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Studies on the Impact of Thermal Stress on Survival and Development of Adaptive Thermotolerance in Immature Stages of *Anopheles culicifacies*

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Abstract

Background & objectives: Not much is known about the impact of temperature on the survival of a number of important vector species of mosquitoes. Most survival analyses of mosquitoes are reported in adults and not in immature stages. Impact of thermal stress and development of adaptive thermotolerance in late third instar larvae of *An. culicifacies* was evaluated in the laboratory.
Methods: Late third instar larvae were exposed to temperatures of 37°C, 39°C, 41°C, 43°C and 45°C and lethal time to cause 50% mortality (LTM_{50}), LTM_{90} values were calculated. To assess the adaptive thermotolerance, larvae were exposed to 37°C and 39°C and these thermally pre-adapted larvae were later re-exposed to higher temperature of 43˚C and 45˚C.
Results: All larvae survived at 37°C up to 360 minutes whereas at 45°C they succumbed within 60 minutes of exposure. Larvae pre-adapted at 37°C and 39°C were re-exposed to 43°C and 45°C and larvae pre-adapted at 39°C were more thermo-tolerant than larvae pre-adapted at 37°C which depended on exposure time.
Interpretation & Conclusions: Pre-adapted larvae derived from higher temperature were more thermotolerant than the larvae pre-adapted at lower temperature. This preliminary study on the impact of temperature on thermotolerance of larvae of *An. culicifacies* has highlighted the importance of the studies on the interaction of abiotic factors specially temperature on the survival of disease vectors.

1. Introduction

Geographical expansion of vector-borne diseases in several continents has been partially associated to changes in global warming [1]. Most of such diseases are transmitted by mosquitoes, whose developmental cycle is affected by temperature [2]. At increased temperature, the rate of digestion of blood meal increases which in turn accelerates the ovarian development, egg laying, reduction in duration of the gonotropic cycle and increased frequency of feeding on hosts thereby increases the probability of transmission [3]. Surprisingly, little is known about the impact of temperature on the survival of a number of important vector species of mosquitoes [4, 5]. So far most survival analyses of mosquitoes are reported on adult [6] and not in immature stages. Lassiter et al. [7] in his studies reported that temperature has a major effect on the rate at which the immature stages of insects develop into adults. Further, it is well known that the transmission of the disease depends on various extrinsic and intrinsic factors including the density of adult mosquitoes which in turn are directly dependant on the survival of the immature stages. In some studies heat induced adaptive thermotolerance in mosquitoes has shown increased tolerance to higher temperatures [8].

Hence, we present results of a preliminary study undertaken to investigate the impact of a range of temperatures on survivability and development of adaptive thermotolerance on immature stages of *An. culicifaies* under laboratory conditions.

2. Materials and Methods

Mosquito rearing

An. culicifacies was maintained in an insectary at temperature of 27±2°C and relative humidity of 75±5% and a photoperiod of 14 h : 10 h (Light : Dark). Larvae were provided a mixture of yeast powder and dog biscuit in the ratio of 4:6 as food.

Exposures to determine sub-lethal temperatures

Each replicate with 25 late third instar larvae in different batches were placed in 250 ml of dechlorinated tap water separately and maintained in thermostatic controlled water bath at different temperatures viz. 37˚C, 39˚C, 41˚C, 43˚C and 45˚C for different time periods. Selection of temperatures was based on a simple algorithm as below and above 40°C which is recognized as the threshold temperature for immature stage survival and later development [9]. Larval mortality was recorded at every 10 minute interval and lethal indices were calculated to assess the impact of variable temperatures on survival rate of mosquito larvae.

Adaptive thermotolerance

To assess the adaptive thermotolerance, larvae were exposed to 37°C and 39°C and the viable larvae were left to recover in an insectary maintained at 27±2˚C and 75±5% RH. These thermally pre-adapted larvae were later re-exposed to higher temperature at 43˚C and 45˚C, as these temperatures have caused maximum larval mortalities in non-adapted larvae. Calculated lethal time (in minutes) to cause 50% mortalities in thermally exposed larvae (LTM_{50}) and LTM_{90} and % viability at different times of exposure were used as parameters.

