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PRELIMINARY STUDIES ON THE EFFECT OF TEMPERATURE STRESS ON THE REPRODUCTIVE PERFORMANCE OF DENGUE VECTOR *AEDES AEGYPTI*, IN SRI LANKA AND ITS SUSCEPTIBILITY TO INSECTICIDES

H.T.R. Jayasooriya [19] and L. Jayawardena

Department of Zoology, Open University of Sri Lanka

INTRODUCTION

Mosquitoes have a complex life cycle where ovi-position, development of larvae and pupae occur in aquatic environments and the adults emerging from pupae disperse to an entirely different ecological niche in the terrestrial environment. Variation in temperature and chemical contaminants are major abiotic factors encountered by both aquatic stages and the adult mosquitoes. Although most studies on disease transmission had been focused on the adult stages of mosquitoes, it has now become clear that conditions experienced by immature stages of vectors may be equally important in determining the transmission of vector borne diseases (Muturi *et al*, 2011).

Studies carried out by Mourya *et al*, (2004) reveal that the adults emerging from heat shock treated larvae of *Aedes aegypti* from India to have lower fecundity, increased longevity and increased susceptibility to Chikungunya virus, which are factors influencing disease transmission. *Aedes aegypti* breeding predominantly in peri-domestic container habitats in Sri Lanka are exposed to high temperatures during day time in their larval stage and the adults emerging are exposed to insecticides.

This study is an attempt to investigate the

- effect of temperature stress on *Aedes aegypti* larvae,
- effect on fecundity and fertility of adults emerging from temperature treated larvae and
- effect on susceptibility to a insecticide of adults emerging from temperature treated larvae.

METHODOLOGY

Heat treatment of mosquitoes

A laboratory strain of *Aedes aegypti* maintained in the insectary at OUSL at $29^0C \pm 2^0$ and at 75%- 80% humidity was used for all the experiments (Aed P strain). To obtain a temperature tolerant strain (Aed T), batches of fourth instar larvae (L_4 larvae) of Aed P strain were exposed to a temperature stress at 45^0 C for 10 minutes (causing 75% - 80% mortality of Aed P larvae) using a water bath (Grant) having continuous circulation of water. The exposed larvae were kept for 24 hours under $29^0C \pm 2^0$ temperature and the surviving larvae were reared up to the adult stage to obtain the next generation of L_4 larvae and the procedure was repeated until the 3rd generation. Thereafter the selection pressure was increased to 12 minutes of heat stress at 45^0C until generation 8 (f_8). At each generation approximately 2000 – 2500 larvae were exposed to heat stress to obtain sufficient surviving adults.

Larval mortality studies

To determine the variations in mortality with temperature, in both Aed P and Aed T, batches of 100 , L_4 larvae were exposed temperatures ranging from 42 ^{0} C to 46 ^{0} C for 10 minutes

[19] Correspondences should be addressed to Prof. T. Jayasooriya, Department of Zoology, Open University of Sri Lanka (email: htjay@ou.ac.lk)

in a water bath. The exposed larvae were transferred to bowls containing 250 ml of tap water and larval food at $29^0C \pm 2^0$ and the mortalities were counted after 24 hours. Batches of larvae from Aed P and Aed T were also exposed to different time periods ranging from 5 min - 20 min at a single temperature of 45^0C. In both the experiments two trials for each strain were conducted and regression analysis was carried out on results obtained.

Studies on adult fecundity

To compare fecundity (number of eggs laid per female) from Aed P and Aed T strains, 20 blood fed females were placed separately in equal size plastic bottles having moistened filter paper for egg laying in the insectory at ($29^0C \pm 2^0$). After 4 days, the eggs laid were counted separately for each adult female of both strains.

Studies on adult fertility

To compare percentage of eggs hatching and producing immature stages (fertility), randomly selected batches of eggs from both Aed P strain and Aed T strain (12 batches from each strain) were counted and placed in plastic bowls containing 250 ml of tap water and larval food in the insectary at $29^0C \pm 2^0$. All such rearing bowls were covered with net. After 7 days emerging adults and pupae were counted.

