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Manuscript title: Studies on the fitness components and comparative oviposition preferences of *Aedes albopictus* in various larval microhabitats of Burdwan, West Bengal

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Abstract
The objective of the study was to record the fitness parameters like gonotrophic cycle, fecundity, hatchability of *Aedes albopictus* mosquito. Blood was fed and the gonotrophic cycle, fecundity rate, hatchability rate and survival rate was calculated. On performing one way ANOVA it was seen that seasonal variation had a highly significant effect on fecundity ($P < 0.05$), no significant effect on survival rate ($P=0.189$), and a significant effect on percent hatchability ($P<0.05$). Oviposition activity index (OAI) was calculated from both natural and artificial breeding habitat water. Positive oviposition activity index was recorded for coconut shell water (0.40) and tyre water (0.14) while OAI was found negative for earthen pots and tree hole breeding water. No household product showed positive OAI, indicating them as repellent and no oviposition was found in red chilli and ginger solution. *Aedes* larvae rearing water was found to be positive for OAI (0.12), indicating it to be more attractant than control water amongst the artificial breeding habitat provided.

Keywords: Hatchability, Fecundity, Gonotrophic cycle, Oviposition activity index, Correlation, *Aedes albopictus.*

1. Introduction
Blood feeding mechanism of the female mosquitoes is one of the most essential survival strategy and the only pathogen transmission method. Via blood feeding on hosts, especially humans, they can transmit the pathogen and carry on with their generation. This also reflects much on their evolutionary success that they have achieved today. Blood feeding in mosquitoes represents phenotypic expression of their reproductive investment [1]. The energy otherwise allocated for the maintenance of the somatic function and daily activities, here gets used in the process of ensuring their reproductive success [2]. So the cost benefit ratio must be perfectly accounted for otherwise it is it's not wise for the female mosquitoes to invest so much of their energy in the egg laying process. Thus after their blood meal they take the least possible time to lay as many eggs as possible and aim on taking as many blood meals as possible during their life span. This also ensures successful transmission of the pathogen within that said time period. In order to understand the vector dynamics and its disease transmission capability, it is essential to study certain fitness components related to the vector.
The vector of interest here is *Aedes albopictus* (Skuse), the asian tiger mosquito, which has colonized many Southern and mid-western states in United States [3, 4]. Used tyres from Japan seem to be there source of their entry, and with the interstate movement and use of tyres it seemed to have spreaded more [5]. Both *Ae. aegypti* and *Ae. albopictus* are invasive species and are responsible for major global outbreaks of Dengue worldwide. Affecting more than 50 million people each year and causing more than 20,000 deaths [6] these flaviviruses are transmitted mainly by *Ae. aegypti*, although *Ae. albopictus* is also responsible for potential transmission [3]. *Ae. albopictus* as the second main vector of the dengue virus is a well-established fact in the Western Hemisphere [7]. Its larvae were found to be naturally infected by dengue virus types 2 and 3 in Reynosa, Mexico [8] and with type 1 virus in State of Minas Gerais, Brazil [9]. On a very alarming note, Nasci *et al.* [10] observed insemination of *Ae. albopictus* male on *Ae. aegypti* female and Forattini [11] alerted that *Ae. albopictus* is able to replace *Ae. aegypti* ecological niche at any moment. Since *Ae. albopictus* is more of an intriguing problem right now, it calls for a solution to eradicate it. Little of the work has been

done in West Bengal on its fitness components which include its gonotrophic cycle, survival rate, percent hatchability, fecundity rate and the relationship of all of these to the environmental parameters like temperature, relative humidity and effect of seasons on them. This mosquito has an eclectic and heterogeneous preference by breeders such as containers made of metal, glass, stone, earthenware, plastic, wood or rubber and tree holes, bamboo stumps, rock pools, leaf axils [3]. So a comparative oviposition preference study has also been carried out on both natural and artificial breeding water and they are forced to lay eggs on these so as to study their oviposition activity index (OAI).

