## ***Short communication***

# Alterations due to Heat Shock in biological and commercial features of the Silkworm, *Bombyx mori*


**ABSTRACT**

In the present study FC2 X FC1 bivoltine hybrid was used to examine the impact of HS on larval growth and development, ERR, cocoon traits and biochemical constituents of carbohydrate content. The FC2 X FC1 bivoltine hybrid was subjected for HS at 30, 35, 40 and 45˚C for 1 hr with 1 hr recovery on day 3rd of fifth instar. 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 and commercial traits showed highest weight at 40°C. Consequently, although, all larvae heat shocked 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. A sequential increase in carbohydrate content was obvious both in control and HS on day 6th of fifth instar. However, the carbohydrate content found reduced in HS of 45˚C as compared to control.



Key words: heat shock, larva, silkworm, *Bombyx mori*




## 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 (Tomita *et al*., 2003) and also as one of the most genetically studied insect apart from the fruit fly, *Drosophila melanogaster*.

In India, where sericulture is predominant in tropical regions, silkworm breeding research is a mixed bag of success and failure. The successful introduction of F1 hybrids of tropical female and temperate male silkworm strains was the major contributory factor for the spread of sericulture in India. However, cocoon and silk yield and quality of silk yarn remained inferior to those of temperate silkworm hybrids. The attempts to spread temperate silkworm strains throughout the sericulture belt of India resulted in extensive crop failures especially during hot and humid environmental condition. Keeping this in view, an attempt is made to develop silkworm hybrids tolerant to high temperature environments. A huge gap still persists as challenging task in developing stress and disease resistance strains in India and in providing high yielding silkworm strains which can survive very well round the year by overcoming environmental stress. HSPs expressed at various temperatures differ in number and relative importance of each HSP family in stress tolerance varies from organism to organism (Parsell and Lindquist, 1993). It has been well established that prior exposure to stress induce tolerance and cross-tolerance to subsequent stress since HSP expression co-related with resistance to stress (Feder and Hofmann 1999). The high temperature thermotolerance in fifth instar larvae of *B. mori* reflects its adaptation to high temperatures that are encountered in the course of their normal life. However, in existing rearing practices, young silkworm larvae are recommended to be reared at high temperature (28˚C) and high relative humidity (RH 80%): whereas older silkworm larvae are reared at lower temperature (24˚C) and humidity (RH 65%). These practices thus leave ambiguity over the impact of heat shock on larval biological and commercial traits. The concerted efforts for the improvement of cocoons characters of domesticated silkworm were aimed at superior quality silk production. Therefore, it becomes imperative or essential to develop bivoltine breeds/ hybrids, which can withstand high temperature stress conditions. With this backdrop, the present investigation was undertaken to assess the effect of high temperature on various quantitative and qualitative traits of bivoltine hybrids of *B. mori*.

**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, 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 (Jolly *et al*, 1987) until spinning under room environment. During rearing, each tray including control was provided with paraffin paper and wet foam pads.

**Induction to heat shock [HS]:**

Fifth instar, 3rd day larvae of FC2 X FC1 were placed in thin walled plastic Petri plates for heat shock treatment 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.

**Analysis of biological and commercial traits:**

**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.

**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,*

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

**Larval weight**

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

**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.

**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.

**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.

**Shell ratio**

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

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

**Carbohydrate estimation, Anthrone method (Sadaasivan *et al.*, 2011):**

For estimation of total glycogen 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 glycogen 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 glycogen present in the sample was estimated following the formula and presented in mg/ml,

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

**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.

**Results**

**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 day Control, 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) (Figure 1). Besides, larvae didn't survive upon HS at 45˚C, so no larvaval weight was observed upon HS at 45˚C.