Studies on adult susceptibility to insecticides

To determine adult susceptibility to permethrin, from Aed P and Aed T strain, 24 hour old adults were exposed to 0.75% permethrin impregnated papers following World Health Organization (WHO) recommended guidelines for adult mosquito susceptibility tests. Each test kit had 20 – 25 adult mosquitoes and 12 trials from Aedes P strain and 7 trails from Aedes T strain were conducted along with a control trial using pyrethroid control papers. The mortalities were counted after 24 hours. Results obtained for the above three experiments were analyzed using Mann Whitney test of Minitab statistical package.

RESULTS AND DISCUSSION

When L4 larvae were exposed to different temperatures, the mortality increased with increasing temperature Figure.1A. Regression analysis showed linear relationships in both Aed P strain (R2 = 0.8942) and Aed T strain (R2 =0.9129) Figure.1B. The lethal temperatures which used a 50% mortality LT_{50}) was 44.46^0C in Aed T and 44.10^0C in Aed P. This indicates heat stressed Aed T strain at generation f_8 shows a 0.36 ^0C tolerance at LT_{50} in comparison to the heat unstressed Aed P strain.

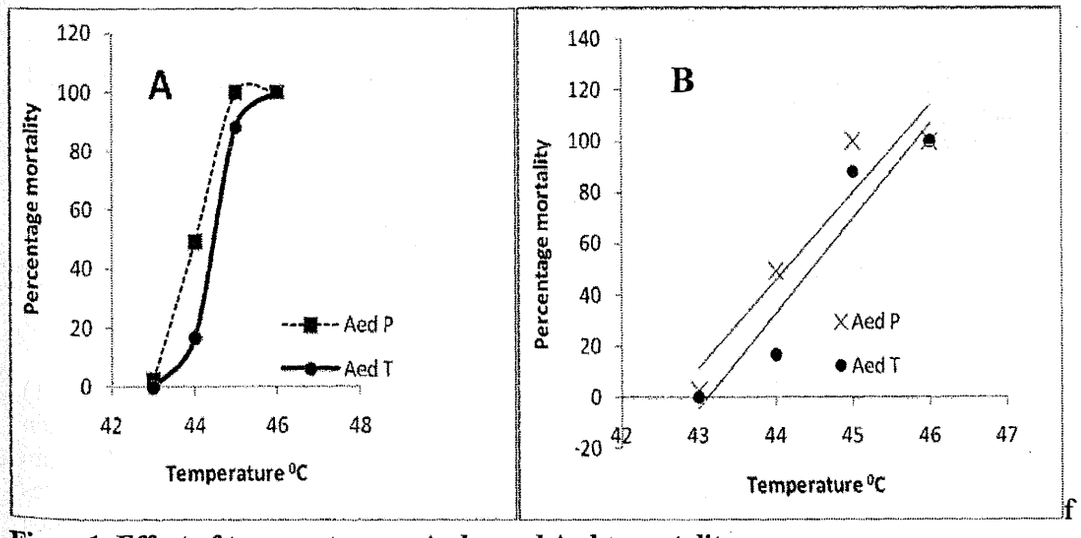

Figure1. Effect of temperature on Aed p and Aed t mortality

Even though the same exposure time was used as in Mourya *et al*, 2004, a selection at a higher temperature of 45^{0}C up to f_8 generation enabled the establishment of a higher heat tolerant Aed T strain in the present study. It was also observed that Aed P strain had a higher tolerance to temperature compared to heat unstressed normal stain from Pune, India, possibly due to different geographical locations of the species

At 45^0 C using different exposure times also indicated marked changes in mortality with exposure time (Figure. 2). LT_{50} value increased from 1.8 minutes in Aed P to 9.49 minutes in Aed T and a wider range of mortalities were observed in Aed T strain compared to Aed P strain. However by 20 minutes Aed P and Aed T strain larvae were all dead at f_8 generation.

