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Heat shock induced changes in the cocoon traits of poly- and bi-voltine silkworm strains of *Bombyx mori*

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Abstract
The manifestation of cocoon characters due to heat shock (HS) during the larval stage of poly- (Daizo and Pure Mysore) and bi-voltine (NB_4D_2 and CSR_2) silkworm strains of *Bombyx mori* was studied. Because, temperature being a major factor that shape distribution of organisms, insects in particular have diverse response to deal with critical temperature and exposure to wide-ranging thermal conditions led to amendment of biochemical and physiological protective mechanisms. To this, different instar larvae of poly- and bi- voltine breeds were subjected to thermal stress and the data generated in respect of cocoon traits were pooled separately for statistical analysis. Interestingly, highest weight of the cocoon was recorded from fifth instar CSR_2 larvae HS at 40°C (1.99 g) with an improvement of 44.20% against control (1.38g). But, high rate of response to HS at 40°C was recorded in NB_4D_2 with an improvement of 46.82% in the weight of the cocoon. Among different instars, the fifth instar larvae of all the breeds HS at 40°C produced good quality cocoon with higher shell weight than control groups. Consequently, although, all instars larvae HS at 35 and 40°C metamorphosed into pupae with better growth than control highest per cent of improvement in it was recorded from fifth instar. However, heat shock temperature of 45˚C was lethal since all the biological and commercial characters were severely affected in all instars. Thus, temperatures of 40°C shall be taken into account to elicit profound response to acquire tolerance to overcome the fluctuated environmental condition, while screening for better parents to develop thermotolerant breeds/hybrids for tropical countries like India.

Keywords: *bombyx mori*, heat shock, thermotolerant, cocoon traits

1. Introduction
The insects are the most abundant creatures on the earth and systematic attention to recognize their reaction to ecological and environmental stresses has improved considerably because of their medical, economical and ecological significance. Due to high commercial value many sericigenous insect species have been widely used for the extraction of silk protein fibers. The silkworm, *Bombyx mori* L., is a domesticated insect, accepted for its silk producing aptitude and as a model system for biological experiments. Longtime domestication has made the silkworm sensitive to abiotic and biotic stresses. Among abiotic factors, temperature is an important determinant of growth and productivity as silkworms which is poikilothermic insect [1]. As a result, organisms make use of varied adjustments at different levels of biological organization to deal with the fluctuating natural thermal environment [2, 3, 4].
Response to heat shock of an organism's habituating tropical environment is differing from those of temperate climate. According to the field rearing of mulberry silkworms this feature has direct significance wherein polyvoltine breeds of *B. mori* in India (Pure Mysore, C. Nichi, Nistari etc.,) display more tolerance to high temperatures compared to the bivoltine breeds (NB_4D_2, NB_{18} etc.,) of temperate origin [5, 6]. However, among different bivoltine breeds NB_4D_2 and NP2 exhibited better survivability and positive effect of HS on commercial traits of cocoons thorough acquired tolerance against control [7]. Among different instars, resistance to HS has increased as the larval development proceeds in the order of first instar > second instar > third instar > fourth instar > fifth instar silkworm along with increased cocoon and shell weight over control [7, 8].
Due to global warming, the impact of high temperature on living organisms has been the central part of scientific investigation in recent days. Moreover, all species has its own choice of temperatures for its usual growth and very high temperature not only slows down the growth also induce developmental malfunction, such as larval ecdysis and adult emergence [10]. In poikilotherms, temperature decides the fate of insect during embryonic and postembryonic development and affects the life-cycle plasticity, physiological thermal traits, immune responses and gene expression.

2. Materials and Methods
Silkworm strains, rearing and maintenance of larvae
The eggs of polyvoltine (Daizo and PM) and bivoltine (NB_4D_2 and CSR_2) strains were incubated under optimum temperature 25±1˚C and 75±5% relative humidity followed by black box until hatching. The larvae were reared on mulberry leaves up to spinning following standard rearing procedures.

Induction of heat shock
Larvae of different strains in each instar were placed separately in thin-walled Petri dishes/test tubes for HS at 35, 40 and 45˚C with 75% ± 5% relative humidity in the water bath for 2 h. After HS induction the larvae were transferred to room temperature for recovery for 2 h [7] and then they were reared under natural fluctuated environmental conditions until spinning.

