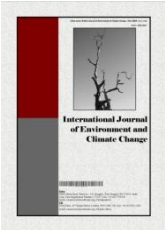

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Alterations Due to Heat Shock in Biological and Commercial Features of the Silkworm, *Bombyx mori*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The impact of HS on larval growth and development, ERR, cocoon characteristics and biochemical elements of carbohydrate content was investigated using FC2 X FC1 bivoltine hybrid silkworm breed. On the third day of the fifth instar, the FC2 X FC1 bivoltine hybrid was treated to HS at 30, 35, 40 and 45ºC for 1 h with 1 h retrieval. Following that, silkworms were maintained in the rearing house under naturally fluctuating environmental circumstances in order to assess their intrinsic potentiality for overcoming temperature changes and producing cocoons in contrast to non-HS *B. mori* larvae. At 40°C, the biological and commercial features gained the most weight. As a result, although all larvae heat shocked at 35 and 40°C metamorphosed into pupae with better growth than controls, the fifth instar showed the greatest improvement in it. 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 should be considered when screening for better parents to develop thermotolerant breeds/hybrids for tropical countries such as India, in order to elicit a profound response and acquire tolerance to overcome the fluctuated environmental condition. Both control and HS showed a sequential increase in carbohydrate content but found declined upon HS of 45˚C.

Keywords: Heat shock; larva; silkworm; Bombyx mori.

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1. INTRODUCTION

The sericulture industry has contributed significantly to the economic development of many countries due to commercial importance of silk in the Textile World and easy to rear silkworms under domestication. Thus, the silkworm, *Bombyx mori* has not only exploited over long period for cocoon production but also widely used in basic research, biotechnology and as a molecular model insect. To date, thermotolerance in *B. mori* evaluated through conventional silkworm breeding programs for the selection of silkworm breeds, which perform better in varied environmental conditions, especially in tropical regions such as southern India is in vain and offers systematic studies following advanced molecular techniques to understand the molecular mechanism underlies in thermal acclamation/adaptation of *B. mori*. The silkworm *B*. *mori* is an economical important insect and it has been exploited by man for the production of silk since quite long time. In recent years it has been recognized as a molecular model insect not merely because of considerable importance in silk production but for the synthesis of recombinant proteins [1] It is also one of the most genetically researched insects. Silkworm breeding research is a mixed bag of success and failure in India, where sericulture is prevalent in tropical regions. The widespread development of sericulture in India was aided by the successful introduction of F1 hybrids of tropical female and temperate male silkworm strains. However, temperate silkworm hybrids produced less cocoon and silk, and the quality of silk yarn was lower. Attempts to propagate temperate silkworm strains over India's sericulture belt resulted in widespread crop failures, particularly in hot and humid conditions.

In light of this, an effort was made to create hybrid silkworms that could survive in hot conditions. Developing stress and disease resistance strains in India and producing high yielding silkworm strains that can survive exceptionally well year-round by surviving environmental stress are extremely difficult tasks. The amount of HSPs expressed at different temperatures varies, as does the relative contribution of each HSP family to stress tolerance [2]. Since HSP expression and resistance to stress are correlated, it is well known that prior exposure to stress results in tolerance and cross-tolerance to subsequent stress [3]. The fifth instar *B. mori* larvae's high temperature thermotolerance is an adaptation to the high temperatures they endure during their typical life cycle. But according to current rearing procedures, younger silkworm larvae should be raised at a high temperature (28 °C) and high relative humidity (RH 80 %), whereas older silkworm larvae should be raised at a lower temperature (24°C) and humidity (RH 65%). Thus, the effects of heat shock on the biological and commercial features of larvae are unclear as a result of these practises. The goal of the coordinated efforts to enhance the domesticated silkworm's cocoon characteristics was to produce silk of the highest grade. Therefore, creating bivoltine breeds or hybrids that can endure high temperature stress conditions becomes critical or needed. In light of this, the current experiment was carried out to determine how high temperatures affected different quantitative and qualitative features of bivoltine hybrids of *B. mori*.

2. MATERIALS AND METHODS

The bivoltine hybrid FC2 X FC1 of silkworm, *Bombyx mori* was used in the present study to evaluate heat shock response at varied temperatures. FC2 X FC1 layings were procured from UT Sericulture Department, Mehander, Poonch, Jammu and Kashmir. FC2 X FC1 disease free layings were incubated under optimum environmental conditions (Temperature 25±1˚C and 75±5% Relative humidity) until hatching. Hatched larvae were reared by adapting the standard procedure [4] until spinning under room environment. During rearing, each tray including control was provided with paraffin paper and wet foam pads.

2.1 Induction to Heat Shock [HS]

Fifth instar, 3rd day larvae of FC2 X FC1 were placed in thin walled plastic boxes for heat shock treatment in B.O.D at 30, 35, 40 and 45˚C for 1 hr in water bath where 90% relative humidity (RH) was maintained. Feeding was resumed immediately after 1 hr of recovery period and reared along with control until spinning under normal environmental conditions.

