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# Influence of temperature variations on cocoon parameters of silkworm, *Bombyx mori* L.

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**Abstract**
Growth and development of the silkworm, *Bombyx mori* L. larvae are strongly influenced by environmental changes such as temperature, humidity etc. The high temperature leads to the poor performance of the bivoltine breeds resulting in poor cocoon characters. The experiment was laid out in completely randomised design with three replications. The larvae of 5$^{th}$ instar were exposed to three temperature regimes (25±1 ºC, 30±1 ºC and 35±1 ºC). Significant variations in cocoon weight, shell weight and shell ratio were noticed. However, the maximum mean values of cocoon weight (1.80g), shell weight (0.38g) and shell ratio (21.10%) was observed at 25±1 ºC whereas the lowest mean values of cocoon weight (0.83g), shell weight (0.06g) and shell ratio (7.21%) was recorded at 35±1 ºC. Among the twelve breeds studied, breeds U-3 and U-4 recorded significant results in terms of cocoon characters at high temperatures indicating their capacity for thermo tolerance and thus these breeds can be reared as promising breeds for commercial exploitation under high temperature.

**Keywords:** silkworm larvae, high temperature, cocoon weight, shell weight, shell ratio

## 1. Introduction

The silkworm, *Bombyx mori* L. (Lepidoptera) is a poikilotherm that is highly sensitive to environmental conditions due to artificial domestication and indoor rearing. Among the environmental conditions, temperature is most vital factor. It is well known that genetic traits, pathogenic traits, economic traits and pathogenic resistance of silkworms are tightly linked to the surrounding temperature (Sun *et al.*, 2017) [6].

Temperature plays a key effect in the development of silkworms. As silkworms are cold-blooded creatures, temperature have a direct impact on several physiological processes. In general, the early instars are high temperature resistant, which also contribute to improve survival and cocoon characteristics. Temperature rise enhances different physiological processes while physiological activity reduces as a result of a drop in temperature. Increased temperature, especially in late instars, increases larval development and reduces the larval stage. On the other hand, at low temperatures the larval growth is sluggish and larval stage extends. The optimal temperature during rearing ranges from 20 to 28 ºC for normal silkworm growth, while ideal temperature is between 23 to 28 ºC for maximum production. Temperature above 30 ºC directly impact silkworm's health.

The effect of temperature higher than 30 ºC on silkworm larvae was reported earlier by Shirota (1992) [5] and Tazima and Ohnuma (1995) [7] while attempting to synthesize high temperature resistant silkworm races. They confirmed the genetic nature of thermo-tolerance, by selecting the silkworm races based on pupation rate which are reared under high temperature conditions during fifth instar. Therefore, it is very much essential to develop bivoltine breeds which can withstand the high temperature stress conditions. The influence of temperature on the growth and development of bivoltine silkworm breeds, with temperature exceeds 35 ºC resulting in poor growth of silkworm and reduction in commercial traits. Fluctuations of temperature prevent insects from attaining their potential physiological performance.

High temperature affects nearly all biological processes including the rates of biochemical and physiological reactions (Wilmer *et al.*, 2004) [8], and can eventually affect the quality or quantity of cocoon crops. The cocoon crop loss due to high temperature leads to disease incidence in bivoltine breeds, thus in order to minimize the loss there is a need to evolve tolerant silkworm breeds which can perform well even under adverse eco-climatic conditions to get sustainable cocoon yield. Thus, emphasis must be given to evolve bivoltine silkworm breeds suitable to high temperature conditions during summer season, in this regard twelve silkworm breeds were tested at high temperature conditions during fifth instar to provide valuable information that will allow identification of thermotolerant bivoltine silkworm breeds based on the silkworm rearing performances relative to three important economical traits.

