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Research Article

Studies on the Survivorship of *Aedes albopictus* (Diptera: Culicidae) *in vitro* in differentiated breeding water from some parts of district Dehradun, Uttarakhand

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Abstract

Survival of arthropod vectors is one of the most important components of transmission of a vector-borne pathogen. In general, any influence on the vector that increases female longevity will increase the likelihood of survival through the extrinsic incubation period of the virus and the risk of disease transmission. Survivability rate of *Aedes albopictus* was determined during the month of April 2015 based on the breeding success on 03 experimental parameters *viz.,* aged tap water, natural water and distilled water, by constructing a cohort (horizontal) life table. Survivability rate of *Aedes albopictus* was higher where eggs were emerged in natural water resources, followed by aged tap water. Basic reproductive rate (R_o) of natural water resources accounted highest followed by aged tap water and distilled water. On performing the single way ANOVA, *p* value found higher than 0.001 concluding the fact that survivability was not independent of water resources. However, while performing Kaplan-Meier survivability test over age dependent parameters no such determining variation was registered. The value of Log rank test (Mantel Cox) abolish the null hypothesis suggesting the fact that there was significant difference in overall survival distribution among the three experimental parameters. *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. Thus, there must be some steps taken in order to analyse the chemicals released by these household deterrents so as to actively utilizing them in the *Aedes* management strategy.

Keywords: *Aedes albopictus*, Experimental waters, Cohort life table, Kaplan-Meir survivability

Introduction

The survivability / longevity of mosquitoes have been the topic of numerous studies, dating back to 1933.[1] In general, any influence on the vector that increases female longevity will increase the likelihood of survival through the extrinsic incubation period of the virus and the risk of disease transmission.[2]

In nature few organisms die of senescence, most being killed by predators, disease and other hazards long before they reach old age[3] and as a corollary of that belief it is sometimes deduced that mortality rates are independent of age.[4] In wild population of female adult mosquitoes the mortality rates are independent of age[5] although it was recognized that mortality rates are some sort of higher in older females.[6, 7] From a theoretical analysis of

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mosquito cycles it was seen that the survival rate is the most important factor determining the stability of the population and total egg production.[8] Mosquito longevity is a limiting factor for the transmission of mosquito-borne pathogens, particularly as the pathogens require a period of time to penetrate the midgut lining from an infectious blood meal, replicate and disseminate through the mosquito and infect the salivary glands to enable transmission. High mosquito survival is therefore critical to transmission cycles and reducing mosquito survival rates is a priority of disease control programs.[9] Model based studies over survivability of *Aedes aegypti* and *Aedes albopictus* mosquitoes were carried out by several workers.[10-12] The combination of higher temperatures and water renewal found to increase egg viability and shortened the incubation period along with quick metamorphosis in *Aedes aegypti* and *Aedes albopictus*.[13-16] Some notable works on immature and adult survivorship of *Aedes albopictus* and *Aedes aegypti* mosquito were Li et al.[17] and Hugo et al.[18] respectively. There are ample of works on the physico-chemical characteristics of mosquitoes breeding water and larval density and population from different areas.[19-25] Moreover, still there is no observation that relates the physico-chemical properties of breeding water with survivorship of *Aedes* mosquitoes. So, the objectives of the present study were to perceive the preliminary idea on survivorship of *Aedes albopictus* mosquitoes that emerged from different breeding water sources of varying chemical compositions.

Materials and Methods

Mosquito Rearing

Aedes albopictus species have been hatched, reared, maintained and cultured for several generations in the mosquito insectaries of the Parasitology Research laboratory, Zoology Department, D.A.V. (P.G.) College, Dehradun, Uttarakhand. All the mosquitoes were maintained in 25±2ºC, 75±5% relative humidity and 12:12 h (light: dark) photoperiod in the insectary. Male and female mosquitoes were housed together in the same cage (30 cm x 30 cm x 30 cm) for about 5-6 days prior to blood meal in order to mate. Freshly laid eggs were monitored and accessed for the survivability experiments during the month of April 2015. Once adults were emerged honey-soaked cotton and albino rat were periodically introduced in the cages for males and blood feeding for gravid females during post mating session.