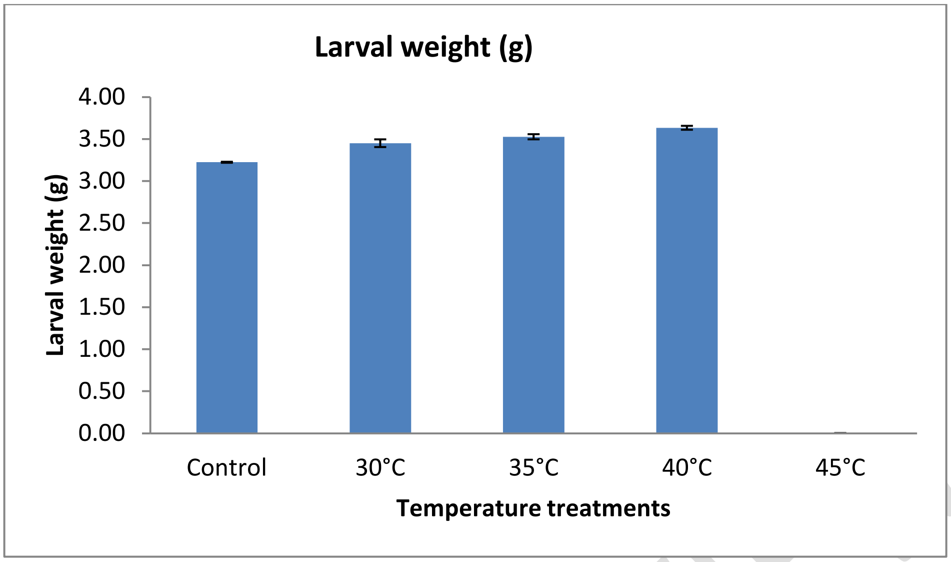


**Figure 1. Larval weight FC2 X FC1 larvae at different temperatures**

**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, Figure. 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.

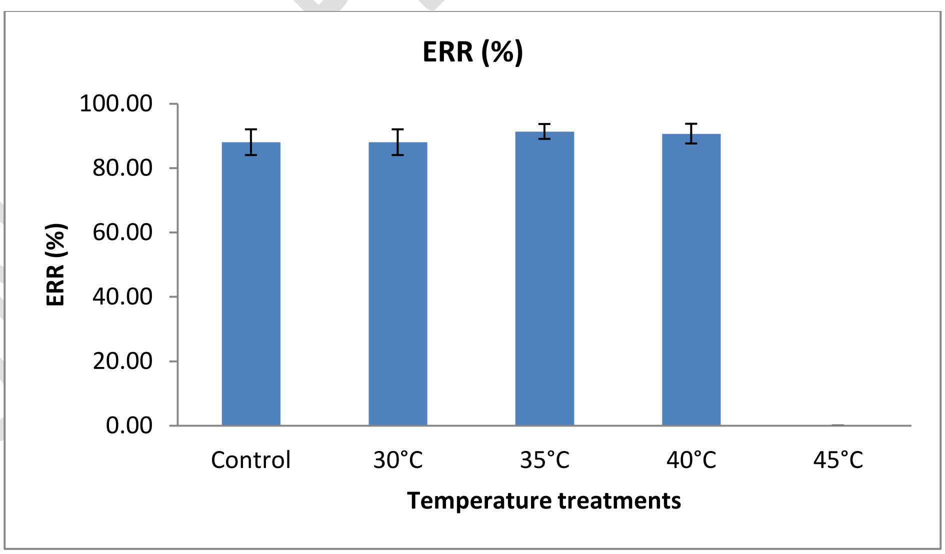


**Figure 2. ERR of FC2 X FC1 larvae at different temperatures**

**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 (Figure 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%.

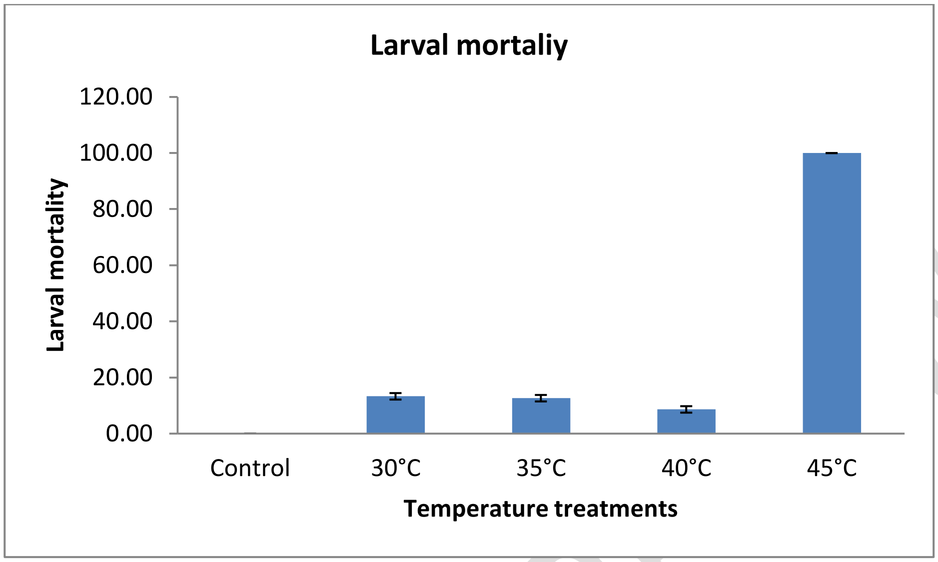


