INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 1, p. 156-164, 2017

RESEARCH PAPER OPEN ACCESS

Impact of thermal stress on survival and induced cross tolerance to toxins of *Bacillus thuringiensis* in wild *Aedes aegypti*

T. Sarita Achari, Usha Rani Acharya, Tapan Kumar Barik*

Laboratory of Applied Entomology, Department of Zoology, Berhampur University, Berhampur, Odisha, India

Key words: Temperature, *Aedes aegypti,* Mosquito, *Bacillus thuringiensis,* Cross tolerance

http://dx.doi.org/10.12692/ijb/11.1.156-164 Article published on July 21, 2017

Abstract

To assess the impact of thermal stress, late third instar larvae of field collected *Aedes aegypti* were exposed to variable temperatures viz. 39°C, 40°C, 41°C, 42°C, 43°C, 44°C and 45°C. All larvae were survived up to 300 minutes exposure at 39°C where as hundred percent larval mortality observed at higher temperature exposure. Further, it was observed that 25.1%, 35.6%, 78.9%, 90% and 100% larval mortality found after *Bti* treatment at 0.5 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm and 2.5 ppm respectively. Similarly, in order to assess the cross tolerance level to *Bti*, larvae pre-adapted at 39°C for 60 min, 90 min and 120 min duration were re-exposed to *Bti* solution of 1.0 and 1.5 ppm. Data suggested that the pre-adapted larvae showed 4.4 and 1.9 fold less larval mortality that indicates temperature play an important role in the development of cross tolerance to toxins of *Bti* in wild *Aedes aegypti*.

* **Corresponding Author:** Tapan Kumar Barik ✉ tkbarik@rediffmail.com

Introduction

Arthropods play an essential role in the global ecosystems. However, some of them are capable of spreading harmful pathogens resulting in devastating consequences. Among these, few mosquito species pose a serious threat and cause a significant economic burden in disease-endemic countries by transmitting major human diseases such as malaria, dengue, chikungunya, and filariasis (Barik *et al.,* 2012).

In the past 50 years, incidence of Dengue has increased 30-fold with increasing geographical expansion to new countries (WHO, 2016). *Aedes* mosquitoes are usually distributed in tropical regions of the globe and vectored the viruses that cause dengue fever, yellow fever, West Nile fever, chikungunya, eastern equine encephalitis and Zika fever etc.

As no specific treatment and effective vaccine exists for most of mosquito borne diseases till today, mosquito vector control at their different developmental stages are the only options to reduce the incidence of mosquito borne diseases.

In the present scenario, mosquito vector control programmes aims at controlling vector population using chemical insecticides due to their rapid action. However, resistance of mosquitoes to various chemical insecticides threaten the efficacy of control programmes throughout insecticide-treated areas.

Therefore, the need of alternate, effective and environment friendly control strategies, which could remain effective even in the face of growing insecticide, is an urgent need. Mosquito vector control using *Bti* and *Bsph* as larvicides proved highly effective (Charles and Nielsen, 2000) and environmentally safe to non-target organisms (WHO, 1997).

Geographical expansion of mosquito-borne diseases in several continents has been partially associated with global warming (Githeko *et al.,* 2000). Increase in the global temperature up to 1.4-5.8°C due to climate change (IPCC, 2007) accelerates the challenge for ongoing efforts to certain vector-borne diseases. In nature, variation in environmental temperature affects the developmental cycle in most organism (Cossins and Bowler, 1987; Worner, 1998; Bale *et al.,* 2002) and mosquitoes in particular (Rueda *et al.,* 1990). *Aedes* mosquitoes are able to adapt to varying environmental conditions, and has shown seasonal increases in the vector population and seasonal variability in vector competence (Paupy *et al.,* 2003).

Advance studies on epidemiology of dengue have demonstrated that marked changes appear in disease transmission patterns (Gubler and Kuno, 1997; Guha-Sapir *et al.*, 2005; Chadee *et al.,* 2007) and change in the behaviour and ecology of the dengue vector mosquitoes due to climate change (Chadee and Martinez, 2000; Chen *et al.,* 2006; Hemme *et al.,* 2010).

Therefore, information related to the effects of temperature on the rate of development and survival of the various stages of mosquitoes is necessary for designing new models for vector-control strategy (Wagner *et al.,* 1975; Moon, 1976; Haile and Weidhaas, 1977; Greever and Georghiou, 1979).

