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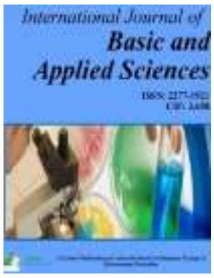

Full Length Research Paper

Effect of Thermal Stress on Heat- Shock Protein Expression of White Nistari Race (M_{12}W) of *Bombyx mori* L.

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ABSTRACT

Mulberry silkworm (Bombyx mori L.) is one of the extensively used animal tool, whose growth and development are significantly affected by ambient temperatures, so regularly used as a type specimen for thermo tolerance experiments on insects An experimental research study was undertaken to consider the thermal sensitivity of silkworm larva (Bombyx mori) of multivoltine white nistari race. Eggs, larvae (4th day of Vth instar), pupae and adults of silkworm Bombyx mori L were subjected to expose under temperatures ranging from 18°C to 44°C during 3 consecutive days with 1 hr 30 min. duration in haemolymph of Bombyx mori expression of some protein bands or appearance of new ban with different exposure time can be considered as desirable traits in imparting thermo-tolerance to the larvae. In the haemolymph of Vth instar larva, the appearance of 72 kDa protein as a result of heat shock after raising temperature at 44°C. Cocoon and shell weight significantly enhanced after heat stress over control respectively which is attributed to the expression of Hsp72 at Vth instar larval stage. This study therefore reflects these larvae may be reared under thermal stress conditions in order to obtain quality cocoons than the non-heat shocked silkworm larvae.

Introduction

Silkworms (Bombyx mori) are those insects which are capable of converting leaf protein into silk protein through a series of biochemical changes and acts as a model organism of Lepidopteran insects. Silkworm is a poikilothermic insect, who's growth and development is greatly affected by environmental temperature. Continuous domestication for prolonged time makes them susceptible to an environment which is always harmful/lethal and produces "stress" or shock to these sensitive insects (Howrelia *et al*, 2011). In order to overcome this shock to a considerable level their body accommodates for a protective mechanism by producing kinds of proteins generally known as "Heat shock proteins (Hsps) with the activation of some "genes" located in their chromosomes (Hong *et al*, 2010; Ponnuvel *et al*, 2010 & Sosalegowda *et al*, 2010). They are called HSPs because of their steady expression during exposure to increased temperatures and under stressful conditions. Heat shock proteins were presumed to ensure survival under stressful conditions by involvement in damage protection or damage repair due to their action as molecular chaperons (Meenashi and v. prabakaran 2013). Heat shock proteins are present in cells under normal conditions, but they can express at high levels when exposed to a sudden temperature jump or other stress (Zhao *et al*, 2012). A number of protein substances are frequently found to accumulate in the haemolymph. Haemolymph is known to be very essential for the metamorphosis of insects including silkworm (Omana and Gopinatha, 1995) have shown change in protein profile in tissues and haemolymph following heat shock of B mori in vivo. Although climatic factors like temperature and humidity have been known to play a key role in growth and productivity of silkworm. Indigenous silkworm races like white nistari (M_{12}W) race in West Bengal showing better productivity traits popularized to harness the better hybrid vigor in the field (Vijaya kumari et al 2019**).** As most of the productive strains likeWhite Nistari of silkworm are not capable to adapt the wide range of higher temperature, generally farmers compel to rear tolerant strains that shows low productivity and provide inferior quality silks. In this context, a study on thermo tolerance in silkworms is very imperative for sericulture industry. Not much information is available on the effect of temperature on silkworm physiology of White nistari races. It is therefore imperative to study the effect of temperature on the different life history stages of *Bombyx mori L* of White Nistari race with special reference to the expression of heat shock protein and its subsequent impact on the improvement of commercial traits of cocoons of the race. Hence the present research article aimed to understand how silkworm responded different thermal stress condition which impacted on silkworm rearing after re-backing to the optimum rearing temperature and simultaneously how thermotolerance effect on their cocoon commercial characters.