Table 1: Schedule for heat shock treatment

I instar	2nd day after hatching
II instar	2nd day after 1st moult
III instar	2nd day after 2nd moult
IV instar	3rd day after 3rd moult
V instar	3rd day of V instar

Cocoon weight
About 6 cocoons selected randomly from each replication on day 6 after spinning were used to record their weight.

Shell weight
The cocoon shell weight was recorded after removing the pupa from the randomly selected 6 cocoons from each replication.

Shell ratio
Shell ratio was calculated using the formula

$$= \frac{\text{Shell weight}}{\text{Cocoon weight}} X100$$

Pupal weight
For pupal weight, the pupae removed from randomly selected cocoons (6) of each replication were used.

Data analysis
All the data were subjected for statistical analysis using two-way ANOVA.

3. Results
Induction of HS at different instar larvae of both poly- and bivoltine breeds of *B. mori* exhibited significant relative influence on the characteristics of cocoons as analyzed through two-way ANOVA at $p<0.05$. Sustained survival following the HS at 35 and 40˚C was significantly higher in all the silkworm strains against their respective controls while 45˚C was lethal as all the commercial traits were insensitively affected in all instars.

Impact of heat shock on the cocoon
Weight of the cocoons spun by the larvae HS at 35 and 40°C was found increased over their respective control populations (Fig.1). An average highest weight of the cocoon was noticed in CSR_2 strain HS 40°C in fifth instar (1.99g) with an improvement of 44.20% against control (1.38g). Concurrently, 46.82% of improvement was recorded in NB_4D_2 at the same stage with an average weight of 1.26 g in control. The cocoons spun by the larvae of NB_4D_2 HS at 35˚C in I, II, III, IV and V instars weighed 1.47, 1.55, 1.68, 1.74 and 1.79g respectively whereas in control it was 1.26g. Whereas in CSR_2 HS at 35˚C during I, II, III, IV and V instars the cocoon weighed was 1.48, 1.56, 1.69, 1.78 and 1.78g respectively against control of 1.38g. The cocoons spun by larvae of Daizo HS at 35˚C in I, II, III, IV and V instars weighed 1.25, 1.31, 1.31, 1.44 and 1.48g respectively as against 1.12g in control. The cocoons spun by the larvae of PM HS at 35˚C in I, II, III, IV and V instars weighed 1.20, 1.21, 1.23, 1.25 and 1.27g respectively but in control the cocoon weight was 1.05g (Fig.1A).
The cocoons spun by the larvae of NB_4D_2 HS at 40˚C during I, II, III, IV and V instars were weighed 1.53, 1.64, 1.76, 1.84 and 1.85g respectively against 1.26g in control. Whereas in CSR_2, 1.51, 1.62, 1.76, 1.89 and 1.99g of cocoons were spun by the larvae HS at 40˚C during I, II, III, IV and V instars respectively but in control it weighed 1.38g. Cocoons spun by the larvae of Daizo HS at 40˚C in I, II, III, IV and V instars weighed 1.26, 1.35, 1.47, 1.57 and 1.65g respectively as against 1.12g cocoon weight in control. The cocoons spun by the larvae of PM HS at 40˚C in I, II, III, IV and V instars were weighed 1.23, 1.25, 1.27, 1.28 and 1.30g respectively but in control the cocoon weight was 1.05g (Fig.1B).