The current study was designed with specific objective to investigate the effect of variable temperatures on survival rate of late third instar larvae of field collected *Aedes aegypti* mosquitoes under laboratory conditions. In addition to this, experiments were also carried out to understand the levels of cross-tolerance to *Bti* in thermally pre-adapted larvae in comparison to non-adapted larvae of *Aedes aegypti* mosquitoes.

Materials and methods

Mosquito collection and mass rearing

Both immature and mature stages of *Aedes aegypti* mosquitoes were collected from various localities near Berhampur city, Odisha. *Aedes aegypti* mosquitoes were taken for culture after their proper identification and maintained in an insectary at a temperature of 25 ± 1° C and relative humidity of 65 ± 5% with 12 hour light and dark photo periods.

Larval food for mass rearing includes mixture of dog biscuit and yeast powder in the ratio of 3:2 (Helinski *et al.*, 2006).

The amount of food depends upon the number and stage of larvae (Thomas and James, 1997) and for adult, 10% glucose solution soaked in raisin was provided as nutrition. Adults were allowed to mate freely in $1ft^3$ cages. Females were blood fed using artificial blood feeder. All experiments were performed against F_1 progeny of field collected *Aedes aegypti* mosquitoes.

Experimental protocol

Exposure to determine sub-lethal temperature

Each replicate with 20-25 late third instar larvae in different batches were placed in 250 ml of dechlorinated water separately and maintained in thermostatic controlled water bath at different temperatures viz. 39°C, 40°C, 41°C, 42°C, 43°C, 44°C and 45°C for different time periods. Selection of this temperature range 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 (Bayoh *et al.* 2004).

To assess the impact of variable temperatures on survival rate of mosquito larvae, larvicidal bioassay were performed according to the protocol of WHO (1998) and larval mortality was scored at every 10 minute interval and lethal indices were calculated.

Exposure to determine the efficacy Bti

To understand the bioefficacy of *Bti*, against *Aedes aegypti* commercially available *Bti* were taken and prepared in solution form using distilled water.

Subsequently, different concentrations of the test solutions were prepared by serial dilutions from the freshly prepared stock solution. Various concentrations ranged from 0.5-2.5gm/lit of *Bti* were tested against late third instar larvae of *Aedes aegypti*. Only water without *Bti* was used for control set.

Determination of Cross tolerance to Bti

Cross tolerance is an acclamatory response to one stress that affords tolerance to similar or other forms of stress (Raghavendra *et al.*, 2010a).

To determine the cross tolerance level if any, the thermally pre-adapted larvae of *Aedes aegypti* mosquitoes were exposed at 39°C for 60 min, 90min and 120min were re-exposed to *Bti* solution of 1.0 ppm and 1.5 ppm using the standard test procedures of WHO and percent larval mortality was observed after 24hr of treatment.

Statistical analysis

Larval mortality data after exposure to range of temperature and *Bti* solution was analyzed using log probit regression analysis to calculate the lethal time to cause 50% mortality (LT_{50}) and LT_{90} in the treated larvae using the statistical software package, SPSS 18.0 version.

Results

Effect of temperature stress on survivability of larvae of Aedes aegypti

Late third instar larvae of *Aedes aegypti* mosquitoes were exposed to a range of temperatures viz. 39°C, 40°C , 41°C, 42°C, 43°C, 44°C, 45°C for different time periods. Negligible mortality of about 1.6% was observed at 39°C, up to 300 minutes exposure, whereas variable mortalities were registered at other higher temperature exposures (Table 1).

It has been observed that in the current investigation, larval mortality was increased with an increase in temperature exposure in a dose dependent manner.

It was observed that, the lethal time of larvae was significantly reduced when 1°C temperature was raised.

The LT_{50} values for larvae exposed to 41°C, 42°C, 43°C, 44°C, 45°C were 56.31, 33.09, 33.29, 6.57, 6.32 minutes respectively. Similarly, the LT_{90} values for larvae exposed to 41°C, 42°C, 43°C, 44°C, 45°C were 96.08, 74.39, 73.03, 10.65, 11.10 minutes respectively.

Table 1. Thermal tolerance of larvae of field collected *Aedes aegypti* mosquito to different temperatures and time periods.

