Dhaka Univ. J. Biol. Sci. **30**(2): 233-241, 2021 (July) DOI: https://doi.org/10.3329/dujbs.v30i2.54649

EXPRESSION OF HEAT SHOCK PROTEIN GENES *AAEHSP26, AAEHSP83* AND *AAEHSC70* IN RESPONSE TO THERMAL STRESS IN *AEDES AEGYPTI* LARVAE

HAFISHA KHATUN ANEE, ASHFAQUL MUID KHANDAKER, ROWSHAN ARA BEGUM* AND REZA MD. SHAHJAHAN

Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Aedes aegypti, Heat shock proteins, Relative gene expression

Abstract

Climate change is responsible to a certain extent for the occurrence and spread of arboviral pathogens worldwide. Temperature is one of the crucial abiotic factors influencing the physiological processes of mosquitoes. Several genes of heat shock protein (*AaeHsp26, AaeHsp83,* and *AaeHsc70*) families are known to be expressed in mosquitoes, which aid in overcoming stress induced by elevated temperature. In this study, the relative expression of heat shock protein genes has been examined using Quantitative Real-time PCR (qPCR). The temperatures used for heat shock treatment were 27(control), 37, and 42°C for 1 hour heat shock period and applied to 3rd instar larvae. Significant up-regulation has been seen at 37, and 42°C. The highest expression level, about 82.43 fold, was reported for the *AaeHsc70* gene at 42°C followed by 78.36 fold for *AaeHsp26* at 37°C and 4.79 fold for *AaeHsp83* at 42°C. The current study has shown that HSPs are important markers of stress and may function as critical proteins to protect and enhance the survival of *Ae. aegypti* larvae and pupae. Biological implications of these findings could impact the vector competencies

Introduction

Aedes aegypti is a well-known vector of different diseases. In southeast Asia, they are mainly responsible for dengue fever and chikungunya. World Health Organization (WHO) has recognized dengue as one of the fastest-growing viral diseases globally. Severe dengue was first recognized in the 1950s. A study on the prevalence of dengue estimates that 3.9 billion people in 128 countries are at risk of infection with dengue viruses(1). Bangladesh reported 1,00,965 cases in 2019, as of 14 December 2019, this is an almost ten-time increase compared to 2018. The peak occurred in August, and the number of cases shows a decreasing seasonal trend. Dengue was known to exist in only 9 countries in the 1970s, but it is now endemic in 128 countries and strikes as many as 96 million people each year, according to global data collected by WHO 2019.

*Author for correspondence: <rowshanbegumdu@yahoo.com>.

Gradual temperature rise in the environment causing morphological, physiological and behavioural changes in *Ae. aegypti* populations[2]. Temperatures can drastically alter the genetic structure and gene expressions and thus affect mosquito development[3]. Heat shock studies indicate that temperature perhaps stimulates the expression of proteins that aid in tiding over the stress and crucial for survival, mediated through heat shock proteins (HSPs)[4]. Heat shock protein genes are members of a highly conserved gene family. They are found in practically all living organisms, from bacteria to humans[5]. Several families of heat shock proteins (HSPs) are known to be expressed in insects, including mosquitoes, and may have a cumulative role in responding to stress induced by elevated temperature[6-8]. In addition to heat shock, their production can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exercise, exposure of the cell to toxins, starvation, hypoxia, nitrogen deficiency (in plants), or water deprivation in human cells and many other animals and insects including mosquitoes[9-12]. For example, in *Anopheles stephensi*, it has been documented that temperatures >39ºC induced HSPs[13]. Post blood meal, a rise in Hsp70 has been observed in *Culex pipiens* and *Anopheles gambiae*[14]. A similar phenomenon has been observed in bed bug, *Cimex lectularius*, suggesting the protective role of HSPs[15]. Thermal stress in *Ae. aegypti* larvae have been found to affect the susceptibility in adults to the chikungunya virus[16]. Overexpression of Hsp70 and Hsp90 to stress like dehydration have been observed in mosquitoes like *Ae. aegypti*, *Ae. gambiae* and *Culex pipiens*. Overexpression of Hsp70 under induced thermal stress in stem borer, *Chilo suppressalis* have been reported[17]. As a consequence, these proteins are also referred to as stress proteins[18]. Therefore, the relative expression of HSP genes in different developmental stages is important to understand the overall response of mosquitoes to heat shock and other types of environmental stress.