2.2 Analysis of Biological and Commercial Traits

2.2.1 Determination of heat sensitivity

Heat sensitivity in terms of larval, pupal and adult mortality was assessed by their inability to enter into succeeding instar or to spin cocoon, metamorphose into pupae and then moth.

2.2.2 Effective Rate of Rearing (ERR)

The ERR was calculated based on the number of cocoons spun by the number of larvae HS and or brushed.

ERR was calculated by the following formula,

$$ERR = \frac{Number\ of\ good\ cocoons\ spun/harvested}{Number\ of\ larvae\ brushed} \times 100$$

2.2.3 Larval weight

About 6 larvae were selected randomly from each replication on day 6 of fifth instar and their weight was recorded.

2.2.4 Cocoon weight

About 6 cocoons were selected randomly from each replication 6 day after spinning and their weight was recorded individually. Average weight of the cocoons was determined.

2.2.5 Pupal weight

For pupal weight, the pupae removed from randomly selected 6 cocoons of each replication were used and their weight was recorded individually. Average weight of the pupa was determined.

2.2.6 Shell weight

For shell weight, 6 cocoons randomly selected from each replication were used and weight of the cocoon shell was recorded after removing the pupa from the cocoons.

2.2.7 Shell ratio

Shell ratio was calculated based on the shell weight of the respective cocoon weight using the formula,

$$Shell\ ratio = \frac{Shell\ weight}{Cocoon\ weight} \times 100$$

2.3 Carbohydrate Estimation, Anthrone Method (Sadaasivan et al., 2011)

For estimation of total carbohydrate content, the larval extract was prepared by homogenizing 1 g in 2 ml of 5% Trichloroacetic acid with the help of micro-pestle and centrifuged at 3000 rpm for 10 min. The supernatant was taken for estimation of carbohydrate content. 1 ml of larval extract sample of the treatment was taken separately in to a test tube and 4 ml of Anthrone reagent was added. All the test tubes were placed in boiling water bath for eight minutes. After cooling the samples optical density was measured at 630 nm against blank using spectrophotometer. The quantity of carbohydrate present in the sample was estimated following the formula and presented in mg/ml,

$$Concentration\ of\ the\ sample = \frac{Optical\ density\ of\ the\ sample \times Concentration\ of\ the\ standard}{Optical\ density\ of\ the\ standard}$$

2.4 Data Analysis

All the data derived from three replications of different treatments were used to draw mean values along with standard deviation and significant variations employing one-way ANOVA using SPSS version 20.

3. RESULTS

3.1 Changes in the Larval Growth Due to Heat Shock

The larval growth as influenced by HS at different temperatures was measured based on their weight on day 3rd of fifth instar. Accordingly, an average weight of the larvae recorded was 3.22, 3.42, 1.40, 3.53, 3.63 and 0.00 g that corresponds to 30, 35 and 45°C larvae of FC2 X FC1 respectively (Table 1) that statistically significant at P<0.01. However, slight improvement of larval weight was observed at all temperatures. Interestingly, increased weight of 3.63 g was observed in the larvae derived from 40°C HS against control (3.22 g) (Fig. 1). Besides, larvae didn't survive upon HS at 45˚C, so no larvaval weight was observed upon HS at 45˚C.

3.2 Changes in the ERR Due to Heat Shock

The ERR denotes for the larvae succeeding to spin cocoons. Eventually, the silkworm larvae derived from different HS induced were reared under natural environmental conditions prevailed in the rearing house. Interestingly, 91% of improvement in ERR was recorded in the population derived from 35°C against control (88%). However, increased ERR of 90.67% was

also observed at 40°C and same ERR of 88% was observed at 35°C and control (Table 1, Fig. 2) which is significant at $P < 0.01$. On the other hand, larvae didn't survived after HS at 45˚C, so no ERR was observed at 45˚C.

3.3 Changes in the Larval Mortality Due to Heat Shock

The sensitivity of different instars larvae of FC2 X FC1 to different heat shock (HS) temperatures is quite significant (Fig. 3). The larvae of FC2 X FC1 were found sensitive to both the HS temperatures 30 and 35˚C than 40˚C. At 30˚C, highest of 13.33% mortality was observed while at 35˚C it was 12.67% (Table 1) which is significant at $P < 0.01$. Further, different instars larvae HS at 45˚C exhibited highest mortality of 100%.

3.4 Changes due to Heat Shock in Relation to Cocoon Characters

3.4.1 Cocoon weight

Weight of the cocoon spun by the FC2 X FC1 silkworm larvae derived from HS induced on day - 6 at 30, 35 and 40˚C was found significantly