Details of temperature exposure	No. of larvae exposed (n)	% larval mortality	LTM_{50} in min(Lower limit-Upper limit)	LTM_{90} in min (Lower limit-Upper limit)	χ^2 (df)
39°Cx300min	60 (03)	1.6	-	-	-
40°Cx30 min	80 (04)	5.0	62.032 (59.218-64.851)	106.140 (99.230-115.236)	37.494 (21)
40°Cx60 min	80 (04)	46.2			
40°Cx90 min	80 (04)	77.5			
40°Cx115min	80 (04)	100			
41°Cx30 min	60 (03)	6.6	56.306 (54.123-58.463)	96.080 (91.028-102.342)	17.799 (20)
41°Cx60 min	60 (03)	55.0			
41°Cx90 min	60 (03)	81.6			
41°Cx100min	60 (03)	100			
42°Cx30 min	60 (03)	58.0	33.097 (29.750-36.266)	74.399 (66.760-85.224)	35.868 (18)
42°Cx60 min	60 (03)	78.0			
42°Cx90 min	60 (03)	95.0			
42°Cx100min	60 (03)	100			
43°Cx30 min	60 (03)	58.0	33.295 (29.779-36.615)	73.031 (65.100-84.662)	37.966 (17)
43°Cx60 min	60 (03)	80.0			
43°Cx95 min	60 (03)	100			
44°Cx15 min	73 (04)	100	6.574 (5.980-7.160)	10.658 (9.594-12.301)	1.752 (1)
45°Cx15min	60 (03)	100	6.328	11.109	2.892(1)

(n)- number of replicates, LTM_{50} and LTM_{90}- Lethal time in minutes to kill 50% and 90% of the mosquitoes exposed, $\chi 2$(df)-Chi square value of heterogeneity (degrees of freedom).

Bioefficacy of Bti against larvae of Aedes aegypti

In this study, only 25.1% larval mortality was registered at 0.5 ppm of *Bti* treatment to *Aedes aegypti* larvae after 24hr exposure. Similarly, 35.6%, 78.9%, 90% larval mortality was recorded, when the larvae were exposed to 1.0 ppm, 1.5 ppm, and 2.0 ppm respectively. Hundred percent larval mortality was found at 2.5 ppm after 24hr exposure (Fig.2). The LD_{50} and LD_{90} values were 0.931 and 2.23 respectively, $\chi 2$ (df)= 36.621 (Table 2).

Table 2. Bioefficacy of *Bti* on late third instar larvae of *Aedes aegypti*.

Dose (ppm)	No.of larvae exposed (n)	No .of larvae dead	% larval mortality	LD_{50} (Lower limit-Upper limit)	LD_{90} (Lower limit-Upper limit)	χ^2 (df)
0 (control)	200(08)	0	0	0.931 (0.399-1.829)	2.23 (1.343-98.488)	36.621 (3)
0.5	199(08)	50	25.1			
1.0	160(08)	57	35.6			
1.5	95(05)	75	78.9			
2.0	80(04)	72	90.0			
2.5	84(04)	84	100			

Development of cross tolerance to Bti

Thermally pre-adapted larvae of *Aedes aegypti* mosquitoes at different temperatures were later re-exposed to *Bti* solution. In the higher concentration of *Bti* treatment, >90% larval mortality was found, therefore, two different concentrations such as 1.0 ppm and 1.5 ppm of *Bti* were selected for the present study to understand the cross tolerance level of *Aedes aegypti*.

When the non-adapted larvae were exposed to toxin of *Bti* at 1.0 ppm and 1.5 ppm, 44.3% and 61.1% larval mortality was found after 24hr exposure.

However, when larvae thermally pre-adapted at 39°C for 60, 90, and 120 minutes were later re-exposed to *Bti* at same concentrations (1.0 ppm and 1.5 ppm), the larval mortality reduced to 15.7%, 12.5%, 10.0% at 1.0 ppm, and 40.0%, 37.5%, 30.8% at 1.5 ppm respectively (Fig. 3).

Therefore, in the present study, it was observed that the larvae pre-adapted at 39°C for 60, 90, and 120 minutes developed cross tolerance to toxin of *Bti* as compared to non-adapted larvae. Furthermore, the tolerance capability was increased with an increase in intensity of temperature exposure.