Statistics

Larval mortality data after exposure to different temperatures was analyzed using log-probit regression analysis to calculate the lethal time to cause 50% mortality(LT_{50}) in the treated larvae and LT_{90} using the statistical software package, SPSS 17.0 version.

3. Results

Thermotolerance

Non-adapted late third instar larvae of *An. culicifacies* were exposed to a range of temperatures viz., 37˚C, 39˚C, 41˚C, 43˚C and 45˚C. All larvae survived at 37˚C up to 360 minutes while variable mortalities were registered to other higher temperatures(Table-1).

Table 1. Thermal tolerance of *An. culicifacies* larvae to different temperatures and time periods

Details of exposure	% Mortality (n)	LTM_{50} (in min) (lower bound-upper bound)	LTM_{90} (in min) (lower bound-upper bound)	χ^2 (df)
37˚C x 120 min	0% (400)	-	-	-
37˚C x 180 min	0% (400)	-	-	-
37˚C x 240 min	0% (400)	-	-	-
37˚C x 360 min	0% (400)	-	-	-
39˚C x 120 min	0% (400)	650.71 (574.1-768.2)	919.79 (796.2-1109.9)	42.48 (30)
39˚C x 240 min	2.8% (400)			
39˚C x 320 min	3.7% (400)			
41˚C x 180 min	72.6% (179)	121.34 (113.2-129.6)	206.48 (193.0-223.5)	147.67 (22)
41˚C x 240 min	100% (179)			
43˚C x 180 min	82.6% (300)	143.54 (137.7-149.5)	209.08 (199.4-220.8)	155.47 (21)
43˚C x 220 min	100% (300)			
45˚C x 30 min	33.5% (200)	32.24 (29.6-34.8)	47.93 (44.4-52.7)	14.34 (5)
45˚C x 60 min	100% (200)			

(n)- number of larvae exposed, LTM_{50} and LTM_{90}- Lethal time in minutes to kill 50% and 90% of the mosquitoes exposed, χ^2 (df)-Chi square value of heterogeneity (degrees of freedom)

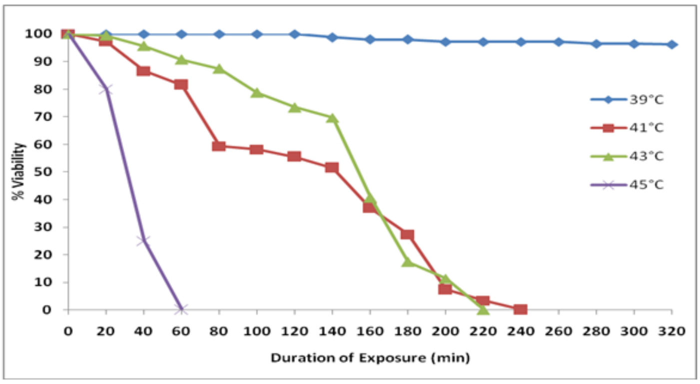
At 39˚C, only 2.8% and 3.7% mortality was observed after 240 and 320 minutes of exposure respectively. Complete mortality was observed after 240 minutes exposure at 41˚C, 220 minutes at 43˚C and 60 minutes exposure at 45˚C. The LTM_{50} values for larvae exposed to 39˚C, 41˚C, 43˚C and 45˚C were 650.7, 121.3, 143.5 and 32.2 minutes respectively (Table.1).

Survival assay

In this study, late third instar larvae were exposed at 37°C, 39°C, 41°C, 43°C and 45°C. A set of larvae without exposure served as control.

All larvae survived up to 360 minutes at 37°C, while at 39°C, all larvae survived up to 120 minutes and 97.2% and 96.3% up to 240 and 360 minutes respectively. At 41°C, there was 81.6%, 55.4%, 27.4% and 3.4% of survival after 60, 120, 180 and 220 minutes of continuous exposure (Fig.1).

Fig 1. Percent viability of the late third instar larvae of *An. culicifacies* at different temperature



Similarly, at 43°C, 90.7%, 73.4% and 17.4% survived after 60, 120, 180 minutes exposure. However, 100% mortality was observed within 60 minutes of exposure at 45°C.