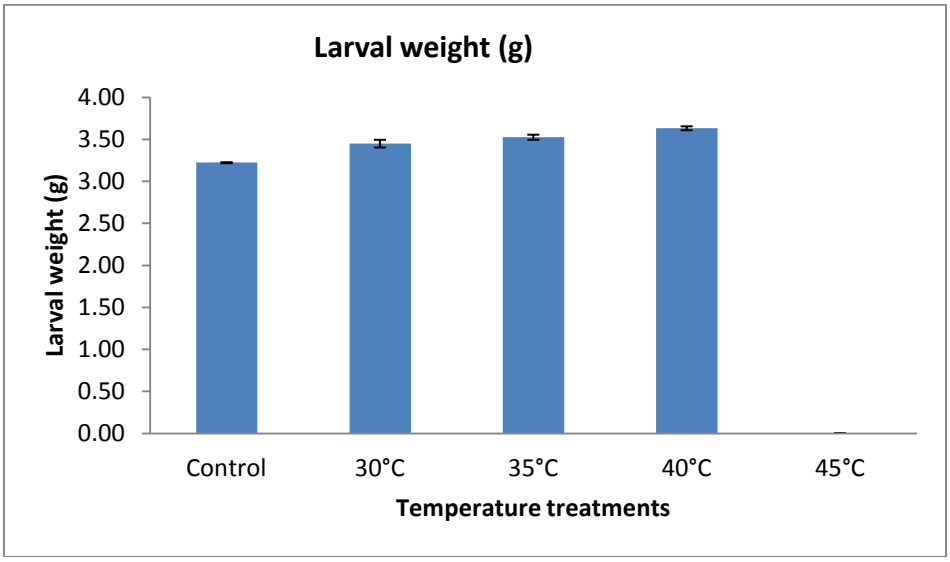

Fig. 1. Larval weight FC2 X FC1 larvae at different temperatures

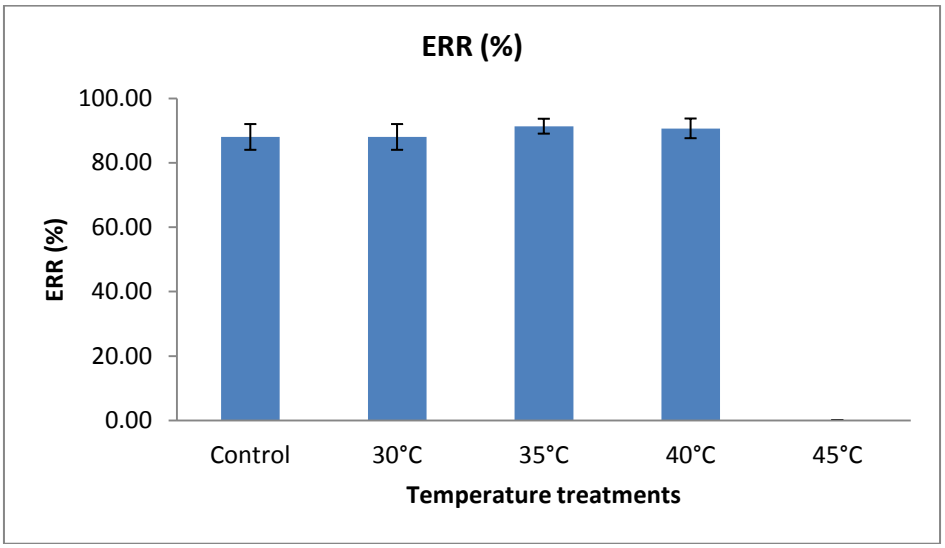

Fig. 2. ERR of FC2 X FC1 larvae at different temperatures

increased compared to control (Table 2, Fig. 4). The highest weight of the cocoon 1.76 g was observed at 40˚C. An average weight of cocoon 1.51, 1.62, 1.76 and 0.00 g was recorded that corresponds to 30, 35, 40 and 45˚C respectively against control (1.38 g) that significant at $P < 0.01$ (Table, 2). Comparatively, the larvae didn't survive upon HS at 45˚C, so couldn't spin the cocoons.

3.4.2 Shell weight

The cocoon shell weight was also obviously affected as that of cocoon weight in control due to fluctuated environmental condition in the rearing house. As a result, the cocoon shell weight in control was 0.23 g. But, significant improvement in the shell weight was noticed in the heat shock induced larvae at 40˚C. The cocoons spun by HS larvae at 30, 35, 40, 45 and control had shell weight of 0.26, 0.28 and 0.30 g respectively (Table 2, Fig. 5) against control 0.28 g. Whereas, whatever the no silkworm larvae survived after heat shock at 45˚C so, no cocoon shell weight was observed at 45˚C.

3.4.3 Pupal weight

Interestingly, weight of the pupa, as an index of its growth, showed highest weight 1.46 and 1.33 g in the population derived the larvae of HS at 35 and 40˚C on day-6 respectively. Whereas, 1.25 and 1.13 g of pupal weight was observed at 30˚C and control during 6th day larval stage of FC2 X FC1 (Table, 2). More importantly the FC1 X FC2 silkworm larvae HS at 45˚C didn't survived and no pupal weight was observed (Fig. 6).

3.4.4 Cocoon shell ratio

The cocoon shell weight ratio was also correspondingly affected as that of cocoon and shell weight due to HS at fifth instar larvae in FC2 X FC1. The cocoon shell ratio recorded as 17.19% in control, highest of 17.55 and 17.44% was recorded in the population derived from larvae of FC2 X FC1 HS at 40 and 35˚C respectively. Concomitantly, none of the larvae survived at 45˚C, so no pupal weight was observed (Table 2, Fig. 7).