Materials & methods

Rearing of silkworm

B. mori larvae (multivoltine race, strain M12W) were reared in the laboratory with locally available mulberry leaves, under standard condition of 24–26°C and 60–85% relative humidity following the standard rearing method of (Krishanaswami *et al* 2003).

Heat- shock method

Eggs, larvae (4th day of Vth instar), pupae and adults of silkworm *Bombyx mori L* were exposed to temperatures ranging from 18°C to 44°C for specified time for 3 consecutive days with 1 hr 30 min. duration (Hightower L E 1991). The heat treatment was always coupled to a relative humidity of 90% (Evgen'ev *et al.* 1987). For each thermal shock experiment, at least 100 eggs, larvae, pupae and adults were exploited for the experiments which replicated thrice. After the thermal acclimation, the samples were re-backing to the optimum rearing temperature and were allowed to recover from heat shock. Survival of eggs by hatchability, larvae by their ability to spin healthy cocoons, pupae by adult eclosions and adult by successful copulation and egg laying was confirmed on observation (Vijaya kumara K, M Saravana Kumar R. Rao, P. Sudhakara, Sailaja B, Vdyunmala, Mishra1 R.K 2019). Effect of exposure to elevated temperature on survivability of life stages of silkworm was studied. In order to study the effect of thermal stress on cocoon parameters and quality of cocoon the parameters viz. single shell weight, single cocoon weight and SR%, were taken into consideration (Morimoto R I 1993).

Extraction and analysis of heat shock proteins

Haemolymph from a punctured proleg of the silkworm larvae was collected in a precooled graduated centrifuge tube containing a few crystals of phenyl thiourea (Gupta 1979). To inhibit the tyrosinase activity of the haemolymph, few crystals of phenylthiourea were added (Gupta 1979). The haemolymph was centrifuged at 3000 gm for 10 min at 4°C. The protein profiles of the hemolymph were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Kajiwara H, Imamaki A; Nakamura M; Mita R; Xia QG; Ishizaka M., 2009 and Laemmli 1970).

Statistical analysis

Each assay was replicated thrice in order to study the differences in thermo tolerance at different heat exposures were subjected to analyse calculating mean with Standard deviation following two-way analysis of variance (ANOVA) (Sheoran and Pannu 1999)

Results

Effects of temperature exposure on the survival of the silkworms

Thermal tolerance of the different morphological stages of the White Nistari race at different temperatures (18 °C, 34 °C and 44 °C), each continuing for 1 hr 30 mins during three consecutive days was indicated as survival percentage (Table 1). The rates of survival of the insect at various heat treatments were low in comparison with the optimum temperature for rearing (Table 1). All the life cycle stages of the respective races were found to be stable at 18°C (Table 1). The races used for experiments are normally reared at 24–26 °C, showed good tolerance for exposures to 34 °C for 1hrs 30 minutes duration (Table 1). However, all the stages viz egg, larvae, pupae and adults when exposed to temperatures of 44 °C the survival rates decline drastically (Table 1). However, larva tolerated higher temperatures in comparison with the egg, pupa or adults (Table 1).

Table-1. Effect of thermal stress on survival of silk worm (white nistari-$M_{12}W$, n=10)

Duration of temperature exposure (For 3 consecutive days) 1hr 30mins	**Survival% of $M_{12}W$**				
	Temperature	**Egg**	**Larvae**	**Pupae**	**Adult**
	24^0C-26^0C Control	99.29	98.86	96.88	95.37
	18^0C	86.73	90.46	77.01	85.54
	34^0C	80.37	88.67	68.32	75.75
	44^0C	1.37	14.63	11.58	9.75
	SEM	1.118	1.419	0.859	0.188
	CD at 5%	2.105	2.671	1.617	0.354

Effects of heat stress on commercial traits

Weight of the cocoons spun by larvae after heat shock treatment was found to increase over their respective control population (Table 2). The weight of cocoons spun by the larvae was 1.008 g at 44^0C, an increase of 11.75% over control. The shell weight also increased up to 0.140 and 0.144 at 34°C and 44°C compared to control (Table 2). However, the highest shell weight was 0.144g in the thermal exposure of 44^0C with 12.5% improvement over control was observed in $M_{12}W$. Noticeably, the increased shell ratio % was recorded to the level of 14.37 at 18°C (Table 2) and 14.43 at 34°C respectively.