Adaptive thermotolerance

Two pre-adaption temperatures at 37°C and 39°C were chosen as at these temperatures, larvae showed normal motility and less mortality monitored up to 360 minutes. Larvae pre-adapted at 37°C and 39°C were re-exposed to 43°C and 45°C to assess the thermal tolerance to higher temperature.

Larvae pre-adapted at 37 °C and 39°C when re-exposed to 43°C, have shown increase in thermotolerance as compared to non adapted larvae and the LTM_{50} values were 113.1(0.7 fold) and 220.1 minutes (1.5) respectively compared to non adapted larvae.

Table 2. Adaptive thermotolerance of pre-adapted larvae of *An. culicifacies* at 43°C and 45°C

Details of re-exposure	% mortality (n)	LTM_{50} (in min) [X] (lower bound-upper bound)	LTM_{90} (in min) [X] (lower bound-upper bound)	χ^2 (df)
37°C x 43°C	85.3% (375)	113.15 (0.78 X) (103.8-122.3)	197.68 (0.94 X) (183.3-216.3)	366.78 (21)
37°C x 45°C	100% (100)	29.6 (0.91 X) (17.4-41.9)	54.98 (1.14 X) (42.4-95.2)	60.03 (5)
39°C x 43°C	83% (100)	220.1 (1.53 X) (212.4-228.0)	339.59 (1.62 X) 325.6-356.0)	79.03 (33)
39°C x 45°C	100% (100)	43.17 (1.33 X) (38.4-47.7)	77.46 (1.61 X) (70.9-86.1)	23.11 (9

(n)-number of larvae exposed, [X]-folds increase in tolerance compared to non-adapted larvae, LTM_{50} and LTM_{90}-Lethal time to kill 50% and 90% of the mosquitoes exposed, χ^2 (df)- Chi square value of heterogeneity (degrees of freedom)

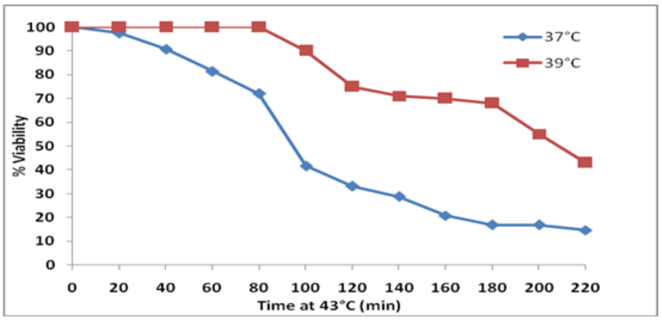
Similarly, larvae pre-adapted at 37°C when re-exposed to higher temperature at 45°C, the LTM_{50} values were 29.6 minutes (0.9 fold) and in larvae pre-adapted at 39°C re-exposed to 45°C registered 1.3 fold increases in thermotolerance as compared to non adapted larvae (Table.2). It indicated that pre-adapted larvae derived from higher temperature were more thermotolerant than the larvae pre-adapted at lower temperature.

Survival assay of adaptive thermotolerance

These studies were carried out on pre-adapted larvae at 37°C and 39°C that were re-exposed to higher temperature of 43°C and 45°C. A set of larvae which had not been pre-exposed served as control.

It was observed that the survival rate of pre-adapted larvae increased over that of the non-adapted larvae. When the larvae pre-adapted at 37˚C were re-exposed to 43˚C, 81.4%, 33.1% and 16.8% larvae survived up to 60, 120 and 180 minutes exposure time respectively. Similarly, larvae pre-adapted at 39˚C when re-exposed to 43˚C, 75% and 68% survived up to 120 and 180 minutes exposures(Fig.2).

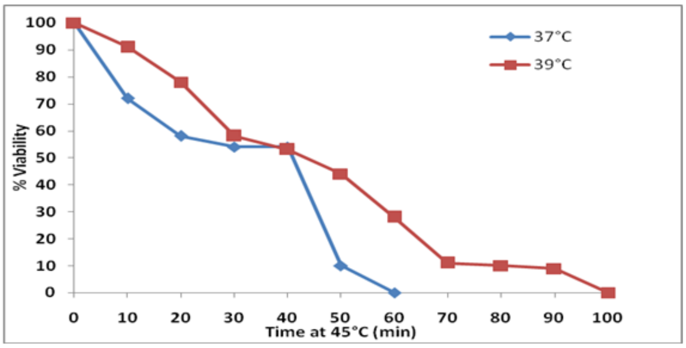
Fig 2. Percent viability of pre-adapted late third instar larvae at 37˚C and 39˚C re-exposed at 43˚C in minutes



In addition, when larvae pre-adapted at 37˚C and 39˚C re-exposed to 45˚C, 54% and 58% survived up to 30 minutes of exposures (Fig. 3).