2. Materials and Methods

2.1 Study period and mosquito cage culture

The entire experiment was conducted during the time period January 2014–December 2014. *Ae. albopictus* mosquitoes were hatched, reared, maintained and cultured for several generations in the mosquito insectary of the Parasitology and Microbiology Research laboratory, Zoology Department, The University of Burdwan. Adult mosquitoes were maintained in optimum conditions of 25±2 ºC temperature, 75±5% relative humidity and 12:12 h (light: dark) photoperiod in the insectary [12]. Male and female mosquitoes were housed together in the same cage (30cm x30cm x 30cm) for about 5-6 days in order to mate. Honey soaked cotton pads were provided as sugar source to them prior to blood meal. Afterwards females were separated and taken to separate cages for blood meal.

2.2 Blood feeding

Mated females were collected and fed upon rabbit blood in order to undergo egg maturation prior to testing. The gravid females were then collected and kept into three different cages. About 100 gravid females were put into cage 1, and 50 gravid female each in cage 2 (natural) and cage 3 (artificial). The date of the blood meal was recorded.

2.3 Setting up of cages

Cage 1: In this cage about 100 gravid females were released. Small white enamel bowls with aged tap water and soaked filter paper were kept to oviposit. Honey soaked cotton pads were also kept. The day of egg laying is noted down and the eggs were collected from the respective filter paper and counted. These eggs were kept in the desiccators for one month in order to study the hatchability rate after 30 days [13]. This process was repeated every month for whole one year. Blood meal was provided only once every month in this cage and again a second batch of gravid females were kept for the second month study. For the entire study, data on fecundity, hatchability, survival rate were noted from this cage. The environmental temperature and the relative humidity were noted down in each month throughout the year.

Cage 2: In this cage, marked as "natural", 50 gravid females were released. Natural ovitraps containing strips of filter paper were kept in small white enamel bowls containing coconut shell water, tree hole water, tyre water, earthen pot water. All these habitat water were collected from the natural breeding habitat of *Aedes* sp. A control ovitrap with a strip of filter paper was also kept which had aged tap water. Occasional shifting of the bowls was done to avoid any kind of positional biasness. Number of eggs laid in each ovitrap and the control were counted and compared.

Cage 3: Marked as "artificial", this cage contained ovitraps that were mainly made up of household products. Various household substances (1gm or 1ml) in 100 ml distilled water were offered to 50 gravid females here along with the control aged tap water bowl. Substances were: a) salt b) sugar c) turmeric d) red chilli powder e) crushed ginger f) *Aedes* larvae rearing water g) distilled water. Occasional shifting to avoid positional biasness was also done here. Eggs were allowed to be laid and were counted on each ovitrap and compared.

2.4. Fecundity, Gonotrophic cycle, Survival rate and Hatchability

Fecundity rate is determined by the number of eggs laid per female, i.e fecundity rate is: total number of eggs laid / total number of gravid females. Gonotrophic cycle is determined the number of days taken to oviposit from the day of the blood meal. Survival rate is determined by the percentage of female mosquitoes that survived for 30 days after the first blood meal. For hatchability studies, randomly 500 eggs were collected and kept in enamel trays for 4 consecutive days. At the end of 4 days number of hatched out larvae were counted and hatchability rate was determined by: total number of hatched out larvae/ total number of eggs tested (n =500) [12]

2.4 Determination of Oviposition Activity Index (OAI)

The comparative oviposition preferences and attractiveness was expressed by oviposition activity index (OAI), calculated according to Kramer and Mulla [13], where

Oviposition activity index = (NT-NS)/ (NT+NS),

Where NT denotes= number of eggs laid in the test water and NS= number of eggs laid in the control water. Index values ranges from +1 to -1, with a positive value indicating that the test water is more of oviposition attractant than the control and a negative value indicating vice-versa, concluding the test water to act as an a repellent.

2.5 Statistical analysis

Statistical analysis was done by SPSS 17.0 software. One way ANOVA was done to see the effect of seasons on the fecundity, survival rate and percent hatchability and to determine any possible interactions that may exist between them. Correlation (Pearson's coefficient) was done between environmental parameters and gonotrophic cycle and number of eggs laid.