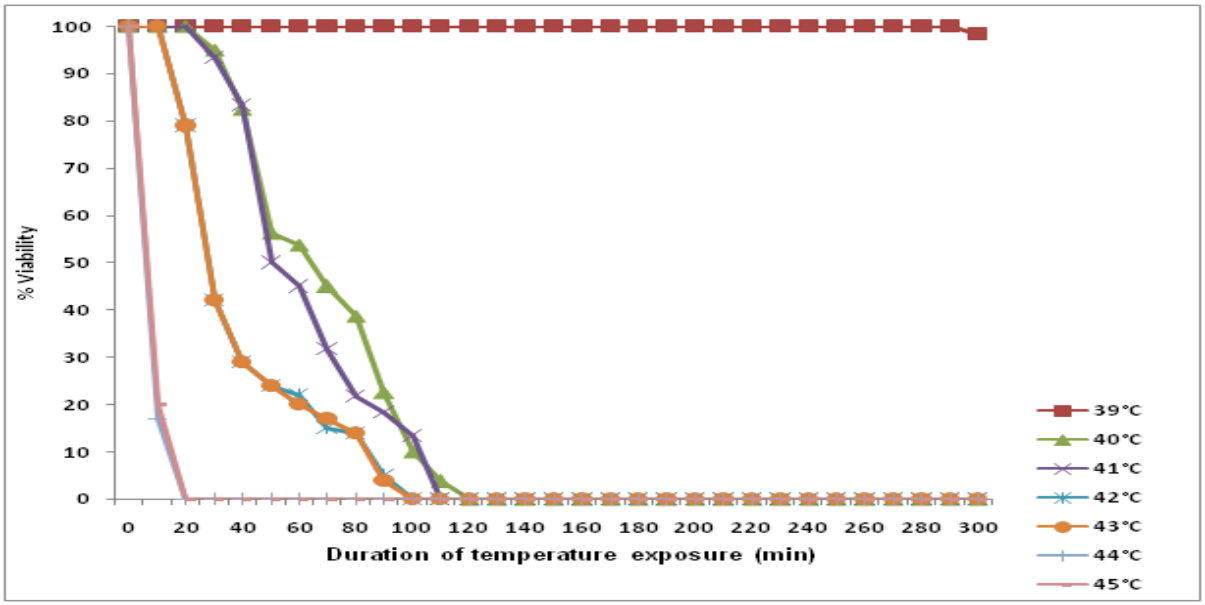

Fig. 1. Percent viability of late third instar larvae of *Aedes aegypti* at different temperature exposure. Percent viability of larvae is plotted against the duration of exposure at different temperature.

Discussion

Currently, the dengue outbreaks become more severe due to its rapid geographical expansion. As no effective vaccine against dengue is currently available, therefore mosquito vector control is the only option. Preventing or reducing dengue virus transmission depends entirely in controlling the mosquito vectors or interruption of human–vector contact. WHO promotes the strategic approach known as Integrated Vector Management (IVM) to control dengue mosquito vector.

The impact of direct exposure to variable temperatures on survival of insects particularly on the immature stages of certain mosquito vector species has been studied for various mosquito species, *Ae. aegypti* (Tun-Lin *et al.*, 2000), *Ae. sollicitans, An. albimanus, Ae. triseriatus, Cx. pipiens, Cx. restuans and Cx. salinarius* (Shelton, 1973), *Cx. quinquefasciatus* (Swain *et al.*, 2008), *An. stephensi* (Raghavendra *et al.*, 2010a) and *An. culicifacies* (Raghavendra *et al.*, 2010b).

The result of the present investigation indicates that the larval mortality increase with an increase in temperature exposure which was in agreement with the previous findings of Mourya *et al.* (2004) on *Aedes* and Raghavendra *et al.* (2010a,b) on *Anopheles* mosquitoes. Further, the current study explains the thermo tolerance capabilities of late third instar larvae of *Aedes aegypti* mosquitoes to various temperatures.