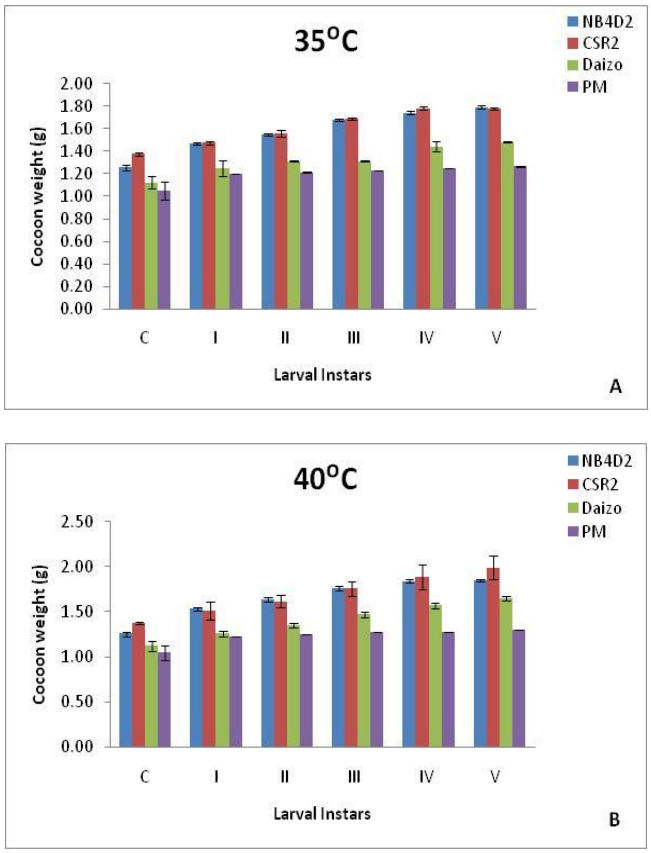

Fig 1: Impact of heat shock on the weight of the cocoon of *Bombyx mori*.

Impact of heat shock on cocoon shell
Cocoon shell weight was also correspondingly found increased upon exposure of different instars larvae of poly- and bivoltine silkworm strains to HS at 35 and 40°C (Fig. 2). Highest cocoon shell weight of 0.33g was recorded in CSR_2 HS at 40°C in fifth instar as against 0.23g in control. Whereas least of 0.20g shell weight recorded in PM was at 40°C against 0.11g in control in fifth instar. At 35˚C HS, the shell weight recorded was 0.24, 0.25, 0.28, 0.29 and 0.29g from I, II, III, IV and V instars HS larvae of NB_4D_2 respectively against 0.20g in control. The shell weight of 0.25, 0.27, 0.28, 0.30 and 0.32g respectively was noticed in I, II, III, IV and V instars larvae of CSR_2 HS at 35˚C. In Daizo, the cocoon shell weight recorded in control was 0.18g but in I, II, III, IV and V instars HS at 35˚C group it was 0.19, 0.20, 0.22, 0.23 and 0.25g respectively. I, II, III, IV and V instars larvae of PM HS at 35˚C produced 0.12, 0.14, 0.15, 0.16 and 0.18g of cocoon shell but in control it was 0.11g (Fig. 2A).
Upon exposure of I, II, III, IV and V instars larvae of NB_4D_2 to 40˚C HS produced cocoon shell weighing 0.25, 0.28, 0.28, 0.31 and 0.31g respectively against 0.20g in control. In CSR_2, it measured 0.26, 0.28, 0.30, 0.30 and 0.33g in I, II, III, IV and V instars respectively against 0.23g in control. The cocoon shell

weight recorded in control was 0.18g, while it was 0.18, 0.22, 0.26, 0.26 and 0.27g in I, II, III, IV and V instars larvae of Daizo HS at 40˚C respectively. The cocoon shell weight of 0.14, 0.15, 0.17, 0.18 and 0.20g was noticed in I, II, III, IV and V instars larvae HS at 40˚C respectively against 0.11g in control group of PM (Fig. 2B).

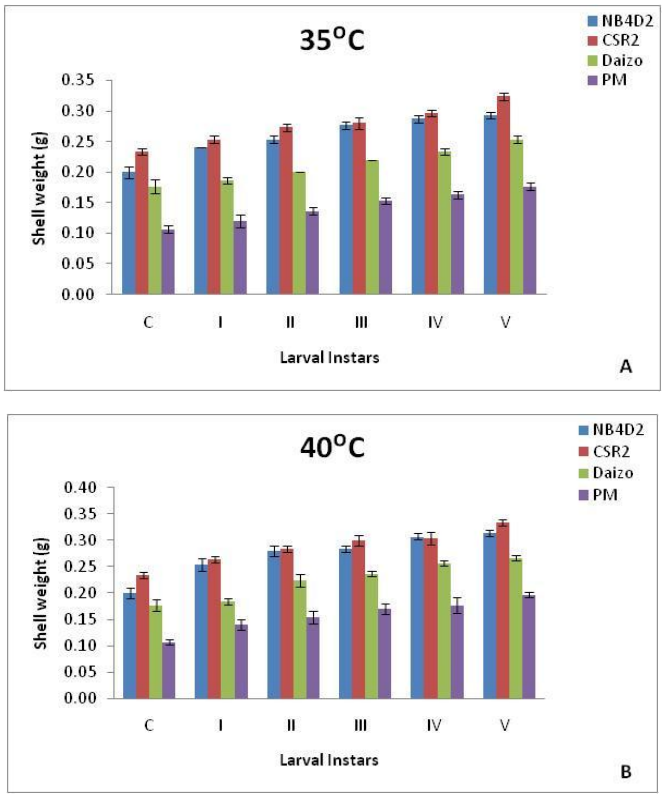

Fig 2: Impact of heat shock on the weight of the cocoon shell of *Bombyx mori*.