The mechanism by which heat shock (or other environmental stressors) activates the heat shock factor has been determined in bacteria. During heat stress, outer membrane proteins (OMPs) do not fold and cannot insert correctly into the outer membrane. They accumulate in the periplasmic space. These OMPs are detected by DegS, an inner membrane protease that passes the signal through the membrane to the sigmaE transcription factor[19]. However, some studies suggest that increased damaged or abnormal proteins bring HSPs into action[20]. HSPs function as molecular chaperones stimulating precise refolding, assisting in establishing proper protein conformation (shape), and preventing aggregation of denatured proteins[21]. These proteins also play crucial roles in assembling multiprotein complexes, cell-cycle control and signaling, and protection of cells against stress or apoptosis. More recently, they have been implicated in antigen presentation to the class I and class II molecules of the major histocompatibility complexes[22]. Heat shock proteins appear to serve a significant cardiovascular role[23].

HSPs constitute a large family of proteins that are often classified based on their molecular weight. In *Ae. aegypti,* three HSP genes have been well characterized and well studied, namely *AaeHsp26, AaeHsp83,* and *AaeHsc70*[4]. In this study, the relative expression of these genes after heat shock application has been studied to unfold the principle behind the thermal adaptation of dengue vector *Ae. aegypti.*

Materials and Methods

The entire research work includes collecting sample (mosquito larvae), rearing mosquitoes, egg collection and storage, RNA extraction and cDNA synthesis, qPCR amplification, and different statistical analyses. Experiments were conducted at Zoological Garden (Dhaka University), Genetics and Molecular Biology Laboratory (Department of Zoology), Center for Advanced Research in Science (Dhaka University), and Invent Technologies Limited. The research was conducted from January, 2019 to July, 2019. Extreme heat shock treatment (37°C and 42°C) was applied to third instar larvae for 60 minutes to optimize the response and facilitate detection of expression of specific genes. Untreated larvae (controls) were held at constant room temperature (27°C) in distilled water. Two replicates were performed for each experiment. About 30 third instar larvae were taken for each RNA extraction sample.

Mosquito Rearing: The larvae of *Ae. aegypti* were collected weekly from their natural oviposition sites (barrels, drums, jars, pots, buckets, flower vases, etc.) and by using artificial ovitraps as well. The larvae of *Ae. aegypti* was identified by morphological methods using the pictorial taxonomic key and molecular method through DNA barcoding using16s rRNA gene[24]. The temperature and relative humidity of the environment of the laboratory of Zoological Garden, Dhaka University, during the mosquito rearing process was maintained at 27-35°C and 55-77%, respectively. During the rearing process, the larvae and adult mosquitoes were provided with chicken liver powder suspension, where about 5 g of chicken liver powder was weighted, and dH_20 was added to a final volume of 500 ml. It was mixed and stored at 4°C and 5% sucrose solution (Around 9.5 g sucrose was combined with 189 ml distilled water, mixed to form the reagent, and stored at 4°C) respectively. At the beginning of the rearing process, the larvae collected were transferred with a transfer pipette to a 3 L large bowl containing 1.5 L water in a quantity of 150 larvae per bowl to avoid overcrowding. Chicken liver powder suspension in an amount of 15 ml was added to each bowl and covered a with lid. Once larvae became pupae, they were transferred from the bowl into 500 ml plastic cups containing 250 ml distilled water, and these cups were placed into rearing cages (constructed with a thin iron rod frame and 18 inch × 18 inch × 18 inches in size). The cups containing pupae were placed inside into a rearing cage and allowed the pupae to become adults. Three to four cotton balls were soaked in 5% sucrose solution, squeezed them together slightly to make one ball, and placed it on the top of a conical flask, and

then around 2/3 conical flasks were placed in each mosquito rearing cage. An anesthetized pigeon was used for blood feeding. For blood feeding, the animal was placed inside the netted cage for 15 min. While adult female mosquitoes became 3 days old, they were deprived of sucrose solution for 12-24 hr before to blood feeding.