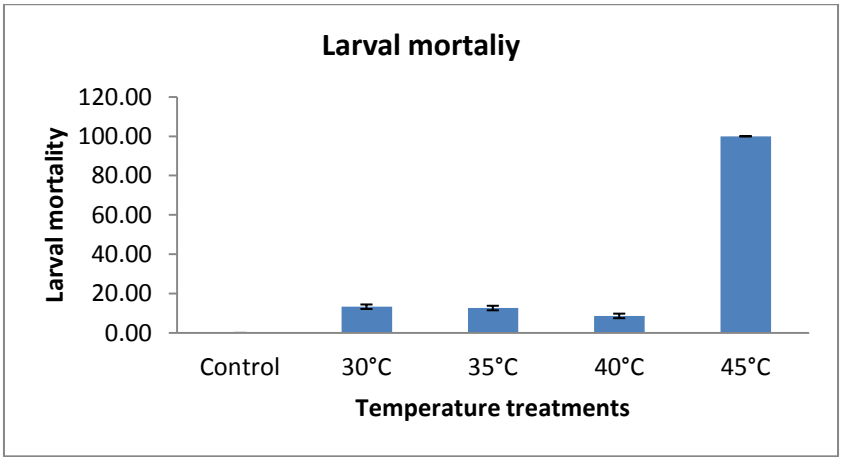

Fig. 3. Larval mortality of FC2 X FC1 larvae at different temperatures

Table 1. Effect of heat shock on biological traits of the silkworm, *Bombyx mori* bivoltine hybrid FC2 X FC1

Treatments	Larval weight		Larval mortality		ERR	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	3.223	0.003	0	0	88	2.309
30°C	3.45	0.026	13.333	0.667	88	2.309
35°C	3.527	0.018	12.667	0.667	91.333	1.333
40°C	3.633	0.013	8.667	0.667	90.667	1.764
45°C	0	0	100	0	0	0
C.D.	0.049		1.648		5.63	
SE(m)	0.015		0.516		1.764	
SE(d)	0.022		0.73		2.494	
C.V.	0.97		3.321		4.267	