## 2. Material and method

Twelve silkworm breeds namely WM, $ND_5$, U-4, $PO_1$, $ND_3$, U-6, SPO, NSP, U-3, $SH_6$, $NB_4D_2$ and $CSR_2$ were selected as materials for the study. Silkworm rearing was carried out in completely randomised design in three replications following the standard method under recommended conditions till 2$^{nd}$ day of 5$^{th}$ instar. On the 3$^{rd}$ day of 5$^{th}$ instar hundred larvae were separated from each bed for thermal treatment *viz.*, 30±1 ºC and 35±1 ºC. Also hundred larvae in the tray were treated as control at ambient temperature (25±1 ºC). For thermal exposure, the larvae of 3$^{rd}$ day of 5$^{th}$ instar were kept in paper trays in BOD incubator at two high temperatures for six hours duration everyday till spinning and were fed with fresh mulberry after recovery period of two hours. When the larvae started spinning, they were shifted to plastic collapsible mountages were used for mounting the ripened larvae. Cocoons were harvested on the 7$^{th}$ day and assessment was made on the subsequent day. Cocoon parameters such as single cocoon weight, single shell weight, and shell ratio were calculated for the batches reared at 30±1 ºC, 35±1 ºC and 25±1 ºC.

### 2.1 Single Cocoon Weight (g)

Ten male and ten female cocoons were randomly selected and weighted on digital balance to determine the single cocoon weight by using the following formula (Kumari *et al.*, 2011):

$$\text{Single cocoon weight} = \frac{\text{Weight of 10 male (g)} + \text{10 female cocoon (g)}}{20}$$

### 2.2 Single Shell Weight (g)

Ten male and ten female cocoon shells from each replicate were weighted on digital balance to determine single shell weight. The formula applied was as under (Kumari *et al.*, 2011):

$$\text{Single shell weight} = \frac{\text{Weight of 10 male (g)} + \text{10 female cocoon shells (g)}}{20}$$

### 2.3 Shell Ratio (%)

The total quantity of silk available from a single cocoon was expressed as a percentage using the following equation (Kumari *et al.*, 2011):

$$\text{Shell ratio} = \frac{\text{Single cocoon shell weight (g)}}{\text{Single cocoon weight (g)}} \times 100$$

## 3. Results

The rearing performance based on single cocoon weight, single shell weight and shell ratio was evaluated under three temperature schedules of 25±1 ºC, 30±1 ºC and 35±1 ºC. The results of breeds are discussed based on the mean of three replications and the data was statistically analysed by employing Analysis of Variance using SPSS 16.0. Perusal of data clearly indicates that the deleterious effect of high temperature was so pronounced on cocoon characters of all the breeds considered for this study.

### 3.1 Single cocoon weight (g)

Significant variations in single cocoon weight of all breeds were recorded at all three temperatures where maximum single cocoon weight was recorded at 25±1 ºC followed by 30±1 ºC and 35±1 ºC. However, breed U-3 recorded the highest single cocoon weight at all three temperatures *viz.*, 30±1 ºC (1.50g), 35±1 ºC (1.33g) and 25±1 ºC (1.80g), whereas lowest single cocoon weight was observed in breed $PO_1$ *viz.*, 30±1 ºC (1.03g), 35±1 ºC (0.83g) and 25±1 ºC (1.42g). At 30±1 ºC, single cocoon weight of all breeds ranged from 1.03 to 1.50g with the highest recorded in $U_3$ followed by U-4 and the lowest in $PO_1$. At 35±1 ºC, it ranged from 0.83 to 1.33g with the maximum cocoon weight in $U_3$ followed by U-4 and the minimum was observed in $PO_1$. At 25±1 ºC, the cocoon weight ranged from 1.42 to 1.80g with the highest recorded for $U_3$ followed by U-4 and lowest recorded for $PO_1$ (Table 1).