Egg Collection, Store and Further Rearing: A piece of brown paper (9 cm × 20 cm) was cut and labeled with the strain type, date, and time. A 500 ml plastic cup was filled with 250 ml distilled water, and the paper was placed in direct contact with the inner wall of the cup along with the water/air interface. The cup was placed in the cage. The brown papers, called egg papers, were collected after 3 days. The collected egg papers were allowed to be dried for 3 days in the insectary. Once dried, those were wrapped with a piece of folded paper towel and placed in a plastic container.

RNA Extraction and cDNA Synthesis: RNA was extracted from *Ae. Aegypti* 3rd instar larvae using 'Purelink™ RNA Mini kit, Invitrogen' (Catalogue number: 12183018A) according to the manufacturer's instructions. RNA samples were quantified by Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific). cDNA synthesis was done from the extracted RNA according to the manufacturer's protocol by using the cDNA synthesis kit, "RevertAid First Strand cDNA Synthesis Kit" (Cat#K1621).by Thermo Fisher Scientific.

Quantitative qPCR Amplification: The qPCR assay for HSP genes was performed using Real-time qPCR machine (qTOWER3, Analytic Jena). The 25 µl reaction volume contained 12.5 µl Syber green Universal master mix, 0.5 µl of each primer and 2 µl of template cDNA of test and control separately. In all qPCR runs, the AeaActin protein gene was used as an internal control to normalize for variation in the amount of the cDNA template. Following PCR, thermal cycling conditions were used: initial denaturation at 95ºC for 3 min; 40 cycles of denaturation at 95ºC for 15 seconds, annealation at 54ºC for 15 seconds, and a final extension at 60ºC for 20 seconds. PCR primers used in this study are shown in Table 1.

Table 1. List of primers used in PCR and qPCR to amplify HSP genes.

Name	Sequence	Name	Sequence
AaeAct4_F	5'-ATC CAT TGT CCA CCG CAA GT-3'	AaeAct4_R	5'-ATG TGA GCA GTT TCA GCC CG-3'
AaeHsp26_F	5'-TTC AGC ATC CTT CTC CTC GT-3'	AaeHsp26_R	5'-CCA CGA ACT TCC AGG TCA AT-3'
AaeHsp83_F	5'-AAG GCC GTT AAG GAT CTG GT-3'	AaeHsp83_R	5'-GCT AGT GTG GGG AAG AGA G-3'
AaeHsc70_F	5'-ATG AAC CCA ACC AAC ACC AT-3'	AaeHsc70_R	5'-TGG AAC TGA TTT CCT CTG GG-3'

Three HSP genes of *Ae. Aegypti* are *AaeHsp26*, encode a small heat-shock protein, *AaeHsp83*, encodes Hsp90 protein, and *AaeHsc70* encodes Hsc70 protein[4].

Relative Gene Expression Analysis by using Delta-Delta C_T Method ($\Delta\Delta C_T$): Relative change in gene expression can be analyzed using the $\Delta\Delta C_T$ method[25]. In this method for calculating relative gene expression following formulas were used -

(i) Calculating ΔC_T:- $\Delta C_T = C_T$ (Target Gene) - C_T (Housekeeping Gene) (1)

(ii) Calculating Average ΔC_T:- To calculate the $\Delta\Delta C_T$ value, average ΔC_T value was calculated from the ΔC_T values of control (27°C) sample, using the formula, Average ΔC_T = (ΔC_T values of control samples)/(Total no. of control samples) (2)

(iii) Calculating $\Delta\Delta C_T$:- $\Delta\Delta C_T$ = Sample ΔC_T - Average ΔC_T (3)

(iv) Calculating fold gene expression:- fold gene expression = 2^-($\Delta\Delta C_T$) (4)

Results and Discussion

To determine whether HSP genes are differently expressed and over dispersed during thermal stress treatment, qPCR analysis (estimation of mRNA of HSPs) was performed after *Ae. aegypti* larvae (3rd instar) were exposed to heat shock (37ºC and 42ºC) and at room temperature (27ºC) for a one hour heat shock period. F1 (first generation) generation larvae were used for the experiment.

