shock period was 2.22 and 3.33% respectively (Table 5). Though viability rate decreases at high temperature but the fact that some larvae still hatched indicates a possible physiological adaptive response to high water temperatures.

The change in the rate of movement decreased gradually with the increase of temperature and heat shock period in pupa. But the change was irregular in case of 2nd and 3rd instar larvae. The highest movement rate for 2nd and 3rd instar larvae were recorded at 32°C (20 min) and 27°C respectively and lowest were recorded at 42°C(1h) and 42°C(2h) respectively (Table 6). From the findings of the present study, it might be predicted that increased temperature in the

environment due to global warming may favor the population growth of *Aedes aegypti* mosquitoes by shortening duration of mosquito's life cycle leading to increase in the disease transmission rates by mosquito vectors.

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