Impact of heat shock on cocoon-shell

The data on cocoon shell ratio in response to HS at 35 and 40°C in different instar larvae revealed discrete variations, which is still higher than their respective controls. In NB_4D_2, the cocoon shell ratio was 15.92% in control, but 16.33, 16.34, 16.47, 16.44 and 16.36% of shell ratio was recorded from I, II, III, IV and V instars larvae HS at 35˚C respectively. 17.16, 17.56, 16.60, 16.67 and 18.16% of shell ratio was recorded in I, II, III, IV and V instars larvae respectively HS at 35˚C, but 16.66% was noticed in control larvae of CSR_2. I, II, III, IV and V instars larvae HS at 35˚C exhibited the shell ratio of 14.97, 15.23, 16.75, 16.20 and 17.08% against 16.92% in control larvae of Daizo respectively. The shell ratio of 10.55% while noticed in control, it was 10.00, 11.26, 12.47, 13.07 and 13.95% after exposure of I, II, III, IV and V instars PM larvae to HS at 35˚C respectively (Fig.3A).

Upon HS at 40˚C, the NB_4D_2 I, II, III, IV and V instars larvae exhibited 16.52, 17.03, 16.13, 16.63 and 16.93% cocoon shell ratio respectively whereas in control it was 16.32%. 17.44, 17.55, 17.11, 16.12 and 16.89% shell ratio was noticed in I, II, III, IV and V instars larvae of CSR_2 HS at 40˚C respectively against 17.19% in control. The cocoon shell ratio of Daizo was 16.92% in control whilst 14.60, 16.53, 16.61, 16.35 and 16.19% was reported from I, II, III, IV and V instars larvae HS at 40˚C respectively. I, II, III, IV and V instars larvae of PM HS at 40˚C showed 11.38, 12.27, 13.38, 13.80 and 15.10% shell ratio respectively as against 10.55% in control (Fig.3B).

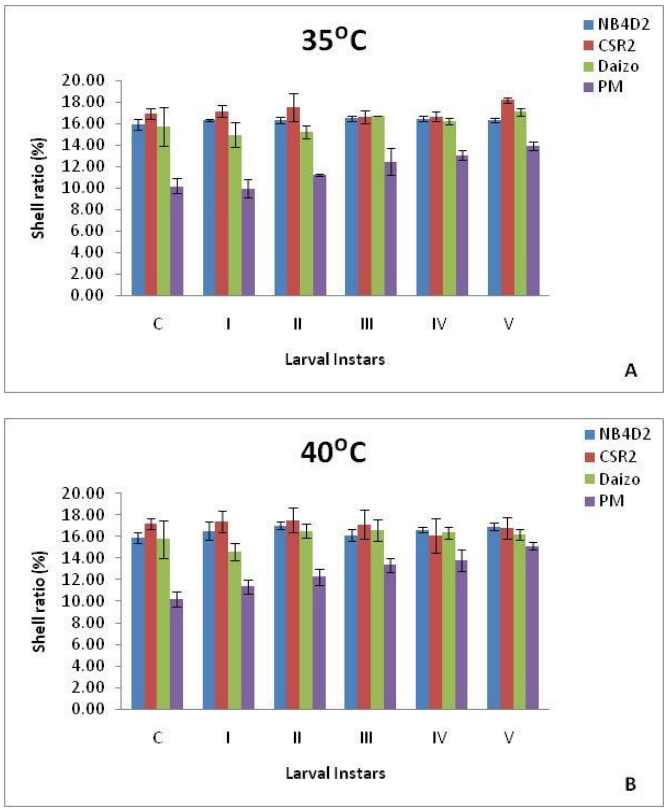

Fig 3: Relative influence of heat shock on the cocoon shell ratio of *Bombyx mori*.