**Figure 3. Larval mortality of FC2 X FC1 larvae at different temperatures**

**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 | |

**Changes due to heat shock in relation to cocoon characters**

**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 increased compared to control (Table 2, Figure, 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.

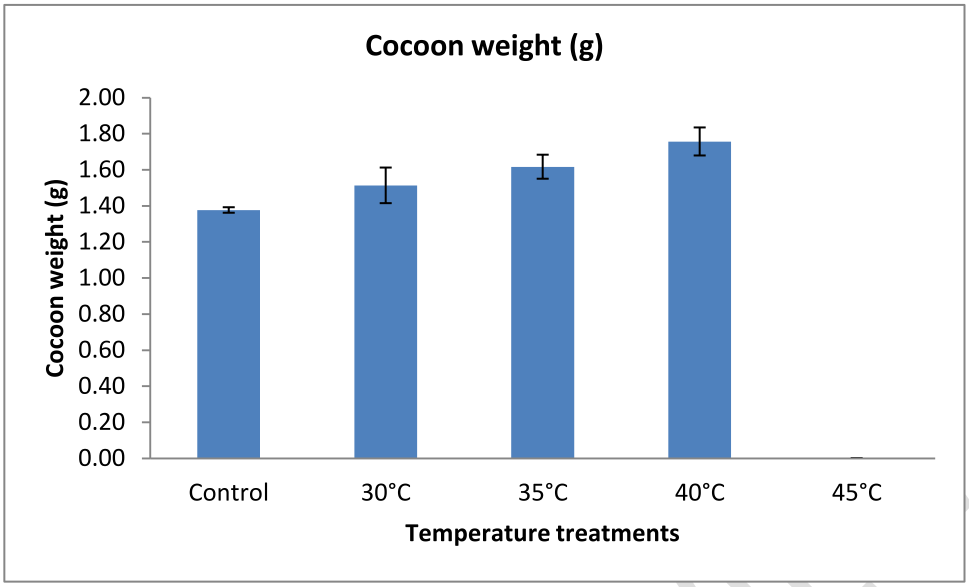


**Figure 4. Cocoon weight of FC2 X FC1 larvae at different temperatures**

## Shell weight

The cocoon shell weight was also unequivocally 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, Figure, 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 .