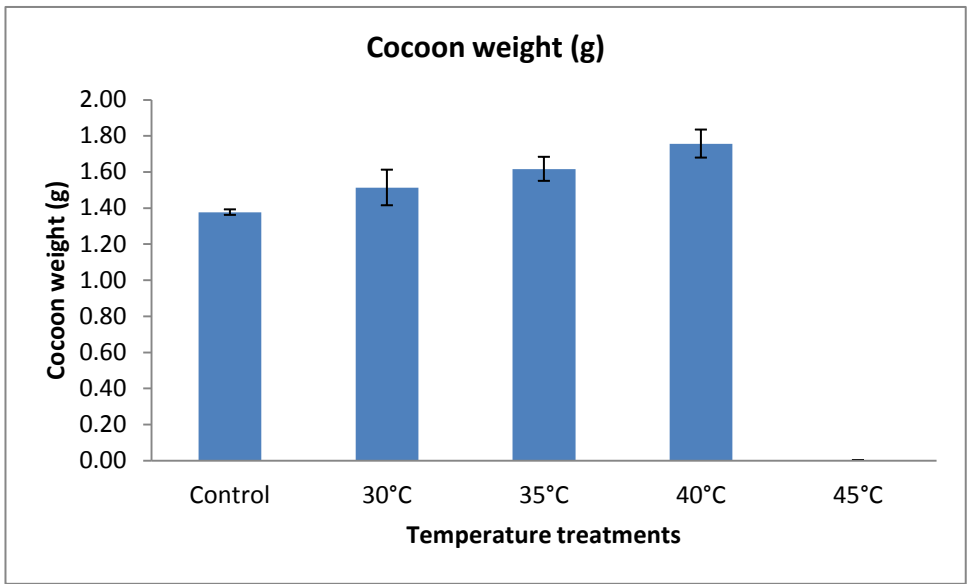

Fig. 4. Cocoon weight of FC2 X FC1 larvae at different temperatures

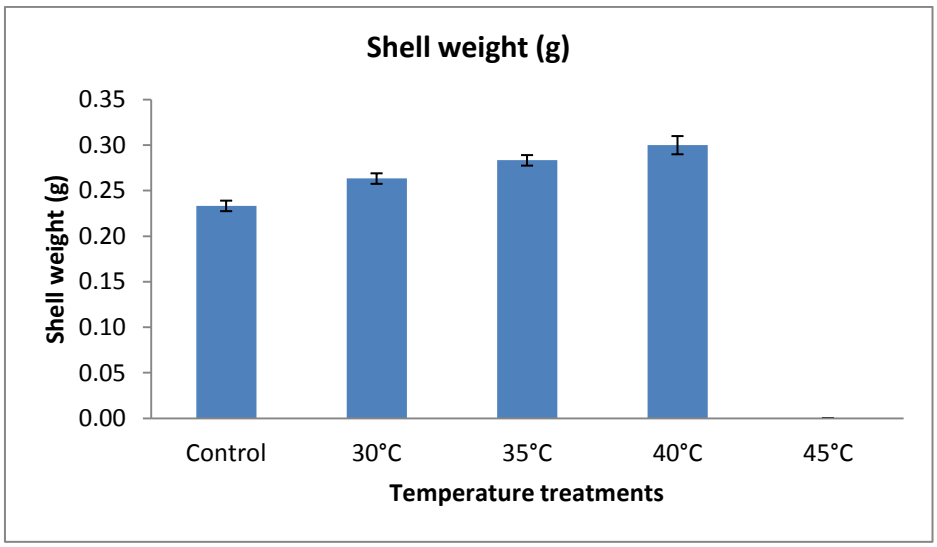

Fig. 5. Cocoon shell weight of FC2 X FC1 larvae at different temperatures

Table 2. Effect of heat shock on cocoon traits of the silkworm, *Bombyx mori* bivoltine hybrid FC2 X FC1

Treatments	Cocoon weight		Shell weight		Pupal weight		Shell ratio	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	1.377	0.009	0.233	0.003	1.13	0.01	17.187	0.285
30°C	1.513	0.057	0.263	0.003	1.25	0.055	17.437	0.579
35°C	1.617	0.038	0.283	0.003	1.333	0.042	17.55	0.641
40°C	1.757	0.045	0.3	0.006	1.457	0.05	17.113	0.771
45°C	0	0	0	0	0	0	0	0
C.D.	0.118		0.012		0.123		1.702	
SE(m)	0.037		0.004		0.039		0.533	
SE(d)	0.052		0.005		0.054		0.754	
C.V.	5.103		2.928		6.454		6.664	

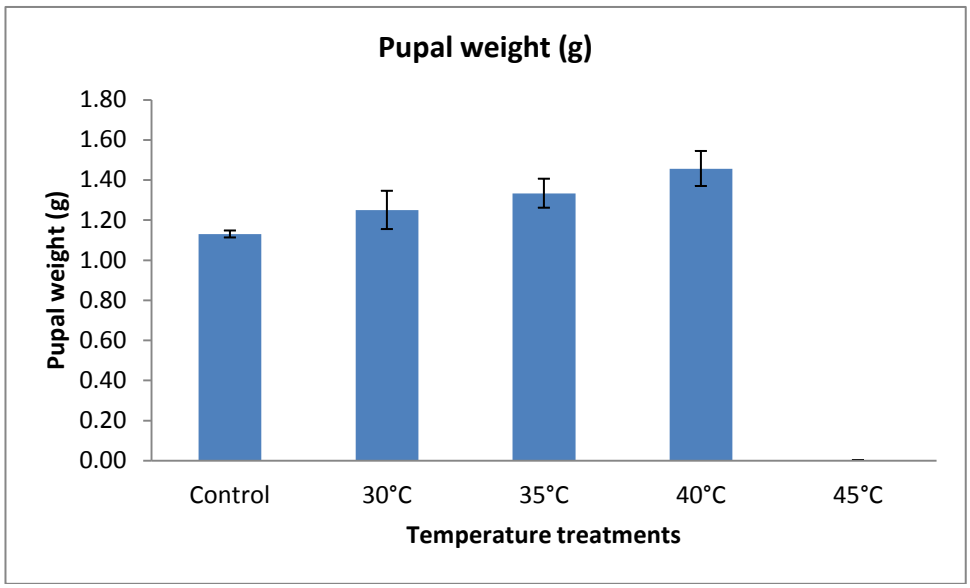

Fig. 6. Pupal weight of FC2 X FC1 larvae at different temperatures

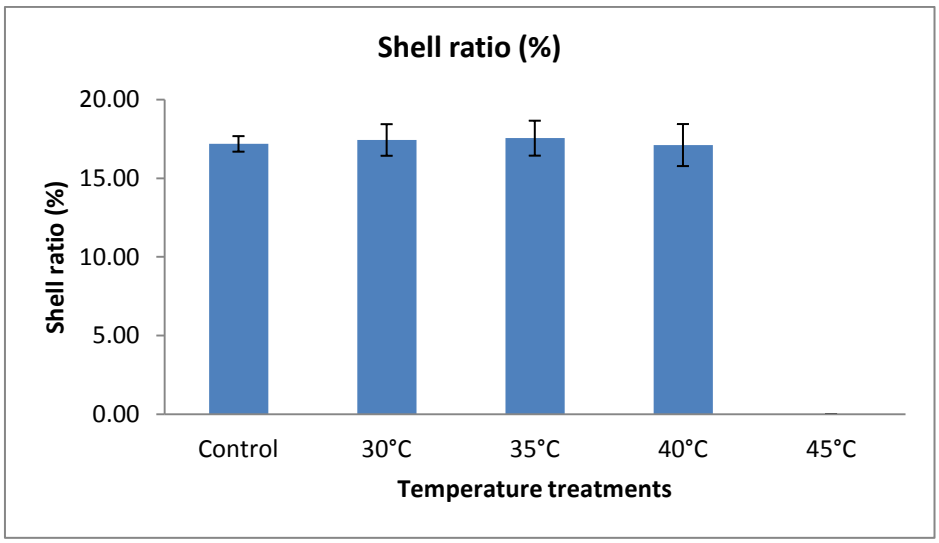

Fig. 7. Shell ratio of FC2 X FC1 larvae at different temperatures

Table 3. Effect of heat shock on Carbohydrate content the of silkworm, *Bombyx mori* bivoltine hybrid FC2 X FC1