The temporal and spatial changes in temperature, precipitation and humidity that are expected to occur due to climate change scenarios will affect the biology and ecology of vectors, intermediate hosts and consequently the risk of disease transmission (Lindsay *et al.*, 1996).We therefore attempted to know the impact of variable temperature exposure on immature stage of F_1 progeny of field collected *Aedes aegypti* and also the level of cross tolerance to toxins of *Bti*. It was observed that, no larvae survived at 40°C, 41°C, 42°C, 43°C, 44°C and 45°C when the

larvae were exposed at 115, 100, 100, 95, 15 and 15 minutes respectively. When larvae of *Cx. annulirostris* and *An. minimus* were exposed to 40°C, 100% larval mortality observed (McDonald *et al.* 1980 and Muirhead-Thomson 1940) which is similar to the current findings where field collected *Aedes aegypti* mosquito achieved 100% larval mortality at 40°C after 115 minutes exposure. Similarly, 100% larval mortality was observed within 80 min of exposure at 43°C in *An. stephensi* (Raghavendra *et al.*, 2010a) whereas in the current investigation, it was observed that field collected *Aedes aegypti* registered 100% mortality after 95 minutes exposure at same temperature exposure.

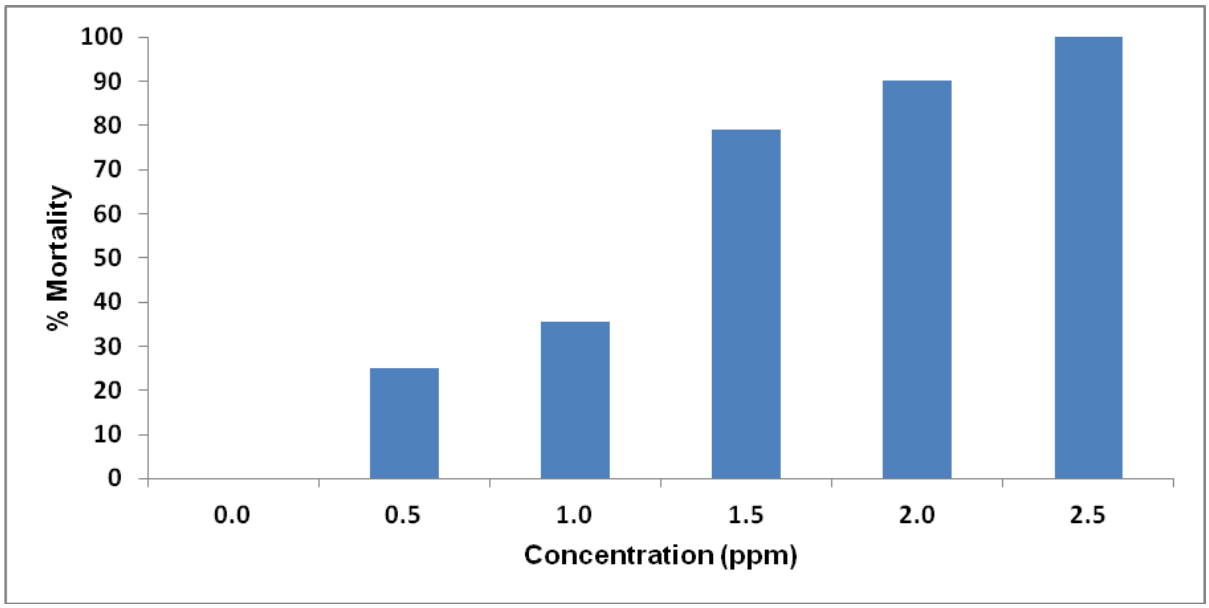

Fig. 2. Bioefficacy of *Bti* on late third instar larvae of *Aedes aegypti*– mosquitoes at different concentrations. Percent larval mortality is plotted against concentration of *Bti.*

In mosquito vector control programme, use of *Bti* has some significant advantages over conventional insecticide. *Bti* can be effective in controlling the immature stages of dengue mosquito vector. The killing efficacy is rapid, typically eliminating all most all immature forms from treated containers within 24 hr, having the residual efficacy of 2 to 4 weeks (Boyce *et al.*, 2013). It has been reported that *Culex* and *Aedes* mosquitoes are highly susceptible to *Bti* than *Anopheles* mosquitoes (Balaraman *et al.*, 1983). *Bti* has an LC_{50} and LC_{95} value of 1.02 and 1.86 ppm for *Ae. aegypti* (Fansiri *et al* 2006). In the present investigation, hundred percent larval mortality was found at the concentration of 2.5 ppm of *Bti* against late third instar larvae of *Aedes aegypti* after 24hr exposure.