The result indicated that larvae pre adapted at 37˚C and 39˚C when re-exposed to higher temperature (43 and 45˚C) and larvae pre-adapted at 39˚C were more thermotolerant than larvae pre adapted at 37˚C and non adapted larvae. This observation is further supported by the calculated lethal indices of Table. 2.

Fig 3. Percent viability of pre-adapted late third instar larvae at 37˚C and 39˚C re-exposed at 45˚C in minutes



4. Discussion

The temporal and spatial changes in climate will affect the biology and ecology of vectors that may also affect the vectorial potential of disease vectors and consequently, disease transmission[1]. We therefore attempted to know the effect of variable temperature and adaptive thermotolerance to higher temperature in *An. culicifacies*.

Good amount of data related to influence of temperature on the development of larvae and adults of order culicidae is available [2, 10, 11]. The influence of temperature on the aquatic stages of mosquitoes has been studied for several species, including mosquito species namely *An. quadrimaculatus* [12], *Toxorhynchites brevipalpis* [13], *Wyeomyia smithii* [14], *Aedes aegypti* [11,15] , *Ae. sollicitans*, *Ae. triseriatus*, *An. albimanus*, *Culex pipiens, Cx. restuans and Cx. salinarius* [16], *Cx. quinquefasciatus* [17] and *An. stephensi* [18,19] and we present the data on the viability and thermotolerance in *An. culicifacies*. Late third instar larvae of *An. culicifacies* were exposed to a range of temperatures and it was observed that larval mortality increased with an increase in temperature as earlier reported by Mourya et al. [8]. Non- adapted larvae of *An. culicifacies* survived at 37˚C up to the observed period of 360 minutes exposure. Almost similar results have been reported on *Ae. aegypti* [18] and *An. stephensi* [18,19]. McDonald et al. [20] and Muirhead–Thomson [21] reported that larvae of *Cx. annulirostris* and *An. minimus* exposed to 40°C have shown 100% mortality. Similarly, in our experiment with *An. culicifacies*, 100% mortality was observed within 240 minutes of exposure at 41˚C. *An. culicifacies* normally died within 60 minutes of exposure at 45°C while *An. stephensi* died within 30 minutes[19]. These observed results on complete lethality at variable temperatures indicated differences in responses to temperature in different species.

Adaptive thermotolerance to temperature in larvae of *An. albimanus* was demonstrated by Benedict et al. [22] whereby they showed that larvae pre-exposed for 30 minutes at 37°C exhibited increased heat resistance. Our experiment on adaptive thermotolerance in *An. culicifacies* indicated that pre-exposure to higher temperatures conferred adaptive thermotolerance as reported earlier by Patil et al. [18]. It was observed that among the larvae pre-adapted at 37°C and 39°C when re-exposed to higher temperature of 43°C and 45°C, larvae pre-adapted at 39°C were more thermo-tolerant than larvae pre-adapted at 37°C in a dose dependant manner with respect to temperature and time of exposure.

It would be necessary to carry out elaborate analysis of the correlation between epidemics and temperatures in specific areas in the summer before the epidemics have occurred with average temperatures of the previous few years. However, there is an urgent need for systematic studies on the influence of temperature on various aspects of the mosquito survival in relation to vector control. This preliminary study on the impact of temperature on thermotolerance of larvae of *An. culicifacies*, the major vector of malaria in India has highlighted the importance of the studies on the interaction of abiotic factors specially temperature on the survival of disease vectors. This gathers more importance in view of the ongoing debate of the influence of global warming on trends of disease transmission. Further, the study also indicates the importance of a retrospective study on the occurrence of outbreaks and epidemics and its possible relations to the present temperature in earlier years.

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