Treatments	Carbohydrate content	
	Mean	S.E.
Control	1.597	0.009
30°C	1.68	0.012
35°C	1.723	0.007
40°C	1.79	0.006
45°C	1.31	0.11
C.D.	0.158	
SE(m)	0.05	
SE(d)	0.07	
C.V.	5.308	

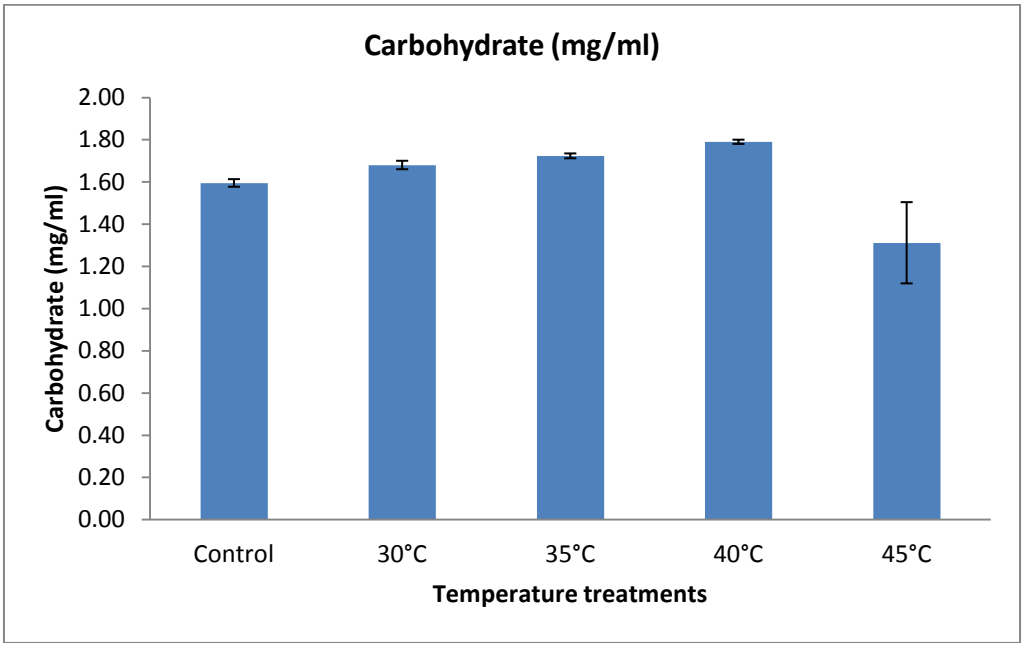

Fig. 8. Carbohydrate content of FC2 X FC1 larvae at different temperatures

3.5 Changes of Carbohydrate estimation Due to Heat Shock

The present investigation revealed that carbohydrate concentration was high in all heat induced larvae as compared to control and was found low at 45°C. In comparison between heat induced larvae carbohydrate concentration was found high at 40°C (1.79 mg/ml) as compared to 30, 35, 45°C and control. The carbohydrate content in FC2 X FC1 larvae on 3rd day of fifth instar was (1.60 mg/ml) in control, (1.68 mg/ml) at 30°C, (1.72 mg/ml) at 40°C and 1.31 mg/ml at 45°C. Although larvae have not survived at 45°C but carbohydrate concentration of 1.31 mg/ml was observed (Table, 3, Fig. 8).

4. DISCUSSION

Since a long time ago, it has been common practise to raise silkworm larvae (*B. mori*) indoors in order to produce cocoons and intern reel silk. As a result of the commercial significance of silk in the textile industry, the sericulture sector has considerably contributed to the economic growth of many countries. As a result of this ongoing domestication, silkworm larvae that were raised in environments with changing environmental conditions lost their ability to tolerate high temperatures and their resistance to illnesses. Additionally, in tropical nations like India, where the temperature and humidity change from season to season, area to region, and throughout the day, the cocoon crop is also impacted. The inconsistent embryonic development in a changing environment leads to weak larvae, poor hatching and inferior cocoons with low silk content. Even a few hours at a temperature of 40°C or higher in the rearing house causes significant harm to *B. mori's* biological and commercial features [5,6]. Therefore, coordinated efforts are made to establish suitable rearing techniques and it is proposed to keep the silkworm eggs from oviposition till hatching under the best climatic conditions, which are 25°C and 75% relative humidity. Therefore, the creation of F1 hybrids with polyvoltine (PM) as the female parent and bivoltine (CSR2) as the male father is primarily responsible for the success of silkworm cocoon crops. *Bombyx mori* bivoltine hybrid FC2 X FC1 silkworm was chosen to study the effect of HS during fifth instar, which determines the post embryonic development and cocoon characteristics for the first time as most studies were restricted to either egg and/or larval stages due to this commercial importance [5]. A correlation study between PM and CSR2 was conducted in connection to the heat shock response of embryos, the growth and development of silkworm larvae, and the characteristics of cocoons because it is well known that temperature has a substantial impact on the productivity and growth of silkworms. It is