Cross tolerance is an acclamatory response to one stress that affords tolerance to similar or other forms of stress (Raghavendra *et al.* 2010a).

To understand the cross tolerance level to *Bti*, late third instar larvae pre-adapted to different temperature were re-exposed to *Bti*. Larvae that pre-adapted at various temperatures showed less mortality and better survivability than non-adapted larvae. Therefore, the results of the present investigation showed a clear indication of development of cross- tolerance to *Bti* by *Aedes aegypti* mosquitoes.

Temperature-induced adaptive thermotolerance in Anopheles mosquitoes has increased tolerance to higher temperatures (Raghavendra *et al.*, 2010a,b) and also tolerance to high-parasite loads of arbovirus in some vectors (Kay *et al.*, 1989; Mourya *et al.*, 2004).

Adaptive cross-tolerance to malathion was also induced by pre-exposing them to different temperatures (Raghavendra *et al.*, 2010a).

Further, experimental studies on vector competence of mosquitoes for several viruses have demonstrated that mosquitoes reared in warmer insectaries are more competent in the transmission of the virus, and they also tolerate higher virus load (Hurlbut, 1973; Watts *et al.*, 1987).

Epidemiological research has shown that temperature is a key "predictor" of dengue infection (Patz *et al.*, 1998).

An increase in temperature above the average temperature in an endemic area would not only enhance the selection of temperature-tolerant mosquitoes in a population having greater longevity, but would also affect both intrinsic and extrinsic factors by reducing extrinsic incubation period and increasing susceptibility of mosquito to viruses (Mourya *et al.*, 2004).

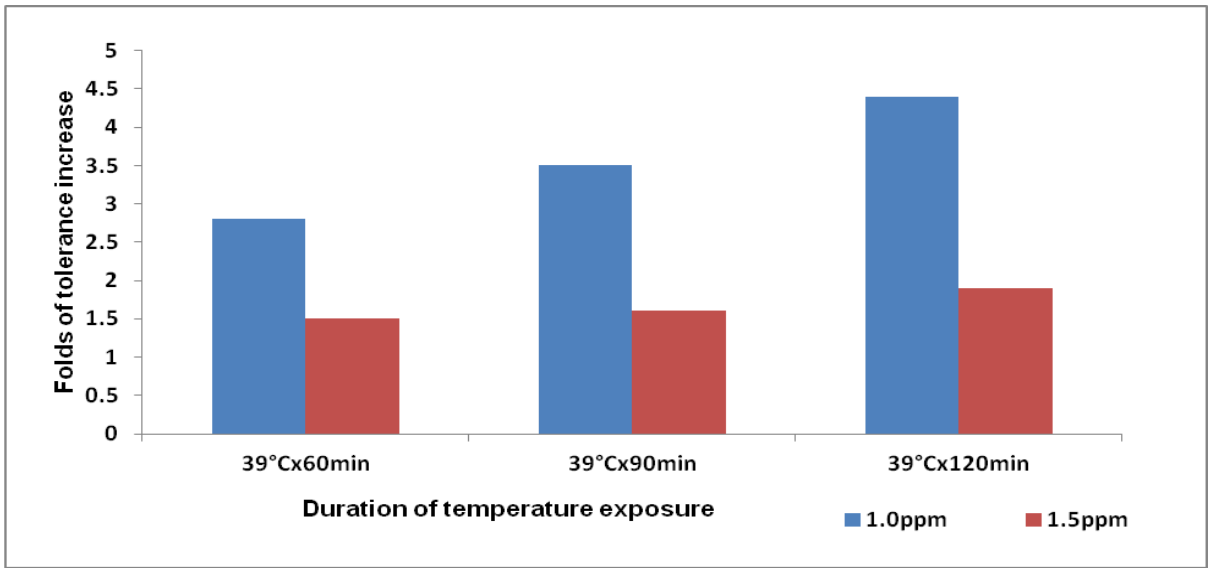

Fig. 3. Level of cross-tolerance to *Bti* of thermally pre-adapted larvae of field collected *Aedes aegypti*.

It has been reported that regions where dengue is already present, a mean temperature increase of about 1°C increases the aggregate epidemic risk by an average of 31%-47% (Patz *et al.*, 1998).