3. Results & Discussions

Table 1 shows the season wise dependence of the fecundity, number of eggs laid, gonotrophic cycle, survival rate, percent hatchability. Variation of the above said parameters with the environmental parameters like environment temperature, relative humidity has also been shown. On performing one way ANOVA it was seen that seasons had a highly significant effect on fecundity ($P < 0.05$), no significant effect on survival rate ($P=0.189$), and a significant effect on percent hatchability ($P<0.05$). Correlation graphs from fig 1 to 4 shows very low positive correlation between environment temperature and no. of eggs laid (Pearson's coefficient, $r=0.11$), a negative correlation between environment temperature and gonotrophic cycle (Pearson's coefficient, $r= -0.49$), a highly positive and significant correlation between relative humidity and no. of eggs laid (Pearson's coefficient, $r= 0.86$) and a negative correlation between relative humidity and gonotrophic cycle (Pearson's coefficient, $r= -0.13$) respectively.

3.1. Tables and Figures

Table 1: Fecundity, survivability, hatchability rate of *Aedes albopictus* throughout the year January 2014 –December 2014

Months	Environment temperature	Relative Humidity (%)	Gonotrophic cycle (in days)	No. of eggs laid	Fecundity (%)	Survival rate (%)	Hatchability (%)
January	18.12 ± 0.20	44	4	1786.2±2.48	17.86	8	13.9
February	19 ± 0.16	67	4	1612.0±2.07	16.12	4	12.15
March	30.7 ±0.18	62	3	1586 ±1.74	15.86	2	12.52
April	33.48± 0.16	48	4	1548.8 ±2.54	15.48	1	11.23
May	38.3 ± 0.17	42	4	1450.8± 3.00	14.50	0	11.21
June	37.52± 0.43	65	3	2046.1±2.42	20.46	6	28.57
July	29.3 ± 0.21	98	3	2325.4±7.52	23.25	1	54.32
August	32.8 ± 0.20	92	2	2450.2±5.77	24.50	1	62.2
September	32.6± 0.29	93	2	2563.2 ±2.69	25.63	3	74.9
October	29.2 ±0.18	87	5	2317.4 ±2.42	23.17	4	43.6
November	25.2 ± 0.15	82	6	2237.8±3.08	22.37	6	33.34
December	22.3 ± 0.15	81	6	1820±8.14	18.20	9	20

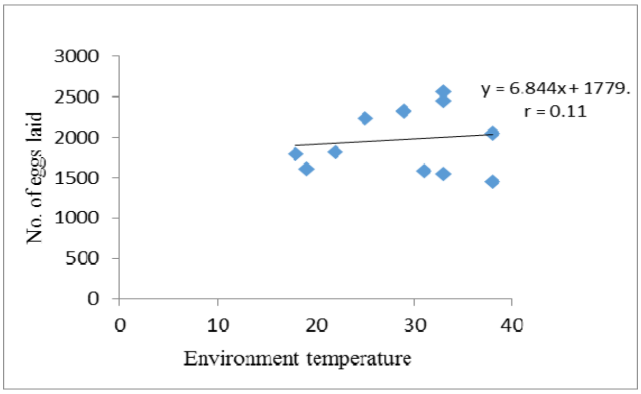

Fig 1: Correlation between no. of eggs laid and environment temperature where r= 0.11.

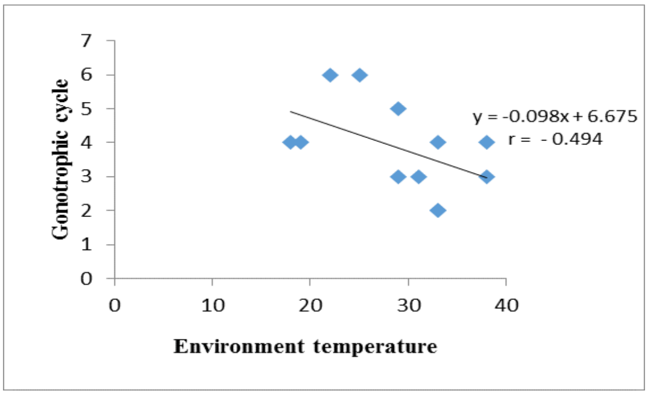

Fig 2: Correlation between environment temperature and gonotrophic cycle where r= -0.49.