clear from prior studies as well that inducing HS for an hour at temperatures between 35 and 45 C and above has a significant impact on embryonic development in terms of hatching [7], however its impact on post embryonic phases was not explored. The goal of the current study was to investigate how FC2 X FC1 responded to heat shock [HS] at various temperatures using the entire organism paradigm. Larvae of the fifth instar of the FC2 X FC1 cross were exposed to heat shock temperatures of 30, 35, 40 and 45˚C for an hour, followed by an hour of recovery (Vasudha et al., 2006) [5]. The FC2 X FC1 interestingly responded significantly to HS temperatures of 30, 35, and 40˚C. At 45˚C, HS caused 100% death. In contrast, death rates of 13.33, 12.67, 22.22, and 8.67 percent were seen at 30, 35 and 40˚C. (Table 1). These results strongly suggest that HS at 45°C is fatal since it results in 100% mortality, which is consistent with the findings of Vasudha et al., (2006) and Manjunatha et al., [5]. Ultimately, the number of cocoons generated was much lower at 35 HS than the control, but was relatively higher at 40˚C HS. As a result, the fifth instar larvae were HS at 40˚C, which led to the highest ERR (91.33 percent), compared to 88.00 percent in the control (Table 1, Fig., 2). It's interesting to note that on day 3 at 40˚C, the weight of the fifth instar larvae was considerably higher than that of control larvae raised under different environmental circumstances. In HS-induced larvae at 45°C, the larval weight was found to be deadly compared to control, demonstrating the lethal effect of high temperature. The biological, biochemical, and physiological response to HS in *B. mori* is significant because of its cytoprotective activity [9], which is connected to the level of tolerance of various silkworm strains but not to the characteristics of the cocoon in FC2 X FC1 bivoltine hybrid. By tracking the larval weight and cocoon characteristics of the FC2 X FC1 bivoltine hybrid as it was raised at various temperatures (30, 35, 40, and 45°C), the thermotolerance of the hybrid was determined. Therefore, in the current investigation, fifth instar larvae of the FC2 X FC1 bivoltine hybrid were raised until they started to spin cocoons before being subjected to 30, 35, 40 and 45°C for 1 hour each, followed by 1 hour of recuperation. The larval weight and cocoon characteristics of the FC2 X FC1 bivoltine hybrid were used to gauge its thermotolerance. Greater larval weight and cocoon characteristics of the bivoltine hybrid FC2 X FC1 were found. Bivoltine breeds with high thermotolerance could be selected using the FC2 X FC1 bivoltine hybrid's significantly different larval weight and cocoon characteristics. The best larval weight and cocoon characteristics from the four temperature treatments examined were found at 40 °C. In general, multivoltine breeds can endure high temperatures better than bivoltine types [10,11]. Following a 1- or 2-hour heat shock at 39°C or 41°C, Joy & Gopinathan [11] report high share survival for the multivoltine silkworm breeds C. nichi and Pure Mysore and low share survival for the bivoltine breed NB4D2. The silkworm larvae were taken into consideration to assess their response to temperature stress with the biological and commercial qualities while keeping all these experimental outcomes in mind [12]. But prior research has demonstrated that early instar silkworm larvae have lower levels of tolerance than late instars (Vasudha et al., 2006). Increased larval weight, cocoon weight, shell weight, pupal weight, and adult survivorship (82 percent) relative to control were all shown to be statistically highly significant responses to 35°C heat shock in all instars. In 2013, Prashant et al. noted that FC2 had demonstrated a profound response to HS temperatures of 35 and 45°C and the inbuilt acquired tolerance to overcome fluctuating environmental conditions. These observations are closely consistent with the current findings and can be used as potent material for the development of thermo-tolerant silkworm strains for the tropics. The fifth instar CSR2 larvae HS at 40°C recorded the highest weight of the cocoon, at 1.99 g, an improvement of 44.20 percent above the control (1.38g). However, NB4D2 showed a high rate of reaction to HS at 40°C, improving by 46.82 percent in the weight of the cocoon. The fifth instar larvae of every breed studied at 40°C formed high-quality cocoons with heavier shells than control groups. However, a heat shock temperature of 45°C proved fatal since it significantly harmed all of the biological and commercial characters across all instars. Thus, while searching for superior parents to create thermotolerant breeds and hybrids for tropical nations like India, temperatures of 40°C shall be taken into consideration to evoke a profound response and acquire tolerance to withstand the fluctuating environmental situation (Muzafar Ahmad Bhat and Hosaholalu Boregowda Manjunatha, 2017). The larvae of FC2 X FC1 were found to contain higher carbohydrates at 40°C, and this quantity also increased at 30 and 35°C. This was also shown in the embryos of the NB4D2 and PM silkworm strains [15], which makes sense given how important carbohydrates are for insect development, morphogenesis, and intermediate