Impact of heat shock on pupa

Weight of the pupae, as an index of physiological status of the larvae comparatively found increased after exposure of different instars larvae to varied HS temperature. Interestingly, third and fourth instar HS induced silkworm larvae at 35 and 40°C metamorphosed into pupae with better growth than control. Eventually, highest pupal weight 1.66g was observed in fifth instar larvae of CSR_2 HS at 40°C as against 1.13g in control. In NB_4D_2, the pupal weight of 1.23, 1.30, 1.40, 1.46 and 1.50g was recorded from I, II, III, IV and V instars larvae HS at 35˚C respectively but in control it was 1.06g. The pupal weight of Daizo was 0.94g in control, whereas 1.05, 1.08, 1.07, 1.20 and 1.24g was noticed from I, II, III, IV and V instars HS induced larvae respectively. 1.07, 1.07, 1.07, 1.08 and 1.08g pupal weight was noticed in I, II, III, IV and V instars larvae HS at 35˚C respectively against 1.93g in control group of PM (Fig. 4A).

After exposure to HS temperature of 40˚C, I, II, III, IV and V instars larvae of NB_4D_2 metamorphosed into pupae were weighed 1.28, 1.36, 1.47, 1.54 and 1.55g respectively, while it was 1.06g in control. Whereas as in CSR_2, the pupal weight in control was 1.13g while HS induced (40˚C) I, II, III, IV and V instars larvae developed as pupae weighed 1.25, 1.33, 1.46, 1.59 and 1.66g respectively. 1.07, 1.12, 1.23, 1.28 and 1.37g of pupal weight was recorded from I, II, III, IV and V instars larvae of Daizo respectively against 0.94g in control. The pupal weight recorded from the control group of PM was 1.93g whereas in HS (40˚C) induced I, II, III, IV and V instars larval group it was 1.10g (Fig.4B). However, since none of the larvae were survived at 45˚C cocoon weight, shell weight, shell ratio and pupal weight was not recorded and presented.

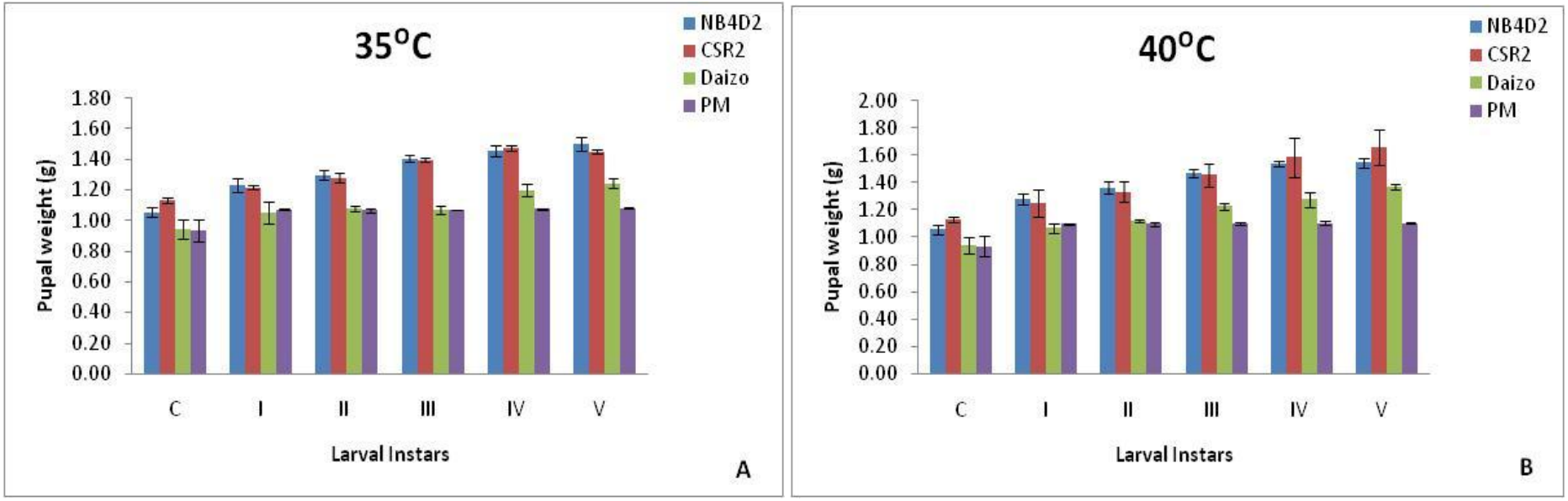

Fig 4: Impact of heat shock on the weight of the pupae of *Bombyx mori*.