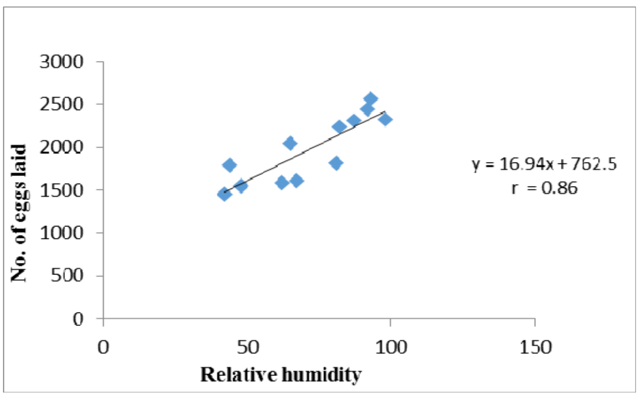

Fig 3: Correlation between relative humidity and no. of eggs laid where r= 0.86

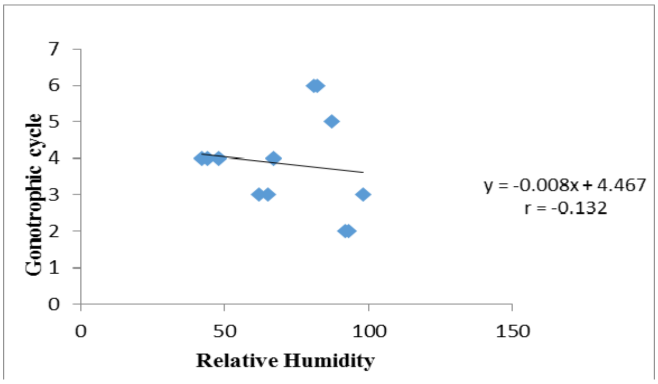

Fig 4: Correlation between relative humidity and gonotrophic cycle where r= - 0.13.

Table 2 depicts the oviposition activity index (OAI) for both natural and artificial breeding water. In case of natural water, the OAI of coconut shell water (0.40) and tyre water (0.14) were found to be positive, which indicates that these test waters are more attractive for oviposition than the control water. Negative OAI was observed for earthen pot water and tree hole water suggesting less oviposition attractant. For artificial breeding water only *Aedes* larvae rearing water was found to be positive for OAI (0.12), indicating it to be more attractant than control water. No oviposition occurred in red chilli and ginger solution, whereas negative OAI was observed for sugar solution, salt solution and turmeric solution.

Table 2: Oviposition activity index of different types of habitat compared to the given control habitat.

Natural Habitat Water **Control habitat: Aged tap water; no. of eggs laid =325**		
Types of habitat water provided	**No. of eggs laid**	**Oviposition Activity Index (OAI)**
Coconut shell water	763± 6.41	0.40
Earthen pot water	225±3.23	-0.18
Tree hole water	147±2.56	-0.37
Tyre water	435±4.20	0.14
Artificial Habitat Water **No of eggs laid in control aged tap water habitat: 302**		
Types of habitat water provided	**No. of eggs laid**	**Oviposition Activity Index (OAI)**
Sugar solution	210.2 ± 1.82	-0.17
Salt solution	66.0 ±2.04	-0.64
Turmeric solution	284.6 ±1.96	-0.03
Red chilly solution	0	0
Ginger solution	0	0
Aedes larvae rearing water	388.4±1.20	0.12
Distilled water	271.8±1.82	-0.05