Conclusion

Vector-borne diseases are emerging as a result of altogether changes in public health policy, insecticide and drug resistance, shift in emphasis from prevention to emergency response, demographic and societal changes, and genetic changes in pathogens. Vaccines are available for only a few diseases and are not widely used. Therefore, mosquito vector control is the only option to interrupt transmission of most vector-borne diseases. Environmentally safe insecticides and research on alternative approaches are still needed to avoid these mosquito borne diseases. Toxins of *Bti* appears to be a safe and efficient bio-insecticide against different mosquito vector and *Aedes aegypti* in particular.

In conclusion, current studies bring to light that temperature stress also plays an important role in the development of cross tolerance to other stress conditions like *Bti* exposure. Result also highlights the understanding of how mosquitoes respond or adapt to increased temperatures due to climate change and thus can contribute to the development of best practice management approaches to control mosquito vectors in future.

Acknowledgments

Authors are thankful to the Head, Department of Zoology, Berhampur University, Berhampur, Odisha for providing necessary facilities and encouragement.

References

Balaraman K, Balasubramanian M, Manonmani LM. 1983. *Bacillus thuringiensis* H-14 (VCRC B-17) formulation as mosquito larvicide. Indian Journal of Medical Research **77,** 33-37.

Bale JS, Masters GJ, Hodkinson ID, Awmak C, Bezemer TM, Brown V, Butterfield J, Buse A. 2002. Herbivory in global climate change research: direct effects of raising temperature on insect herbivores. Glob Chang Biol **8,** 1–16.

Barik TK, Raghavendra K, Goswami A. 2012. Silica nanoparticle: a potential new insecticide for mosquito vector control. Parasitol Res **111,** 1075–1083.

Bayoh MN, Lindsay SW. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. Medical and Veterinary Entomology **18,** 174-179.

Boyce R, Lenhar A, Kroeger A, Velayudhan R, Roberts B, Horstick O. 2003. *Bacillus thuringiensis israelensis (Bti)* for the control of dengue vectors: systematic literature review. Tropical Medicine and International Health **18,** 564-577.

Chadee DD, Martinez R. 2000. Landing periodicity of *Aedes aegypti* with implications for dengue transmission in Trinidad West Indies. J. Vector Ecol **25,** 158–163.

Chadee DD, Shivnauth B, Rawlins SC, Chen AA. 2007. Climate variability, mosquito density and epidemiology of Dengue fever in Trinidad (2002–2004): A prospective study. Ann. Trop. Med. Parasitol **101,** 68–77.

Charles JF, Nielsen-LeRoux C. 2000. Mosquitocidal bacterial toxins: diversity, mode of action and resistance phenomena. Memorias Do Instituto Oswaldo Cruz **95,** 201–206.

Chen AA, Chadee DD, Rawlins SC. 2006. Climate Change Impact on Dengue: The Caribbean Experience: START Publication (ISBN 976-41-0210-7).

Cossins AR, Bowler K. (Eds) 1987. Temperature Biology of Animals. Chapman and Hall, New York, pp 125–157.

Federici BA, Park HW, Bideshi DK, Wirth MC, Johnson JJ. 2003. Recombinant Bacteria for Mosquito Control. The Journal of Experimental Biology **206,** 3877-3885.

Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. 2000. Climate change and vector-borne diseases: a regional analysis. Bull. World Health Organ **78,** 1136–1147.

Greever J, Georghiou GP. 1979. Computer simulation of control strategies for *Culex tarsalis* (Diptera: Culicidae). J Med Entomol **16,** 180–188.

Gubler DJ. 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: DJ Gubler, G Kuno (Eds.) Dengue and dengue hemorrhagic fever. CAB International, Wallingford, UK; 1–22.

Guha-Sapir D, Schimmer B. 2005. Dengue fever: new paradigms for a changing epidemiology. Emerg. Themes Epidemiol **2,**1–10.

Haile DG, Weidhaas DE. 1977. Computer simulation of mosquito populations (*Anopheles albimanus*) for comparing the effectiveness of control strategies. J Med Entomol **13,** 553–567.

Helinski MEH, Parker AG, Knols BGJ. 2009. Radiation biology of mosquitoes. Malaria Journal, **8**(Suppl. 2), S6.