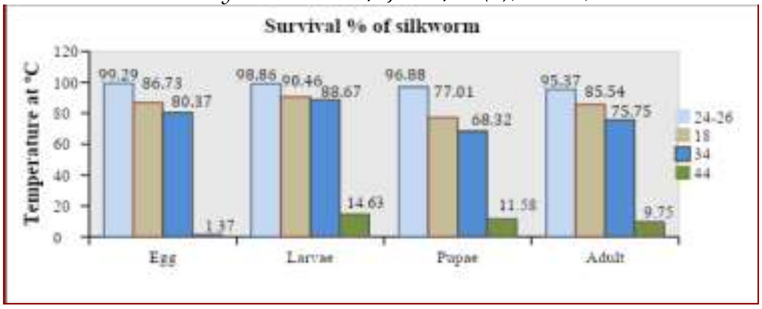


Fig.-1 Effect of thermal stress on survival of silk worm

Table-2 Effect of thermal stress on cocoon parameters of silk worm (white nistari-$M_{12}W$, n=10)

Duration of temperature exposure (For 3 consecutive days) 1hr 30mins	Cocoon parameters of $M_{12}W$			
	Temperature	**Wt. Of single cocoon(gm.)**	**Wt. Of single cocoon shell(gm.)**	**Shell Ratio%**
	24^0C-26^0C Control	0.902	0.128	14.19
	18^0C	0.960	0.138	14.37
	34^0C	0.970	0.140	14.43
	44^0C	1.008	0.144	14.28
	SEM	0.003	0.0005	0.046
	CD at 5%	0.006	0.001	0.088
	CD at 1%	0.009	0.001	0.028

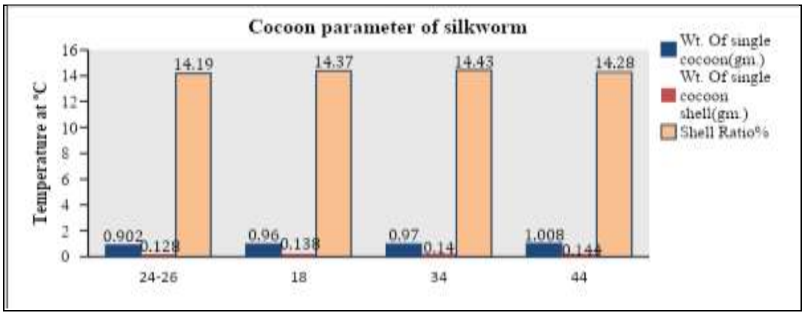
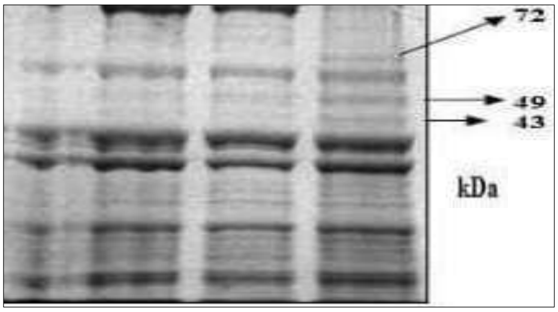


Fig. 2 . Effect of thermal stress on cocoon parameters of silk

Ethological changes

Exposure to temperatures at 18 °C for 1 hour during 3 consecutive days Vth instar larvae of White Nistari race showed solitary habit. no significant behavioural changes appeared when temperature increased up to 34 °C whereas with elevated temperature at 44 °C larvae showed upright position of posterior extreme end and some sort of sidestepping behaviour.