Experimental Set up

Three parameters of breeding water were categorized for the experiments as aged tap water, natural water and distilled water. The categorized water was also collected from 4 varying conditions and analysed for temperatures, pH, total hardness, conductivity, chloride, dissolved oxygen by following standard APHA procedures.[26]

- **Control (Eggs in aged tap water):** Small white enamel bowls with aged tap water of varying time period were kept inside the insectary. 120 eggs were kept in the 4 bowls with 30 eggs each and treated as replicate I, II, III and IV. Occasional shifting of the bowls were done to avoid any kind of positional biasness. After emerging periodical changes of water was performed. This experiment set up was considered as "control".
- **Natural (Eggs in natural water resources):** An insectary was marked as "natural" and water from natural breeding habitats of *Aedes* like tree holes, tires, earthen pots and coconut shells were used for this experiment as replicate I, II, III and IV. Shifting of bowls and periodical changes of natural water were also performed as such.
- **Artificial (Eggs in distilled water):** Here white enamel bowls were filled up with distilled water with same number of eggs in each bowl and marked as "artificial" and followed the experimental techniques.

Survivability Rate Determination

Survivability rate of *Aedes albopictus* was determined by constructing a cohort (horizontal) life table following the standard protocols.[27] To make a life table for the *Aedes* species, we count (or estimate) the population size of at each life stage and the number of eggs produced. The developmental stages were divided into 7 stages starting from egg, with 4 stages of larval development, followed by pupa and adult. The basic reproductive rate (R_0) was calculated to determine population replacement rate using the formula of Begon et al.[27]

Basic reproductive rate (R_0) = Proportion surviving (l_x) x Individual fecundity (m_x)

Where $l_x = a_x/a_o$ a_x = No. of individuals living at the beginning of each age

a_o = Initial no. of eggs

$m_x = F_x/ a_x$ F_x = Eggs produced at each stage

Statistical Analysis

Statistical analysis was done by SPSS 17.0 software. One-way ANOVA was done to observe the variations in the physico-chemical factors and the effect over the survivability of *Aedes albopictus*. The Kaplan-Meier method[28] also known as the "product-limit method", a nonparametric method used is to estimate the probability of survival past given time points (*i.e.,* it calculates a survival distribution) used for survival distribution among the three experiment parameters.

Ethics

Since during the research work utmost care was taken about the mosquitoes being confined only inside the cages in the research laboratory and did not involve any direct human interaction, it was cleared that no ethical issues were involved.

Results

Table 1 shows the means and the standard deviations (SD) for the physico-chemical factors being associated with different experimental breeding waters. Total hardness and conductivity did not vary significantly by the experimental waters. There were significant differences along temperature, pH, chloride, fluoride and dissolved oxygen between aged tap water and natural breeding water. In distilled water except temperature and pH, all