Hemme RR, Thomas CL, Chadee DD, Severson DW. 2010. Influence of urban landscapes on population dynamics in a short-distance migrant mosquito: evidence for dengue vector *Aedes aegypti*. PLoS Neglected Trop. Dis. **4,** e634.

Hurlbut HS. 1973. The effects of environmental and physiological conditions of *Culex tritaeniorhynchus* on the pattern of transmission of Japanese encephalitis virus. J Med Entomol **10,**1–12.

IPCC. 2007. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (Eds.), Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kay BH, Fanning ID, Mottram P. 1989. Rearing temperature influences flavivirus vector competence of mosquitoes. Med Vet Entomol **3,** 415–422.

Lindsay SW, Birley MH. 1996. Climate change and malaria transmission. Annals of Tropical Medicine and Parasitology **90,** 573–588.

McDonald G, McLaren IW, Shelden GP, Smith IR. 1980. The effect of temperature on the population growth potential of *Culex annulirostris* Skuse (Diptera: Culicidae). Austral Ecology **5,** 379-384.

Moon TE. 1976. A statistical model of the dynamics of a mosquito vector (*Culex tarsalis*) population. Biometrics**32,**355–368.

Mourya DT, Yadav P, Mishra AC. 2004. Effect of temperature stress on immature stages and susceptibility of *Aedes aegypti* mosquitoes to *Chikungunya* virus. Am. J. Trop. Med. Hyg. **70,** 346-350.

Muirhead-Thomson RC. 1940. Mosquito behaviour in relation to malaria transmission and control in the tropics. Edward Arnold, London, viii + 219 p.

Patz JA, Martens WJM, Focks DA, Jetten TH. 1998. Dengue epidemic potential as projected by general circulation models of global climate change. Environ Health Perspect **106,** 147–152.

Paupy C, Chantha N, Vazeille M, Reynes JM, Rodhain F, Failoux AB. 2003. Variation over space and time of *Aedes aegypti* in Phnom penh (Cambodia) genetic structure and oral susceptibility to a dengue virus. Inf. Genet. Res. Camb **82,** 171–182.

Raghavendra K, Barik TK, Adak T. 2010a. Development of larval thermotolerance and its impact on adult susceptibility to malathion insecticide and *Plasmodium vivax* infection in *Anopheles stephensi*. Parasitology Research **107,** 1291–1297.

Raghavendra K, Barik TK, Swain V. 2010b. Studies on the Impact of Thermal Stress on Survival and Development of Adaptive Thermotolerance in Immature Stages of *Anopheles culicifacies*. Journal of Ecobiotechnology **5,** 25-30.

Rueda LM, Patel KJ, Axtell RC, Stinner RE. 1990. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol **27,** 892–898.

Shelton RM. 1973. The effect of temperatures on development of eight mosquito species. Mosq News **33,** 1–12.

Swain V, Seth RK, Mohanty SS, Raghavendra K. 2008.Effect of temperature on development, eclosion, longevity and survivorship of malathion-resistant and malathion-susceptible strain of *Culex quinquefasciatus*. Parasitology Research **103,** 299–303.

Thomas G, James P. 1997. Collecting, Rearing, Mounting and Shipping Mosquitoes, The Walter Reed Biosystematics Unit, Division of Entomology, Walter Reed Army Institute of Research 503 Robert Grant Avenue, Silver Spring, MD 20910-7500 USA.

Tun-Lin W, Burkot TR, Kay BH. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Med Vet Entomol **14,** 31–37.

Wagner VE, Tully GA, Goodman ED, Newson HD. 1975. A computer simulation model for population studies of woodland pool *Aedes* mosquitoes. Environ Entomol **4,** 905–919.

Watts DM, Burke DS, Harrison BA, Whitmire RE, Nisalak A. 1987. Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. Am J Trop Med Hyg **36,** 143–152.

World Health Organization. 1997. Techniques to detect insecticide resistance mechanisms (field and laboratory manual). Geneva, (WHO/CDS/CPC/MAL/98.6).

World Health Organization. 2016. Dengue and severe dengue Factsheet. www.who.int/mediacentre/factsheets/fs117/en/

Worner SP. 1998. Ecoclimatic assessment of potential establishment of exotic pests. J Econ Entomol **81,** 973–983.