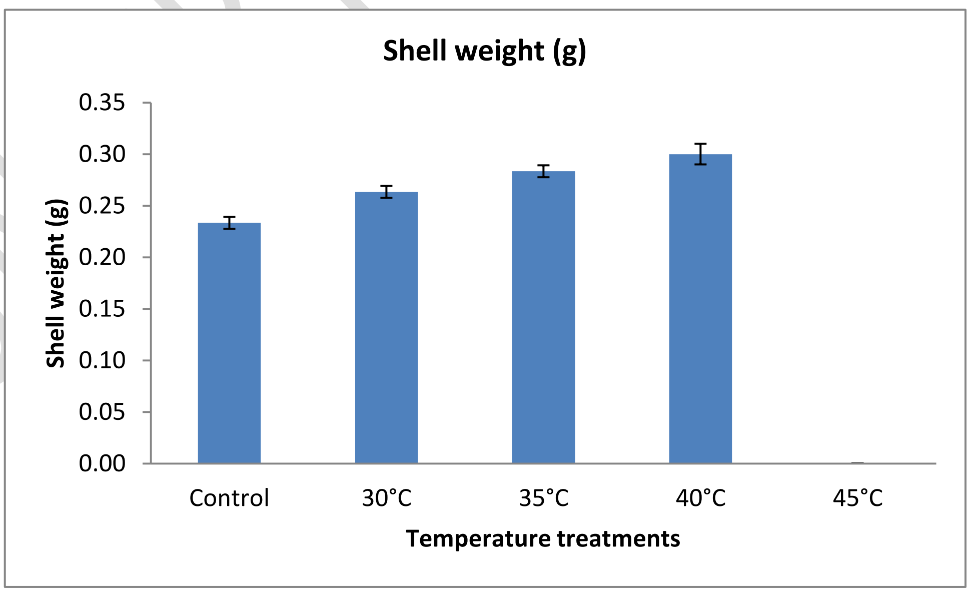


**Figure 5. Cocoon shell weight of FC2 X FC1 larvae at different temperatures**

## 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 PM and CSR2 silkworm larvae HS at 45˚C didn't survived and no pupal weight was observed (Figure, 6).