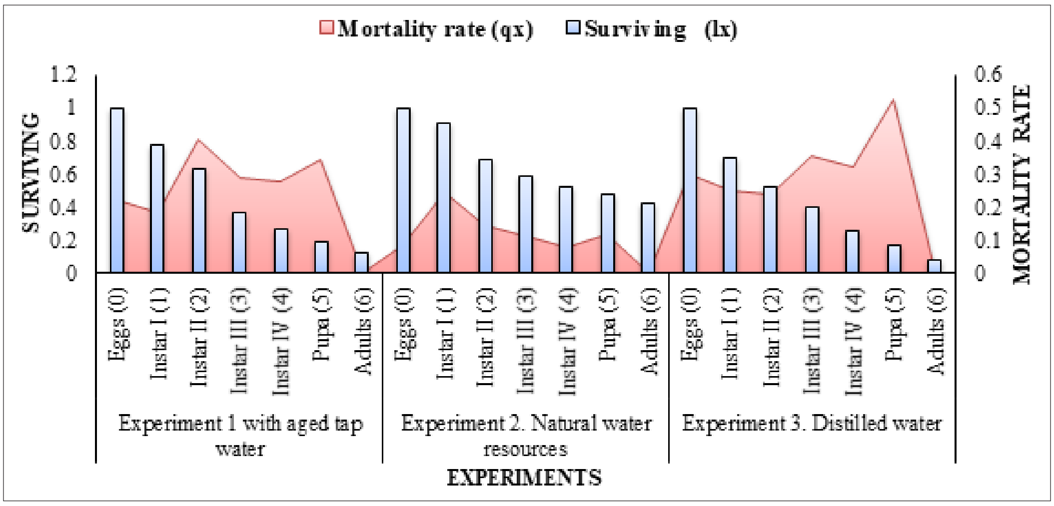

Figure 1.Comparison between mortality rate and surviving values between three experiment set ups

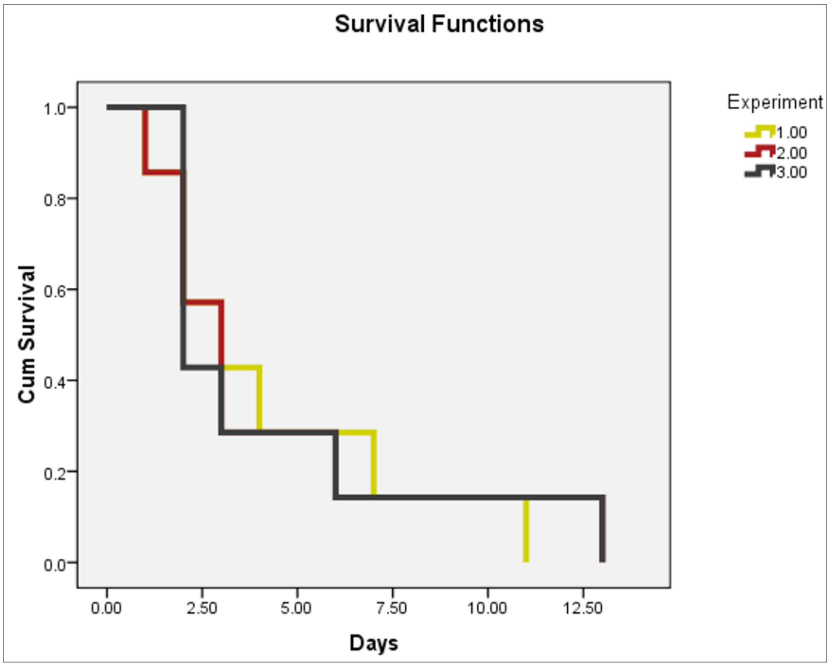

Figure 2.Kaplan-Meier survivability table

1= Aged tap water
2= Natural water resources
3= Distilled water

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Table 1.Cohort (horizontal) life table of *Aedes albopictus*