The relative expression of the small HSP gene *AeaHsp26* was up-regulated after 1 hour at 37°C and 42°C treatment of third instar *Ae. aegypti* larvae (78.36 ± 5.76 and 3.04 ± 0.28), more than about 78 and 3-fold increase over that found in the untreated control (1.001 ± 0.067) respectively (Table 2, Fig. 1a). According to Shivan[26] when late third instar larvae were subjected to heat shock at 37°C and 39°C, elevations in the expression of *AeaHsp26* about 7.49 ± 2.91 and 9.75 ± 3.28 folds respectively. In the study of Zhao[4] the relative expression of *AeaHsp26* was up-regulated after 15 min at 42°C treatment of first instar *Ae. aegypti* larvae (275.03 ± 2.154), more than a 1,300-fold increase over that found in the untreated control (0.1985±0.008) and as the time increased (30, 60, and 180 min), the relative gene expression level also increased and reached 3,000-fold after 3 h at 42°C treatment compared with the control.

The expression of Hsp90 protein, encoded by *AeaHsp83,* was up-regulated after 1h at 37°C and 42°C treatment of *Ae. aegypti* larvae (4.03 ± 0.098 and 4.79 ± 0.61) more than about 4 and 4.79-fold relative increase respectively, over that found in 27°C control (1.001 ± 0.074) (Table 3, Fig. 1b). In case of *AaeHsp83* gene for 42°C, 1 hour heat shock period, no significant result was found. Whereas the study of Shivan[26] shows that the expression level of *AaeHsp83* in the late 3rd instar larvae subjected to heat shock 37°C and 39°C was 655.16 ± 243.0 and 4938.57 ± 1908.1 folds respectively, which do not correspond with the present study. When Zhao[4] experimented with the 1st instar larvae, the expression of *AeaHsp83* was up-regulated after 1 h at 42°C treatment of *Ae. aegypti* larvae (21.28 ± 0.119), more than a 44-fold relative increase over that found in the 1 h 23°C control (0.458 ± 0.023) and after 3 h gene expression (8.296 ± 1.254) increased significantly (50-fold) when compared with the untreated control (0.156 ± 0.016).

The *AeaHsc70* expression level increased significantly after 1 hour at 37°C and 42°C treatment (46.21 ± 0.68 and 82.43 ± 0.81) about 45 and 80-fold, respectively in *Ae. aegypti* larvae compared with the control (1.008 ± 0.13) (Table 4, Fig. 1c). In turn, the study of

Shivan[26] has been concluded that the late 3rd instar larvae subjected to heat shock (37°C and 39°C) also showed up-regulation of *AaeHsp70* about (5.25 ± 1.99 and 18.6 ± 6.95-folds, respectively). In study of Zhao[4] where 1st instar was used, *AeaHsc70* expression level increased significantly but at a much lower level (fourfold) after 15 min at 42°C treatment

Table 2. Expression of *AaeHsp26* gene under heat shock treatment in *Ae aegypti* 3rd instar larvae exposed to 27, 37, and 42°C for one hour.

Temperature	Cyclic threshold (C_T)		ΔC_T	$\Delta\Delta C_T$	$2^{-\Delta\Delta C_T}$	Mean ± SD
	Actin	Hsp26				
27°C	17.45	21.28	3.83	-0.065	1.046	1.001 ± 0.067
	17.34	21.30	3.96	0.065	0.956	
37°C	17.04	14.57	-2.47	-6.365	82.424	78.355 ± 5.755
	16.79	14.47	-2.32	-6.215	74.285	
42°C	16.81	19.20	2.39	-1.505	2.838	3.038 ± 0.283
	16.62	18.82	2.20	-1.695	3.238	

Table 3. Expression of *AaeHsp83* gene under heat shock treatment in *Ae aegypti* 3rd instar larvae exposed to 27, 37, and 42°C for one hour.