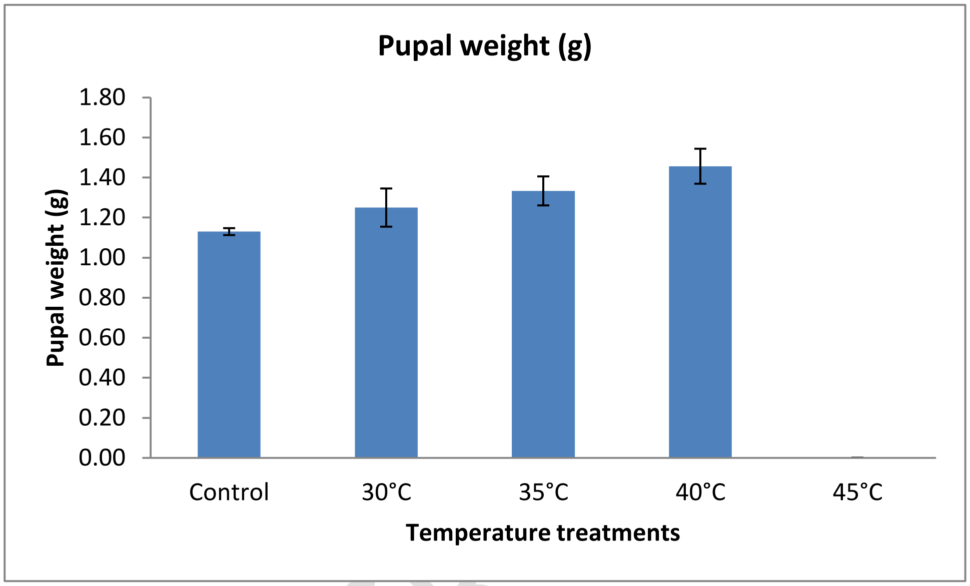


**Figure 6. Pupal weight of FC2 X FC1 larvae at different temperatures**

## 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, Figure, 7).

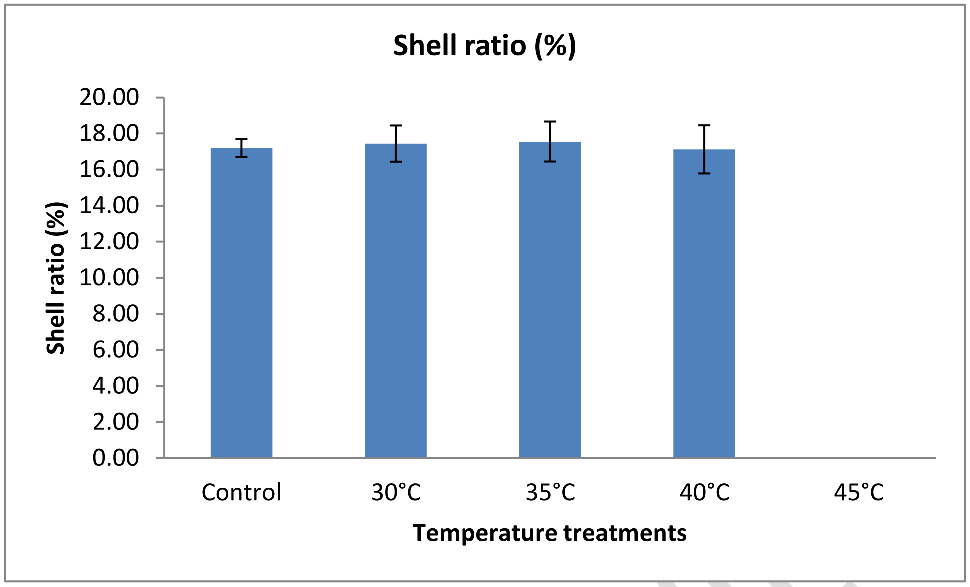


**Figure 7. Shell ratio of FC2 X FC1 larvae at different temperatures**

**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 | |

**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, Figure, 8).

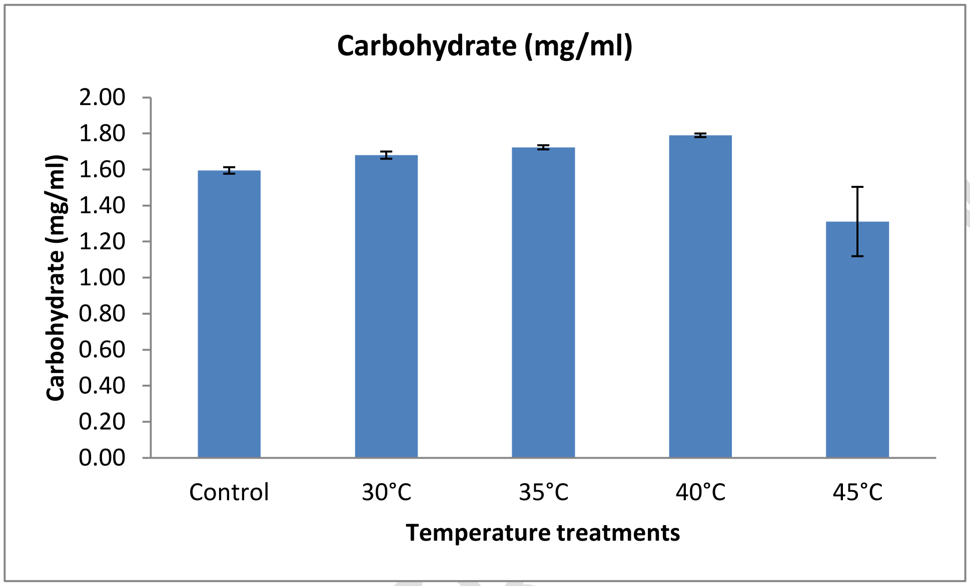


**Figure 8. Carbohydrate content of FC2 X FC1 larvae at different temperatures**

**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 | |

**Discussion**

Rearing of the silkworm, *B. mori* larvae under indoor condition is in practice since long time for production of cocoons and intern reel silk. Hence, the sericulture industry has contributed significantly the economic development of many countries due to commercial importance of silk in the textile world. This continuous domestication leads to loss of tolerance to high temperature and resistance to diseases in silkworm larvae that intern affected cocoon crop while reared under fluctuated environmental condition. In addition, tropical countries like India, the temperature and humidity that vary from season to season, region to region and diurnally also affect the cocoon crop. The erratic embryonic development in a fluctuating environment results in poor hatching, weak larvae, spinning inferior cocoons of poor quality and silk content. Even, a few hours of elevated temperature ~40°C and above in the rearing house causes considerable damage in biological and commercial traits of *B. mori* (Manjunatha *et al*., 2005; Vasudha *et al.*, 2006). Hence, to avoid such short comings and exploit breed potential, concerted efforts are made to develop appropriate rearing techniques and suggested to preserve the silkworm eggs from ovipostion until hatching under optimum environmental conditions that 25±1°C and 75±5% relative humidity. Thus, the success of silkworm cocoon crops is mainly due to production of F1 hybrids using polyvoltine (PM) as female parent and bivoltine (CSR2) as male parents.

In view of this commercial importance, silkworm, *Bombyx mori* bivoltine hybrid FC2 X FC1 was selected to examine the impact of HS during fifth instar, which determine the post embryonic development and cocoon characteristics for the first time as most of the studies were confined to either egg and/or larval stages (Manjunatha *et al.*, 2010). Since, it is a well known fact that temperature plays a significant role on growth and productivity of silkworm a correlation study was carried out between PM and CSR2 in relation to heat shock response of embryos, growth and development of silkworm larvae and cocoon characteristics. It is evident from the earlier studies also that induction of HS for an hour at temperature ranging from 35 to 45˚C and above has great impact on embryonic development (Manjunatha *et al*., 2005) in terms of hatching but its influence on post embryonic stages was not studied.