Experiment	Stage (x)	No. of living at each stage (a_x)	Proportion of original cohort surviving to each stage (l_x)	Proportion of original cohort dying during each stage (d_x)	Mortality rate (q_x)	Eggs produced at each stage (F_x)	Eggs produced per surviving individual at each stage (m_x)	Eggs produced per original individual in each stage ($l_x m_x$)= R_0
Experiment 1 with aged tap water	Eggs (0)	120	1	0.225	0.225	-	-	-
	Instar I (1)	93	0.775	0.142	0.183	-	-	-
	Instar II (2)	76	0.633	0.258	0.407	-	-	-
	Instar III (3)	45	0.375	0.109	0.290	-	-	-
	Instar IV (4)	32	0.266	0.075	0.281	-	-	-
	Pupa (5)	23	0.191	0.066	0.345	-	-	-
	Adults (6)	15	0.125	-	-	315	21	2.625
Experiment 2. Natural water resources	Eggs (0)	120	1	0.084	0.084	-	-	-
	Instar I (1)	110	0.916	0.225	0.245	-	-	-
	Instar II (2)	83	0.691	0.1	0.144	-	-	-
	Instar III (3)	71	0.591	0.066	0.111	-	-	-
	Instar IV (4)	63	0.525	0.042	0.08	-	-	-
	Pupa (5)	58	0.483	0.058	0.120	-	-	-
	Adults (6)	51	0.425	-	-	624	12.23	5.19
Experiment 3. Distilled water	Eggs (0)	120	1	0.3	0.3	-	-	-
	Instar I (1)	84	0.7	0.175	0.25	-	-	-
	Instar II (2)	63	0.525	0.125	0.238	-	-	-
	Instar III (3)	48	0.4	0.142	0.355	-	-	-
	Instar IV (4)	31	0.258	0.083	0.321	-	--	-
	Pupa (5)	21	0.175	0.092	0.525	-	-	-
	Adults (6)	10	0.083	-	-	134	13.4	1.11

other physico-chemical parameters were found negligible due to the typical nature of distilled water.

Overall cohort life tables of the single generation mosquito were represented in Table 2 and Figure 1 showing survivability rate was higher in eggs those were emerged from natural water resources, followed by aged tap water whereas overall minimum survivability was found in distilled water. Moreover, the mortality rates were just in opposite phenomenon showing distilled water > aged water > natural water resources. In view of basic reproductive rate (R_0), natural water resources accounted highest (5.19) followed by aged tap water (2.625) and distilled water (1.11) (Table 2). On performing the single way ANOVA, *p* value found higher than 0.001 concluding the fact that survivability was not independent of water resources. However, while performing Kaplan-Meier survivability test over age dependent parameters, no such determining variation was registered (Figure 2). Log rank test (Mantel Cox) was run to calculate the survival functions. To test this null hypothesis, the log rank test calculates a χ^2-statistic (the "Chi-Square" column), which was compared to a χ^2-distribution with two degrees of freedom (the "df" column). The value ($\chi 2$ = 0.057; Sig. 0.972) abolish the null hypothesis suggesting the fact that there was significant difference in overall survival distribution among the three experimental parameters.

Discussion

Knowledge about vector dynamics is very much essential for effective management strategy. In Argentina a study on fitness components of *Aedes aegypti* 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.[29] The present findings

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showed higher larval survivability rate in natural and aged tap water having significant relation of physico-chemical parameters like temperature, pH, chloride, fluoride and dissolved oxygen with larval development were supported by the earlier studies.[19, 22, 25] The larval density of *Aedes* mosquitoes species were found positive relationship with temperature and dissolved oxygen and negative impact with pH as mentioned in the earlier studies.[21, 23, 25]

The earlier observation[13, 15] on influence of temperature on the development of *Aedes albopictus* found that the minimum period of immature development and intrinsic rate of growth were found at 30ºC and also highest immature survival rates were encountered within 20-30ºC. The study focus on role of habitat on immature development and adult survivorship and concluded that immature development time was significantly shorter and adult lifespan was significantly longer in urban in comparison to suburban habitats.[17] *Aedes* species were observed high in tropical countries due to the prolonged existence of dry season.[18] With the surge of urbanization in Dehradun the utilities of stored natural water and tap water increases which harmonized with the *Aedes albopictus* species breeding efficiency probably due to highly rich in nutrient composition along with prevent against desiccation.[30, 31] Generally number of eggs laid depended a lot on post emergence factors like amount of blood meal, mating time, quality of blood intake and ovipositional water quality.[32-34] Thus there must be some steps taken in order to analyse the chemicals released by these household deterrents so as to actively utilizing them in the *Aedes* management strategy.

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Conflict of Interest: None

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