Temperature	Cyclic threshold (C_T)		ΔC_T	$\Delta\Delta C_T$	$2^{-\Delta\Delta C_T}$	Mean ± SD
	Actin	Hsp83				
27°C	17.45	13.64	-3.83	0.075	0.949	1.001 ± 0.074
	17.34	13.38	-3.96	-0.075	1.053	
37°C	17.04	11.12	-5.92	-2.035	4.098	4.029 ± 0.098
	16.79	10.92	-5.87	-1.385	3.959	
42°C	16.83	15.07	-1.76	2.125	4.362	4.793 ± 0.609
	16.81	15.31	-1.50	2.385	5.223	

Table 4. Expression of *AaeHsc70* gene under heat shock treatment in *Ae aegypti* 3rd instar larvae exposed to 27, 37 and 42°C for one hour.

Temperature	Cyclic threshold (C_T)		ΔC_T	$\Delta\Delta C_T$	$2^{-\Delta\Delta C_T}$	Mean ± SD
	Actin	Hsc70				
27°C	17.30	18.74	1.44	-0.135	1.098	1.008 ± 0.128
	17.45	19.16	1.71	0.135	0.917	
37°C	16.79	12.85	-3.94	-5.515	45.728	46.209 ± 0.680
	17.04	13.07	-3.97	-5.545	46.689	
42°C	16.81	12.03	-4.78	-6.355	81.855	82.427 ± 0.808
	16.62	11.82	-4.80	-6.375	82.998	

(2.132±0.959) compared with the control (0.536±0.189). A slight but insignificant increase in expression of *AeaHsc70* mRNA was found after heat-shock at 1 h 42°C treatment (1.370±0.160) compared with the control (1.169±0.050).