Table 2: Two-way statistical analysis for the cocoon characters of different silkworm strains of *Bombyx mori* Exposed to heat shock.

		Cocoon weight				Shell weight				Shell ratio				Pupal weight			
		NB4D2	CSR2	Daizo	PM	NB4D2	CSR2	Daizo	PM	NB4D2	CSR2	Daizo	PM	NB4D2	CSR2	Daizo	PM
F- Value	Control	1.26	1.38	1.12	1.05	0.20	0. 23	0.18	0.11	16.32	17.18	16.92	10.55	1.06	1.13	0.94	0.93
	Rep.	0.038	0.554	0.160	1.098	0.356	0.017	0.016	0.169	6.319	2.742	1.408	0.038	0.015	0.679	0.198	1.415
	Temp.	15.877	11.622	12.798	1.145	15.961	12.406	10.375	13.423	7.451	0.175	0.228	9.437	15.702	11.369	12.569	15.828
	Instars	1.836	3.274	2.879	1.269	1.569	3.301	4.089	2.653	0.535	1.480	2.429	0.038	1.929	3.114	2.584	0.017
	Temp. XInstars	0.310	0.617	0.572	0.879	0.283	0.566	0.753	0.448	0.510	0.848	0.638	0.651	0.326	0.616	0.564	0.005
Significance	Temp.	***	***	***	---	***	***	***	***	***	---	---	---	***	***	***	***
	Instars	---	*	*	---	---	*	*	*	---	---	---	*	*	*	---	---
	Temp. X Instars	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SE	Temp.	0.06	0.05	0.04	0.41	0.01	0.01	0.01	0.01	0.13	0.22	0.29	0.45	0.05	0.05	0.04	0.02
	Instars	0.07	0.06	0.05	0.46	0.01	0.01	0.01	0.01	0.14	0.24	0.33	0.51	0.05	0.05	0.04	0.02
	Temp. X Instars	0.13	0.12	0.10	0.92	0.02	0.02	0.02	0.02	0.29	0.49	0.66	1.02	0.11	0.10	0.08	0.05
CD 5 %	Temp.	0.17	0.15	0.13	NS	0.03	0.03	0.02	0.02	0.37	NS	NS	1.30	0.14	0.13	0.10	0.06
	Instars	NS	0.17	0.14	NS	NS	0.03	0.03	0.03	NS	NS	NS	1.46	NS	0.15	NS	NS
	Temp. X Instars	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

4. Discussion

The present study is exclusive compared to the reports available for the reason that both polyvoltine, which are known for its tolerance to high and fluctuated environment and bivoltine that has low potential for high temperature were exposed to HS from I to V instars. Thereafter they were reared under natural fluctuated environmental conditions prevailed in the rearing house in order to measure their innate potentiality that facilitate the larvae to overcome this changing temperature and produce cocoons in comparison with non-HS larvae of *B. mori*. The biological, biochemical and physiological response to HS in *B. mori* is important due to cytoprotective action of heat shock proteins (HSPs) that expressed during changing temperatures, toxic substances and microbes infection[11], which are correlated to the tolerance level of different silkworm strains but not cocoon traits of poly- and bivoltine strains/breeds.

Thus, it is indispensable to measure the larval growth in terms of its weight and cocoon characters after HS, where in the response of HS induced and control larvae being reared at fluctuated environment instead of optimum temperature (25±1ºC) and relative humidity (75±5%) be different [12, 13]. Consequently, day-2 young age (I, II, and III instars) and day-3 of late age (IV and V instar) instar larvae of NB_4D_2, CSR_2, Daizo, and PM exposed to 35, 40 and 45°C for 2 h followed by 2 h recovery period were exhibited contrasting response to HS as measured based on weight of the cocoon, shell and pupae in comparison with their respective control group. Among, silkworm breeds tested, very good response to HS was exhibited by NB4D2 silkworm strain followed by Daizo in terms of improvement upto 46 and 47% in cocoon weight respectively. Concomitantly, high sensitivity and tolerance was recorded from CSR2 and PM with declined cocoon shell ratio and marginal improvement in other traits respectively. Generally, multivoltine breeds are better able to tolerate the HS than bivoltine breeds [14, 15] wherein Nistari [16] is identified as the most tolerant one. Further, varied multivoltine silkworm strains were differentiated tolerant, moderately tolerant and inclined based on their survivability under controlled environmental conditions [17]. Moreover, high rate of survival in multivoltine silkworm breeds, C. Nichi and Pure Mysore and low share survival for the bivoltine breed, NB4D2 was reported after HS at 39 or 41°C for 1h or a pair of hours [14, 15]. By keeping all this collective impact in view, the present study revealed that all the instars larvae should be taken into account to measure their response to thermal stress with the expression of HSPs and its encoding genes along with their biological and biosynthetic traits of the silkworm strain [18]. However, the present study substantiate the previous report that the level of tolerance to high temperature in young silkworms is low compared to late age silkworm [7, 18] and this biological response should be systematically investigated among different silkworm breeds/hybrids of commercial significance, keeping global warming in view, while most commonly the whole larvae exposed to warm events at field over a period of 22 to 30 days that occur either for short or long duration in a day or days.