Knowledge about vector dynamics is very much essential for effective management strategy. Studies have been done regarding height specific oviposition responses [14] but very little literature is present on the fitness components of *Ae. albopictus* and ovipositional site preferences. Maciá [15] studied fitness components like pupal mass, fecundity, body weight, development time, survivorship in relation to larval density and container type and suggested intraspecific competition and dependence of the larval density on the container type. Present study showed seasonal temperature fluctuations to have a highly significant effect on the fecundity of female mosquitoes whereas the female longeivity or the survival rate after the first haematophagic activity was shown to be insignificant with temperature variations. In contrast to this, female longevity and fecundity both were seen to be directly affected by increased temperatures in some piece of work [16]. Fecundity of *Ae. albopictus* females were directly correlated with their body size and various work showed a positive relationship between them. However the regression may vary among the strains of the same species [17-25]. Larger females were seen to lay more number of eggs after their first gonotrophic cycle [26]. Size of females limits the maximum egg laying capability in some mosquitoes, as was shown by some workers [27-28]. A complex relationship happens to exist between blood meal size, time of meal, gonotrophic cycle, number of eggs laid and body size [28]. Gonotrophic cycle is negatively related to the temperature fluctuations and relative humidity, thus suggesting temperature has nothing to do with when to lay eggs. They probably lay eggs whenever their maturation occurs. However number of eggs laid was highly correlated with the relative humidity, but low on relation with temperature. Number of eggs laid depended a lot on post emergence factors like amount of blood meal, mating time, quality of blood intake [19, 20, 22, 29, 27, 28]. Also body size controlled numbers of eggs laid, maximum the size more the potential for higher fecundity. Larger females were supposed to accumulate more resources from the blood meal for oogenesis and thus lay more eggs [22].

Hatchability tends to be temperature and rainfall specific. With decrease in temperature or the photoperiod, there tends to be a sharp fall in the egg hatching process. Hatchability percent was highest in monsoon and post monsoon period. Hatchability as low as 11% and as high as 74% surely does have some valid significance in the bionomics of the vector. Failure in egg hatching and factors responsible for it had been an intriguing topic for a lot of workers. Major reason cited out for egg hatch failure is the immaturity due to non-insemination of a teneral female [30]. Adequate and appropriate flooding showed to be essential for egg hatchability. Khatchikian *et al.* [31] suggested that hatchability increases with consistent rainfall and egg hatches out faster in low desiccation areas. Present finding was consistent with his study, proving the truth of the fact once again. In fact, eggs of *Aedes* mosquitoes remain dormant for a very long time until and unless appropriate environmental stimuli and flooding activate the pharate larvae [32]. Only a few of the eggs hatch on obtaining the first stimuli, the rest of it requires more stimuli and environmental push to hatch further [30]. This erratic hatching strategy is thought to be the evolutionary protocol to safeguard themselves from the adversities of the nature [33] and further competitive interactions [34]. Moreover, increase in temperature seems to enhance the risk of hatch failure. An area where rainfall is high, hatchability was also seen to be higher, irrespective of the environment temperature. But in places where rainfall was low or months when rainfall was minimum, high temperature tended to hatchability failure and caused a delay in larval eclosion. Eggs tended to wait for the right stimulus at that time and shifted towards the diapause stage.

Water logged coconut shell and tyres had shown to have high oviposition activity index. They are more attracting to oviposit than aged tap water, as the experiment had shown. It may be probably due to the fact that coconut water was highly rich in nutrient composition. They are rich in minerals like phosphate, calcium, magnesium, nitrate etc. Tyre water also was also found to be rich in nutrient composition and moreover they prevent desiccation [35-36]. In a work done by Ponnusamy L *et al.* [37], bamboo leaf infusions and white oak leaf infusions were shown to be acting as an attractant for gravid female *Aedes* sp, compared to plain water. On the other hand, artificial breeding water like salt solution, sugar solution, and turmeric solution showed negative for oviposition, acting as a deterrent. Though sugar solution may act as an oviposition attractant, but here compared to the aged tap water its oviposition index has dropped significantly. *Aedes* mosquitoes are known to use their olfaction and visual cues to judge the chemical and physical factors present in the breeding water prior to egg laying [38]. Some of the commonly used cues are colour and optical density of water, oviposition substrate, temperature, olfactory cues and chemical cues provided by mosquito larvae [39]. Yap HH *et al.* [40] showed that visual cues form to be a very important part in the egg laying process. They studied and found out that dark coloured containers attracted more females than the light coloured ones. Moreover, eggs were not laid in saline water as compared to seasoned tap water. Conclusion can be drawn that there must be certain factors released from the deterrent chemicals that does not allow the female mosquitoes to choose them as their suitable oviposition substrate [41]. Thus there must be some steps taken in order to analyze the chemicals released by these household deterrents so as to actively utilizing it in the *Aedes* sp management strategy.