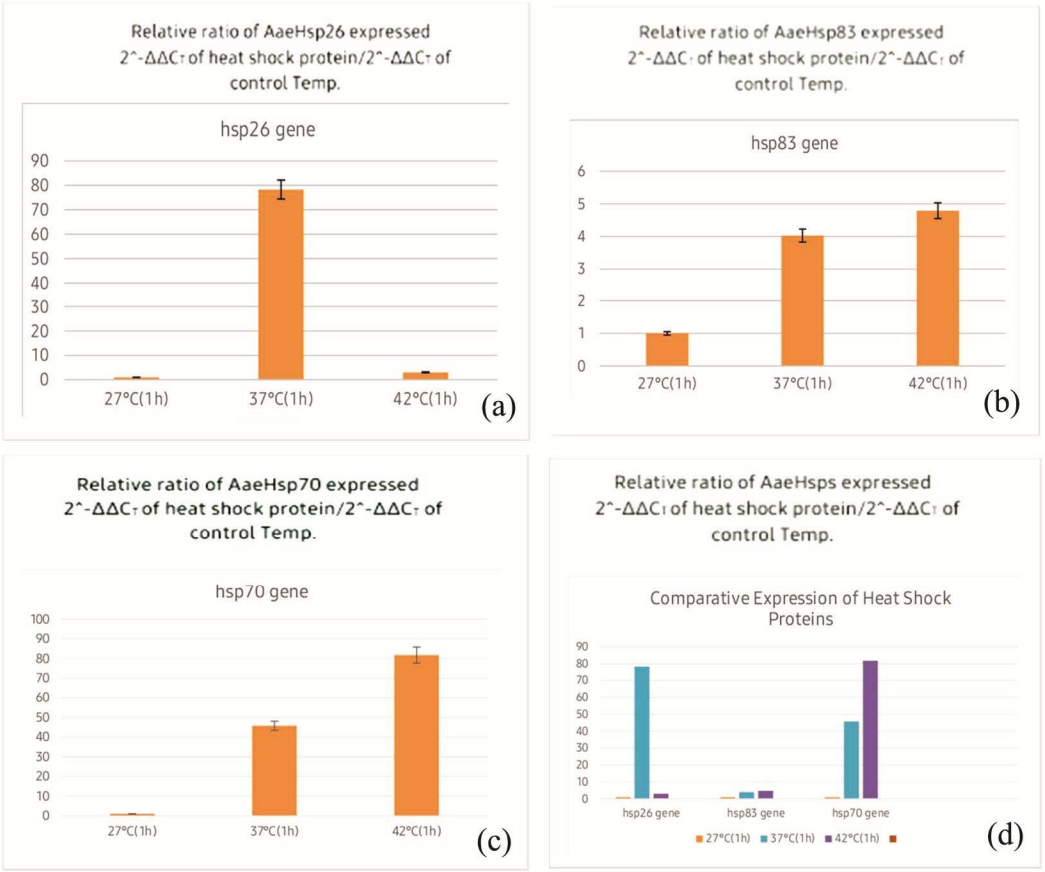

Fig. 1. (a) qPCR results are showing relative expression of *AaeHsp26*, (b) *AaeHsp83*, (c) *AaeHsc70* genes, and (d) Comparative expressions of all the three HSP genes after 37 and 42°C heat shock treatment compared with the 27°C control respectively.

Here it has been seen that the present study does not correspond to the previous literature in different aspects. This may occur due to the use of different instar stages. For example, Zhao[4] used 1st instar, and Shivan[26] used late 3rd instar, whereas, in this study, early 3rd instar larvae were used. Again, culture generation is an important factor. Like in this study, F1 generation larvae were used (culture generation of larvae used in the study of Shivan[26] and Zhao[4] was not mentioned), other reasons can be different environmental factors (nutrient, moisture, day-night period, temperature, etc.). Moreover, in this study, the control temperature was 27°C, whereas, in the study of Zhao, the control temperature was 23°C.

These observations indicate that the HSP genes viz. *AeaHsp26, AeaHsp83,* and *AaeHsp70* were significantly up-regulated at elevated temperatures, and thus these HSPs could be used as thermal stress markers. This upregulation of HSP genes when the water temperature of the containers rises could be the reason for the survival mechanisms in *Ae. aegypti* through the extremely hot summer days. This rise in temperature above the average temperature in an endemic area could probably enhance the selection of temperature-tolerant mosquitoes in a population that could have enhanced longevity, eventually affecting intrinsic and extrinsic factors by a reduction in the extrinsic incubation period and increase in susceptibility of mosquitoes to arboviral pathogens[27].

References

1. Brady OJ, PW Gething, S Bhatt, JP Messina, JS Brownstein and AG Hoen 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. Plos Negl. Trop. Dis. **6:** 1760.
2. Gakhar SK and H Shandilya 1999. Heat shock response during development of theamalaria vector *Anopheles stephensi* (Culicidae: Diptera). Cytobios **99:** 173-182.
3. Zhao L, JW Pridgeon, JJ Becnel, GG Clark and KJ Linthicum 2009. Identifcation of genes differentially expressed during heat shock treatment in *Aedes aegypti*. J. Med. Entomol. **46**: 490-495.
4. Zhao L, JJ Becnel, GG Clark and KJ Linthicum 2010. Expression of aeahsp26 and aeahsp83 in *Aedes aegypti* (diptera: Culicidae) larvae and pupae in response to heat shock stress. J. Med. Entomol. **47**: 367-375.
5. Walter S and J Buchner 2002. Molecular chaperones-cellular machines for protein folding. Angewandte 0 Chemie. **41**(7): 1098-1113.
6. Mahroof R, KY Zhu, I Neven, B Subramanyam and J Bai 2005. Expression patterns of three heat shock protein 70 genes among developmental sstages of the red flour beetle, *Tribolium castaneum* (coleoptera: Tenebrionidae). Comp. Biochem. Physiol. A Mol. Integr. Physiol. **141:** 247-256.
7. Robich RM, JP Rinehart, IL Kitchen and DL Denlinger 2007. Diapause-specific gene expression in the northern houseamosquito, *Culex pipiens* L., identified by suppressive subtractive hybridization. J. Insect. Physiol. **53**: 235-245.
8. Rinehart JP, RM Robich and DL Denlinger 2006. Enhanced cold and desiccation tolerance in diapausing adults of *culex pipiens*, and a role for hsp70 inaresponse to cold shock but not as a component of the diapause program.aJ. Med. Entomol. **43**: 713-722.
9. Mosser DD, NG Theodorakis and RI Morimoto 1988. Coordinate changes in heatashock element-binding activityaand hsp70 gene transcription rates ainahuman cells. Mol. Cell. Biol. **8:** 4736-4744.
10. Yamuna A, V Kabila and P Geraldine 2000. Expression of heat shock protein 70 in freshwater prawn *Macrobrachium malcolmsonii* (h. Milne edwards) following exposure to Hg and Cu. Indian J. Exp. Biol. **38**: 921-925.
11. Boone AN and MM Vijayan 2002a. Constitutive heat shock protein 70 (hsc70) expression in rainbow troutahepatocytes: Effect of heat shock and heavy metal exposure. Comp. Biochem. Physiol. Ctoxicol. Pharmacol. **132**: 223-233.