In view of this commercial interest and as FC2 X FC1 was not tested for its response to environmental insults, the present study was undertaken in order to examine the response of $FC_2$ to heat shock [HS] at a varied temperatures following whole organism concept. Towards 3rd day fifth instar larvae of FC2 X FC1 were exposed to heat shock temperatures of 30, 35, 40 and 45˚C for 1 hr with recovery period of 1 hr (Vasudha *et al*., 2006; Aparna *et al*., 2010). Interestingly, FC2 X FC1 showed significant response to HS temperatures of 30, 35 and 40˚C. HS at 45˚C induced 100% mortality. Whereas, 13.33, 12.67, 22.22 and 8.67 % mortality was recorded at 30, 35 and 40˚C (Table 1). These findings clearly indicate that HS at 45˚C found to be lethal temperature as it induces 100% mortality which is in conformity with the findings of Vasudha *et al.*, 2006 and Manjunatha *et al.*, 2010. Eventually, number of cocoons produced significantly low at 35˚C HS, but comparatively more at 40˚C HS than control. Accordingly, the highest ERR (91.33 %) was observed when the fifth instar larvae was HS at 40˚C as against 88.00 % in control (Table 1, Figure, 2). Interestingly, weight of the fifth instar larvae on day 3 was comparatively more at 40˚C than control larvae reared under some environmental conditions. The larval weight proved to be lethal in HS induced larvae at 45˚C compared to control indicating the lethal effect of high temperature. The biological, biochemical and physiological response to HS in *B. mori* is important due to cytoprotective action (Zhao and Jones, 2012), which are correlated to the tolerance level of different silkworm strains but not cocoon traits of FC2 X FC1 bivoltine hybrid. The thermotolerance of FC2 X FC1 bivoltine hybrid were measured by recording their larval weight and cocoon characters being reared at different temperatures (30, 35, 40 and 45°C). Therefore in the current study, fifth 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 and reared till they began to spin cocoons. The thermotolerant ability of FC2 X FC1 bivoltine hybrid was measured in terms of larval weight and cocoon characters. The larval weight and cocoon characters recorded from FC2 X FC1 bivoltine hybrid were greater. The larval weight and cocoon characters of the FC2 X FC1 bivoltine hybrid varied markedly which could be used to pick the bivoltine breeds for high thermotolerance. From the 4 temperature treatments analyzed, the very best larval weight and cocoon characters were recorded at 40°C. Generally, the multivoltine breeds can able to survive better in high temperatures than bivoltine breeds (Hsieh *et al*., 1995; Joy & Gopinathan, 1995). Joy & Gopinathan (1995) report high share survival for the multivoltine silkworm breeds, C. nichi and Pure Mysore and low share survival for the bivoltine breed, $NB_4D_2$, once a heat shock of 1 h or a pair of hours at 39°C or 41°C. By keeping all these experimental results in view, the silkworm larvae were taken into account to measure their response to thermal stress with the biological and commercial traits (Chandrakanth *et al* ., 2015). However, previous studies have shown that the level of tolerance in early instars of silkworm larvae is low compared to late instars (Vasudha *et al*., 2006). Response to 35˚C heat shock in all the instars found quite significant in terms of increased larval weight, cocoon weight, shell weight, pupal weight , and adult survivability (82 %)compare to control, which are statistically highly significant. With this, we inferred that FC2 had shown profound response to HS temperatures 35 and 45˚C and the inbuilt acquired tolerance to overcome fluctuated environmental condition, which can be exploited as potent material for development of thermo-tolerant silkworm strains for the tropics (Prashant *et al*., 2013). Highest weight of the cocoon was recorded from fifth instar CSR2 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 NB4D2 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. 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 (Muzafar Ahmad Bhat and Hosaholalu Boregowda Manjunatha, 2017). The carbohydrate content was found more at 40˚C in the larvae of FC2 X FC1 was also increased as 30 and 35˚C. This was also observed in the embryos of NB4D2 and PM silkworm strains (Manjunatha *et al.*, 2008), since carbohydrates play a vital role in the development, morphogenesis and intermediary metabolic pathway of insects. However, the glycogen content found variable in all the HS induced larvae while it was found increased at 30 (1.68 mg/ml), 35 (1.72 mg/ml), 40 (1.79 mg/ml) and 45°C (1.31 mg/ml) HS induced day-3 larvae of FC2 X FC1 compared to control (1.60 mg/ml). The storage of fatty acids and glucose is essential in insects for survival, and they are vital for many physiological functions. Fatty acids serve as precursors in the synthesis of eicosanoids and pheromones, and they are needed in substantial amounts for the synthesis of phospholipids and waxes (Arrese and Soulages, 2010). The biochemical process in increased content of carbohydrate in the HS induced larvae is unclear and offers detailed investigation. Meanwhile, the glycogen content was found decreased at 45°C as compared to control, which can be attributed that the HS induced larvae might have utilized more energy to overcome the thermal stress that resulted in larval death as evident in figure 8. Thus, it is suggested that since the silkworm larvae are highly sensitive to fluctuated environmental conditions they should be preserved under optimum conditions or even 1 hr of thermal stress above threshold cause death of the larvae which intern might affect other cocoon traits. This may be the reason for the utilization of carbohydrates during heat acclimation with the corresponding increase of cuticular lipids for their survival for a longer time as well as to reduce the water loss due to heat. Whereas an increase in carbohydrates during rapid heat hardening (RHH 1 hr) corresponds to available energy for immediate requirement since carbohydrate is known to increase the fat content of the flies (Mayntz *et al.*, 2005). Thus the present study revealed that the carbohydrate content has been utilized by the larvae as they grew due to intense metabolic activities which require constant energy for growth and cellular homeostasis. Besides, altered biochemical composition in the embryos as observed in the present study due to thermal stress for shorter period cause larval death. This observation opens an ample scope for evolution of thermotolerant silkworm breeds for either silkworm breeding program or commercial exploitation.

## 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. Based on these findings we conclude that the larvae of FC2 X FC1 bivoltine hybrid when exposed to critical temperature even for 1 hr affect the biological and commercial characters, carbohydrate content and in turn ERR. However, mild HS either at 35 or 40˚C at specific stage of the larvae might facilitate to exhibit acquired tolerance to fluctuated environmental conditions.

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