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5. References

1. Roitberg BD, Sircom J, Roitberg CA, Vanalphen JJM, Mangel M. Life expectancy and reproduction. Nature (Lond.) 1993; 364:108.
2. Leisnham PT, Sala LM, Juliano SA. Geographic variation in adult survival and reproductive tactics of the mosquito *Aedes albopictus*. Med Entomol 2008; 45:210-221.
3. Hawley WA. The biology of *Aedes albopictus*. J Am Mosq Control Assoc (Suppl.) 1988; 1:1-40.
4. Rai KS. *Aedes albopictus* in the Americas. Annual Review of Entomology 1988; 36:459-484.
5. Hawley WA, Reiter P, Copeland RS, Pumpuni CB, Craig GB. *Aedes albopictus* in North America: probable introduction in used tyres from northern Asia. Science 1987; 236:1114-1116.
6. Burke DS, Monath TP. Flaviviruses. Fields Virology, 2001, 1043-1125.
7. Knudsen AB, Romi R, Majori G. Occurrence and spread in Italy of *Aedes albopictus*, with implications for its introduction into other parts of Europe. J Am Mosq Control Assoc. 1996; 12:177-183.

8. Ibáñez-Bernal S, Briseño B, Mutebi JP, Argot E, Rodríguez A, Martínez-Campos C *et al*. First record in America of *Aedes albopictus* naturally infected with dengue virus during an outbreak of Reynosa, Mexico in 1995. Med Vet Entomol 1997; 11:305-309.
9. Serufo JC, Montes Oca de H, Tavares VA, Souza AM, Rosa RV, Jamal MC *et al*. Isolation of dengue virus type 1 from larvae of *Aedes albopictus* in Campos Altos City, State of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 1993; 88:503-504.
10. Nasci RS, Hare SG, Willis FS. Interspecific mating between Lousiana strains of *Aedes albopictus* and *Aedes aegypti* in the field and laboratory. J Am. Mosq. Control Assoc. 1989; 5:416-421.
11. Forattini OP. Mosquitos como vetores emergentes de infecções. Rev. Saúde Pública 1998; 20:497-502.
12. Deng L, Koou SY, Png AB, Ng LC, Lam-Phua SG. A novel mosquito feeding system for routine blood-feeding of *Aedes aegypti* and *Aedes albopictus*. Tropical Biomedicine 2012; 29(1):169-174.
13. Kramer WL, Mulla MS. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. Environ Entomol 1979; 8:1111-1117.
14. Williges E, Faraji A, Gaugler R. Vertical Oviposition Preferences of the Asian Tiger Mosquito, Aedes albopictus, In Temperate North America. J Am Mosq Control Assoc. 2014; 30(3):169-74.
15. Maciá A. Differences in performance of *Aedes aegypti* larvae raised at different densities in tyres and ovitraps under field conditions in Argentina. J Vector Ecol. 2006; 31:371-377.
16. Calado DC, Navarro-Silva MA. Influência da temperatura sobre a longevidade, fecundidade atividade hematofágica de *Aedes (Stegomyia) albopictus* Skuse, 1894 (Diptera, Culicidae) sob condições de laboratório Revta. Bras. Ent 2002; 46:93-98.
17. Livdahl TP, Sugihara G. Non-linear interactions of populations and the importance of estimating per capita rates of change. J Anim. Ecol. 1984; 53:573-580.
18. Packer MJ, Corbet PS. Size variation and reproductive success of female *Aedes punctor* (Diptera: Culicidae). Ecol. Entomol 1989; 14:297-309.
19. Briegel H. Fecundity, metabolism, and body size in Anopheles (Diptera: Culicidae), vectors of malaria. J Med. Entomol. 1900a; 27:839-850.
20. Reeves WC. Epidemiology and Control of Mosquito-Borne Arboviruses in California, 1943- 1987. California Mosquito and Vector Control Association, Sacramento, CA, 1990, 508.
21. Bradshaw WE, Holzapfel CM. Reproductive consequences of density-dependent size variation in the pitcher plant mosquito, Wyeomyia smithii (Diptera: Culicidae). Ann Entomol. Soc. Am 1992; 85:274-281.