12. Boone AN and MM Vijayan 2002b. Glucocorticoid mediated attenuation of the hsp70 response in troutahepatocytes involves the proteasome. Am. J. Physiol. Regul. Integr. Comp. Physiol **283**: R680-r687.
13. Spees JL, SA Chang, MJ Snyder and ES Chang 2002. Osmotic induction of stress-responsive gene expression in thealobster homarus americanus. Biol. Bull. **203**: 33-337.
14. Benoit JB, Lopez-Martinez G, Phillips ZP, Patrick KR and Denlinger DL 2010. Heat shock proteins contribute to mosquito dehydration tolerance. J. Insect Physiol. **56**(2): 151-156.
15. Benoit JB, G Lopez-martinez, KR Patrick, ZP Philips, TB Krause, L David and DLaDenlingera 2011. Drinking a hot blood meal elicits a protective heat shock response in mosquitoes. Proc. Natl. Acad. Sci. USA **108**: 8026-8029.
16. Mourya DT, P Yadav and AC Mishra 2004. Effect of temperature stress onaimmature stages and susceptibility of aedes aegypti mosquitoes toachikungunya virus. Am. J. Trop. Med. Hyg. **70**: 346-350.
17. Ya-dong C, Yu-zhou du1a ming-xing lu., Cheng-kuiqiang 2010. Cloning of the heat shock protein 60 geneafrom the stem borer, chilo suppressalis, and analysis of expression characteristics under heat stress. J. Insect. Science **10**: 100.
18. Santoro MG 2000. Heat shock factors and the control of the stressaresponse. Biochem. Pharmacol. **59**(1): 55-63.
19. Walsh NP, BM Alba, B Bose, CA Gross and RT Sauer 2003. OMP peptide signals initiate the envelope-stressaresponse by activating Deg S aprotease via relief of inhibition mediated by its PDZ domain. Cell **113**(1): 61-71.
20. Narberhaus F 2010. Translational control of bacterial heat shock and virulenceagenesaby temperature-sensing mRNAs. RNA Biology **7**(1): 84-89.
21. Johnson BD, RJ Schumacher, ED Ross and DO Toft 1998. Hop modulatesahsp70/hsp90 interactions in protein folding. J. Biol. Chem. **273:** 3679-3686.
22. Li Z, A Menoret and P Srivastava 2002. Roles of heat-shock proteins in antigen presentation and cross-presentation. Curr. Opin. Immunol. **14:** 45-51.
23. Benjamin IJ and DR Mcmillan 1998. Stress (heat shock) proteins: Molecularachaperones in cardiovascular biology and disease. Cir. Res. **83**(2): 117-132.
24. Leopoldo MR 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Zootaxa **589**: 1-60.
25. KJ Livak and Schmittgen TD 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods **25**(4): 402-408.
26. Sivan A, AN Shriram and NMR Thamizhmani 2017. Expression of heat shockaproteins (hsps) in *Aedes aegypti* (l) and *Aedes albopictus* (skuse) (diptera: Culicidae) larvae in response to thermal stress. Acta Tropica. **167**: 121-127.
27. Muturi EJ, K Costanzo, B Kesavaraju and BW Alto 2011. Can pesticides and larval competition alter susceptibility of *Aedes* mosquitoes (Diptera: Culicidae) to arbovirus infection? J. Med. Entomol. **48**: 429-436.

(Manuscript received: 22 April, 2021; accepted: 30 May, 2021)