metabolism. The glycogen content was variable in all HS-induced larvae, but it was higher in day-3 FC2 X FC1 HS-induced larvae at 30°C (1.68 mg/ml), 35°C (1.72 mg/ml), 40°C (1.79 mg/ml), and 45°C (1.31 mg/ml) compared to control (1.60 mg/ml). In order to survive, insects must be able to store fatty acids and glucose, which are also required for numerous physiological processes. A significant number of fatty acids are required for the synthesis of phospholipids and waxes, as well as serving as precursors in the production of eicosanoids and pheromones [16]. The molecular mechanism causing the increased carbohydrate content in the HS-induced larvae is obscure and invites thorough investigation. HS-induced larvae may have expended more energy to combat the thermal stress that led to larval death, as seen in figure 8, because the carbohydrate content was found to be lower at 45°C as compared to control and other HS temperatures. As a result, it is advised that because silkworm larvae are extremely sensitive to changing environmental conditions, they should be kept in ideal settings. Even one hour of heat stress beyond threshold will result in the larvae dying, which may later alter other characteristics of the cocoon. This could be the cause of how carbs are used during heat acclimation, increasing cuticular lipids to ensure their survival for a longer period of time and lowering water loss from heat. However, since carbohydrates are known to increase the flies' fat content, an increase in carbohydrates during rapid heat hardening (RHH 1 hr) corresponds to energy that is immediately available [17]. Therefore, the current study showed that the larvae used the carbohydrate content as they grew as a result of strong metabolic activities that need constant energy for growth and cellular homeostasis. The development of thermotolerant silkworm breeds, the development of breeding programmes, and commercial exploitation are all made possible by this work [18].

5. CONCLUSION

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+1°C) and relative humidity (75+5%) be different. Day-3 of fiftth instar larvae of FC2 X FC1 bivoltine hybrid were exposed to 30, 35, 40 and 45°C for 1 hr followed by 1 hr recovery period were exhibited contrasting response to HS as measured based on weight of the larval and cocoon parameters in comparison with their respective control group. On the basis of their weight on day 3 of the fifth instar, the weight of the larvae as affected by HS at various temperatures was measured. As a result, the average weight of the larvae was 3.22, 3.42, 1.40, 3.53, 3.63, and 0.00 g, which corresponds to FC2 X FC1 larvae at 30, 35, and 45°C. Interestingly, the larvae from the 40°C HS were found to weigh 3.63 g more than the control larvae (3.22 g). In addition, larvae couldn't live on HS at 45°C, therefore no larval weight was seen there. The population formed from 35°C showed a 91% improvement in ERR compared to the control (88 percent). However, an enhanced ERR of 90.67 percent was also noted at 40°C, while the control temperature and 35°C both had the same ERR of 88 percent. However, no ERR was seen at 45 °C since the larvae died after HS there. The FC2 X FC1 larvae were shown to be more sensitive to the HS temperatures of 30 and 35 C than 40 C. At 30°C, the highest death rate of 13.33 percent was noted, while at 35°C, it was 12.67 percent. At 40 °C, the cocoon's greatest weight of 1.76 g was recorded. In comparison to the control, an average cocoon weight of 1.51, 1.62, 1.76, and 0.00 g was reported. These values correspond to 30, 35, 40, and 45 C, respectively (1.38 g). The control's cocoon shell weighed 0.23 g. However, the heat shock-induced larvae at 40 C showed a considerable improvement in the weight of their shell. The shell weight of the cocoons spun by HS larvae at 30, 35, 40, 45°C and control was 0.26, 0.28, and 0.30 g. Intriguingly, the pupa's weight, which serves as a measure of its growth, revealed the highest weights of 1.46 and 1.33 g on day 6 in the population formed from HS larvae at 35 and 40°C, respectively. At 30°C and the control, pupal weights of 1.25 and 1.13 g were found on the sixth day of the FC2 X FC1 larval stage, respectively. Due to HS at fifth instar larvae in FC2 X FC1, the cocoon shell weight ratio was similarly impacted as that of cocoon and shell weight. The population formed from FC2 X FC1 HS larvae was documented to have a cocoon shell ratio of 17.19 percent in control, with the greatest percentages being 17.55 and 17.44 percent at 40 and 35°C, respectively. The results of the current study showed that the content of carbohydrates was low at 45°C and high in all heat-induced larvae as compared to controls. When comparing heat-induced larvae, it was discovered that the carbohydrate concentration was higher at 40°C (1.79 mg/ml) than it was at 30, 35, 45, and the control. On the third day of the fifth instar, FC2 X FC1 larvae had a

carbohydrate content of 1.60 mg/ml under control conditions, 1.68 mg/ml at 30°C, 1.72 mg/ml at 40°C, and 1.31 mg/ml at 45°C. Despite the fact that larvae did not survive at 45°C, a concentration of carbohydrates of 1.31 mg/ml was detected. According to these results, the bivoltine hybrid FC2 X FC1 larvae are affected by the biological and commercial characteristics, carbohydrate content, and ERR when exposed to critical temperatures for even a short period of time, 1 hour. However, mild HS at 35 or 40 C at particular larval stages may help the larvae show acquired tolerance to changing environmental circumstances.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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