22. Clements AN. The Biology of Mosquitoes: Development, Nutrition, and Reproduction. Chapman & Hall, London 1992; 1:509.
23. Siegel JR, Novak R, Lampman, Steinly B. Statistical appraisal of the weight-wing length relationship of mosquitoes. J Med. Entomol. 1992; 29:711-714
24. Renshaw M, Service MW, Birley MH. Size variation and reproductive success in the mosquito *Aedes cantans*. Med. Vet. Entomol 1994; 8:179-186.
25. Hogg JC, Thomson MC, Hurd H. Comparative fecundity and associated factors for two sibling species of the *Anopheles gambiae* complex occurring sympatrically in The Gambia. Med. Vet. Entomol 1996; 10:385-391.
26. Suleman M. Intraspecific variation in the reproductive capacity of *Anopheles stephensi* (Diptera: Culicidae). J Med. Entomol. 1990; 27:819-828.
27. Hurd H, Hogg JC, Renshaw M. Interactions between blood feeding, fecundity and infection in mosquitoes. Parasitol. Today 1995; 11:411-416.
28. Frankino WA, Juliano SA. Costs of reproduction and geographic variation in the reproductive tactics of the mosquito *Aedes triseriatus*. Oecologia 1999; 120:59-68.
29. Shieh JN, Rossignol PA. Opposite influences of host anaemia on blood feeding rate and fecundity of mosquitoes. Parasitology 1992; 105:159-163.
30. Aida HN, Dieng H, Ahmad AH, Satho T, Nurita AT, Salmah MRC, *et al*. The biology and demographic parameters of *Aedes albopictus* in northern peninsular Malaysia. Asian Pac J Trop Biomed. 2011; 1(6):472-477.
31. Khatchikian CE, Dennehy JJ, Vitek CJ, Livdahl T. Environmental effects on bet hedging in *Aedes* mosquito egg hatch. Evol Ecol 2010; 24(5):1159-1169.
32. Novak RJ, Shroyer DA. Eggs of *Aedes triseriatus* and *A. hendersoni*: a method to stimulate optimal hatch. Mosq News 1978; 38:515-521.
33. Khatchikian CE, Dennehy JJ, Vitek CJ, Livdahl T. Climate and geographic trends in hatch delay of the treehole mosquito, *Aedes triseriatus* Say (Diptera: Culicidae). J Vector Ecol. 2009; 34(1):119-128.
34. Dieng H, Boots M, Tamori N, Higashihara J, Okada T, Kato K, *et al*. Effects of food, embryo density and conspecific immatures on hatchability in the dengue vector *Aedes albopictus*. House Househ Insect Pest 2007; 28:117-127.
35. Medlock JM, Avenell D, Barrass I, Leach S. Analysis of the potential for survival and seasonal activity of Ae. albopictus (Diptera: Culicidae) in UK. J Vector Ecol. 2006; 31:292-304.
36. Straetemans M. Vector related risk mapping of the introduction and establishment of *Ae. albopictus* in Europe. Euro Surveill 2008; 13:14.
37. Yap HH, Lee CY, Chong NL, Foo AE, Lim MP. Oviposition site preference of Aedes albopictus in the laboratory. J Am Mosq Control Assoc. 1995; 11(1):128-32.
38. Muir DA. Anopheline mosquitoes: Vector reproduction, life cycle and biotype. *In: Wernsdorfer WH, McGregor I, editors. Malaria, principals and practice of malariology*. London: Churchill Livingstone, 1988, 431-51.
39. Jonathan DT, Charles SA, Coby S. Laboratory and field evaluations of oviposition responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. J Med Entomol. 1998; 35:967-76.
40. Ponnusamy L, Xu N, Nojima S, Wesson DM, Schal C, Apperson CS. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. Proceedings of the National Academy of Sciences April, 17, 2008; 105(27):9262-9267.
41. Tilak R, Gupta MJ, Suryam MV, Yadav JD, Dutta Gupta BKK. A Laboratory Investigation into Oviposition Responses of *Aedes aegypti* to Some Common Household Substances and Water from Conspecific Larvae. MJAFI 2005; 61:227-229.