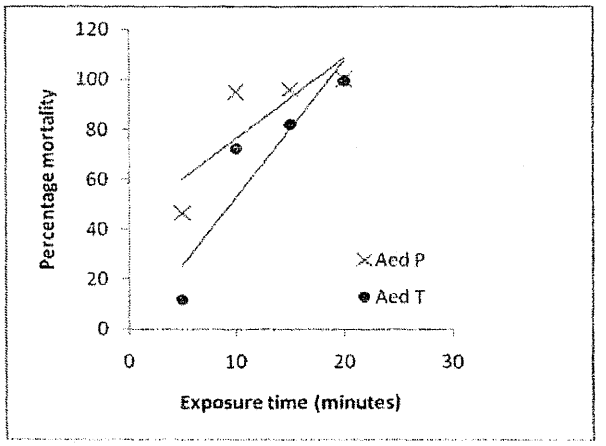

Figure 2. Temperature tolerance of Aed P and Aed T at 45°C when exposed to different time periods

Experiments carried out with Aed P and Aed T strains of adult mosquitoes revealed a variation in fecundity, fertility and in the susceptibility to permethrin (Table 1). The mean fecundity of adult females appeared to be less in Aed T compared to the unstressed Aed P but statistical analysis using Minitab, Mann Whitney test indicates that data does not prove the hypothesis that temperature treatment of larvae reduces adult fecundity, unlike in studies carried out in India. However the mean percentage of eggs hatching to produce immature stages was significantly lower ($P \geq 0.05$) in heat stressed Aed T strain and indicates that temperature effects hatching of eggs.

Mosquito strain	Mean number of eggs laid per female ± SE (fecundity)	Mean Percentage of eggs hatched ± SE (fertility)	Mean Percentage mortality to permethrin ± SE (susceptabilty)
Aed P	81.95 ± 7.40	48.52 ± 4.52	63.82 ±3.81
Aed T	67.89 ± 7.42	32.80 ± 3.91	44.32 ± 4.95
P value	P = 0.0920	P = 0.0097	P = 0.0044

Table 1 – Variations in fecundity fertility and susceptibility to permethrin of adult mosquitoes

A significant reduction ($P \geq 0,05$) in the susceptibility to permethrin in Aed T (Table 1) indicates that the temperature tolerance has influenced a tolerance to permethrin. When exposed to permethrin there was a higher percentage of survivors in Aedes T strain (approximately 20%) compared to Aed P strain. Pyrethroid insecticides and other insecticides are being used to control dengue vectors in Sri Lanka. Personal experiences also show that

running water in taps to be approximately 40^0C during midday indicating that peri-domestic water collections where larvae and pupae are found may have almost the same temperature or even higher temperatures during mid day in the dry season. Exposure to such high temperature may influence temperature tolerant adults to emerge that may survive insecticide treatment in field situations.

Environmental stressors like extremes of temperature are known to change the expression of various heat shock proteins (Hsp) in living organisms, under stressful conditions. These proteins stabilize denatured proteins and refold proteins that have been already denatured, (Muturi *et al*, 2011) and can be used as biomarkers of environmental stress. Mourya *et al*, (2004) reports the expression of heat shock proteins in the temperature tolerant strain that is believed to have increased susceptibility to a Chickungunya virus strain. Such studies in understanding the molecular mechanism involved in temperature tolerance of Aed T strain will be useful for further studies on temperature tolerance of dengue vectors under field situations.

CONCLUSIONS

A temperature tolerant strain of *Aedes aegypti* was established in the laboratory. It has been characterized with respect to larval mortality, adult fecundity and fertility and adult susceptibility to permethrin. Further studies on several aspects, longevity of larvae, pupae, males and females, adult and larval susceptibility to other insecticides, mechanisms involved in heat tolerance, susceptibility to pathogens etc. will provide a model to understand what may happen during disease transmission under field situation which will be of considerable importance to Sri Lanka.

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