L1 L2 L3 L4

Fig: 3. Silkworm larvae Vth instar were exposed to 18°C, 34 °C and 44 °C for 1 h 30 minutes in 3 consecutive days. After a recovery period of 24 hours, the hemolymph was collected and the proteins were separated on SDS-PAGE (10%) and stained with Coomassie brilliant blue (lanes 1–4). In this figure, Lane 1: control (no heat shock); Lane2: 18 °C; Lane 3: 34°C; Lane 4: 44°C respectively. Tissue specific expression of 72 kDa heat shock protein is indicated by an arrow.

Discussion

The silkworm *Bombyx mori* showed varying levels of thermal tolerance at the different stages of life cycle viz. the egg, larva, pupa and adult. The rates of survival of the silkworm at various thermal treatments were low in comparison with those reared at room temperature. Except for the pupae, all the developmental stages of the respective silkworm race were more or less stable at 18^0C and 34^0C. (Table 1). However, on exposure to temperatures of 44^0C or above, the survival rates were more intensely reduced, especially the eggs showed a scarce survival rate of 1.37 % (Table 1, Fig. 1). The white nistari strain normally reared at 24–26^{0}C, showed good tolerance for exposures to higher temperature. The late age worm tolerated higher temperature for longer durations as compared to the eggs or adults. The effect of varied levels of temperature (18^{0}C, 34^{0}C and 44^{0}C) for 3 consecutive days with 1 hr 30 mints of duration was studied on cocoon parameters of Vth instar larval stage. (Table 2, Fig. 2). The weight of cocoons spun by the larvae was 1.008 g at 44^{0}C, an increase of 11.75% over control. Weight of the shell of the cocoon after heat shock was found to be significantly increased at the same temperature over their respective control (Table2, Fig.2). The highest shell weight was 0.144g in the thermal exposure of 44^{0}C with 12.5% improvement over control was observed in M_{12}W. The increased shell ratio was registered 14.43 in response to heat shock upto 34^{0}C. It was clear by the analysis of heat shock proteins of V th instar larval hemolymph that continued expression of heat inducible polypeptide of 72 kDa even after recovery time of heat exposure at 44^{0}C. It was also noticed that the same protein (72 kDa Hsp) disappeared remarkably in haemolymph after recovery from 18^{0}C and 34^{0}C. This result suggests that the acquired thermo tolerance against heat shock occurred due to the induction of Hsp72 in larval hemolymph. An early consequence of heat shock is the appearance of a set of highly conserved proteins, the HSPs, ubiquitous to all organisms examined so far. The HSPs are considered to confer protection against the adverse effect of heat shock (Hightower 1991). Differential expression of some protein bands viz 49 and 43 kDa protein consequent to heat shock (Figure 3) was evident at heat shock temperatures of 44^{0}C. InVth instar larval hemolymph appearance of 72 kDa protein consequent to heat shock (Fig.3) was evident to heat shock temperatures of 44^{0}C and the observations are more or less similar with the studies of Evgenev *et al*. 1987. The increased cocoon weight and shell weight over control is actually attributed to the expression of Hsp72 at V th instar larval stage. Similar findings were also reported by Sinha and Sanyal (2013). Further due to induced tolerance, heat shock larvae spun better cocoons in comparison to their respective control. This study therefore focuses that after thermal stress, in order to get the desirable features, these larvae may be reared under optimum temperature in relation to obtaining quality cocoons than the non-heat shocked individuals. However, in these aspects further research to determine species-oriented responses to heat shock is necessary.

Conclusion

The experiment again proved that Nistari, the indigenous multivoltine race of West Bengal can tolerate even unfavourable environmental conditions and give at least few returns to the silkworm rearers. In this perspective, the present experimental research revealed that after exposure to thermal stress, if these larvae are subjected to rear under ideal temperature for rearing where frequent fluctuations occur; they will accomplish better and spin good quality cocoons than the non-heat shocked silkworm larvae.

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