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6. References

1. Benchamin KV, Jolly MS. Principles of silkworm rearing. Proceedings of the Seminar on Problems and Prospects of Sericulture. Vellore, India, 1986, 63-108.
2. Hochachka PW, Somero GN. In: Biochemical adaptation, Oxford University Press, New York, 2002.
3. Bhattacharjee S. Triadimefon pretreatment protects newly assembled membrane system and causes up-regulation of stress proteins in salinity stressed *Amaranthus lividus* L. during early germination. Journal of Environmental Biology, 2008, 29.
4. Kumar G, Tripathy R. Influence of heat stress on genome of grass pea (*Lathyrus sativus* L.). Journal of Environmental Biology. 2009; 30:403-408.
5. Jolly MS. Appropriate Sericultural Techniques. International Centre for Training and Research in the Tropical Sericulture, Mysore, 1987, 176.
6. Pershad GD, Datta RK, Vijayakumar HV, Bhargava SK, Jolly MS. Performance of some multivoltine races of *Bombyx mori* L. Sericologia, 1986; 26:295-301.
7. Vasudha BC, Aparna HS, Manjunatha HB. Impact of heat shock on heat shock proteins expression, biological and commercial traits of *Bombyx mori.* Insect Science, 2006; 13:243-250.
8. Howrelia HJ, Bharat BP, Selvanayagam M, Rajakumar S. Impact of temperature on heat shock protein expression of *Bombyx mori* cross-breed and effect on commercial traits. Journal of Environmental Biology. 2011; 2(1):99-103.
9. Prashanth J, Bhat MA, Punyavathi, Manjunatha HB. Heat Shock Response of FC2 - A bivoltine hybrid of the mulberry silkworm, *Bombyx mori*. International Journal of Biotechnology and Bio-engineering Research, 2013; 4:73-88.
10. Chapman RF. The Insects: Structure and Function. Cambridge University Press, Cambridge, 2002; 242-386.
11. Zhao L, Jones WA. Expression of heat shock protein genes in insect stress responses. Invertebrate Survival Journal. 2012; 9:93-101.
12. Huang PJ, Chen JH, Hong DH, Chen CN. Preliminary study on the inheritance of tolerance to high temperature in some silkworm strains. Journal of the Agricultural Association of China. 1979; 105:23-39.
13. He Y, Oshiki T. Study on cross breeding of a robust silkworm race for summer and autumn rearing at low latitude area in China. Journal of Sericultural Science of Japan. 1984; 53:320-324.
14. Hsieh FK, Yu SJ, Su SY, Peng SJ. Studies on the thermotolerance of the silkworm, *Bombyx mori*. Chinese Journal of Entomology, 1995; 15:91-101.
15. Joy O, Gopinathan KP. Heat shock response in mulberry silkworm races with different thermotolerances. Journal of Biosciences, 1995; 20:499-513.
16. Koundinya PR, Kumaresan P, Sinha RK, Thangavelu K. Screening of promising germplasm of polyvoltine silkworm (*Bombyx mori* L.) for thermotolerance. Indian Journal of Sericulture, 2003; 42:67-70.
17. Srivastava PP, Kar PK, Awasthi AK, Raje Urs S. Identification and association of ISSR markers for thermal

stress in polyvoltine silkworm, *Bombyx mori*. Russian Journal of Genetics. 2007; 43:858-864.
18. Manjunatha HB, Rajesh RK, Aparna HS, Silkworm thermal biology: A review of heat shock response, heat shock proteins and heat acclimation in the domesticated silkworm, *Bombyx mori*. Journal of Insect Science. 2010; 10:189.