**Table 1:** Single cocoon weight (g) of silkworm breeds reared at three different temperatures

| Breeds / Temperature | WM | $ND_5$ | U-4 | $PO_1$ | $ND_3$ | U-6 | SPO | NSP | U-3 | $SH_6$ | $NB_4D_2$ | $CSR_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30±1ºC | 1.15±0.01$^b$ | 1.45±0.01$^b$ | 1.47±0.01$^b$ | 1.03±0.01$^b$ | 1.30±0.01$^b$ | 1.43±0.01$^b$ | 1.10±0.01$^b$ | 1.07±0.01$^b$ | 1.50±0.01$^b$ | 1.27±0.01$^b$ | 1.13±0.01$^b$ | 1.32±0.01$^b$ |
| 35±1ºC | 1.05±0.00$^a$ | 1.26±0.01$^a$ | 1.29±0.01$^a$ | 0.83±0.01$^a$ | 1.14±0.01$^a$ | 1.23±0.01$^a$ | 0.87±0.00$^a$ | 0.85±0.00$^a$ | 1.33±0.01$^a$ | 1.08±0.01$^a$ | 1.01±0.00$^a$ | 1.17±0.01$^a$ |
| 25±1ºC | 1.54±0.00$^c$ | 1.73±0.01$^c$ | 1.76±0.01$^c$ | 1.42±0.02$^c$ | 1.59±0.00$^c$ | 1.70±0.00$^c$ | 1.49±0.01$^c$ | 1.44±0.01$^c$ | 1.80±0.02$^c$ | 1.57±0.01$^c$ | 1.52±0.01$^c$ | 1.64±0.01$^c$ |

Each value is a mean±standard error of three replications.
Figures followed by same letters in column are non-significant by Tukey HSD test.

### 3.2 Single shell weight (g)

All the silkworm breeds recorded maximum single shell weight at 25±1ºC followed by 30±1ºC and 35±1ºC but no significant variations were observed in breeds WM and $NB_4D_2$ at 30±1ºC and 35±1ºC temperature. However, U-3 breed observed highest single shell weight at all three temperatures *viz.*, 30±1ºC (0.30g), 35±1ºC (0.24g) and 25±1ºC (0.38g) and breed $PO_1$ recorded lowest shell weight *viz.*, 30±1ºC (0.09g), 35±1ºC (0.06g) and 25±1ºC (0.23g). At 30±1ºC, single shell weight of all breeds ranged from 0.09 to 0.30g with the highest in U-3 breed followed by U-4 and least in $PO_1$, whereas at 35±1ºC it was ranged from 0.06 to 0.24g with the highest single shell weight in $U_3$ followed by U-4 and the lowest value was recorded in $PO_1$. At 25±1ºC, the values for single shell weight ranged from 0.23 to 0.38g and the breed U-3 recorded the highest single shell weight followed by U-4, whereas breed $PO_1$ observed the lowest values (Table 2).

**Table 2:** Single shell weight (g) of silkworm breeds reared at three different temperatures

| Temperature \ Breeds | WM | $ND_5$ | U-4 | $PO_1$ | $ND_3$ | U-6 | SPO | NSP | U-3 | $SH_6$ | $NB_4D_2$ | $CSR_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30±1ºC | 0.15±0.01$^a$ | 0.26±0.00$^b$ | 0.28±0.01$^b$ | 0.09±0.00$^b$ | 0.19±0.01$^b$ | 0.23±0.01$^b$ | 0.11±0.00$^b$ | 0.11±0.00$^b$ | 0.30±0.01$^b$ | 0.16±0.01$^b$ | 0.13±0.01$^a$ | 0.20±0.01$^b$ |
| 35±1ºC | 0.11±0.01$^a$ | 0.19±0.02$^a$ | 0.22±0.02$^a$ | 0.06±0.01$^a$ | 0.13±0.02$^a$ | 0.16±0.02$^a$ | 0.07±0.01$^a$ | 0.07±0.01$^a$ | 0.24±0.02$^a$ | 0.11±0.02$^a$ | 0.10±0.01$^a$ | 0.14±0.02$^a$ |
| 25±1ºC | 0.26±0.01$^b$ | 0.33±0.00$^c$ | 0.35±0.00$^c$ | 0.23±0.00$^c$ | 0.27±0.01$^c$ | 0.32±0.00$^c$ | 0.25±0.01$^c$ | 0.27±0.01$^c$ | 0.38±0.01$^c$ | 0.28±0.00$^c$ | 0.27±0.01$^b$ | 0.29±0.00$^c$ |

Each value is a mean±standard error of three replications.
Figures followed by same letters in column are non-significant by Tukey HSD test.

### 3.3 Shell ratio (%)

Shell ratio of all breeds recorded maximum at 25±1ºC and found to be decreased with increased in temperature. However, no significant variations were observed in breeds WM, $PO_1$, SPO and $SH_6$ at 30±1ºC and 35±1ºC whereas breed $CSR_2$ recorded no significant variation at 25±1ºC and 30±1ºC. Breed U-3 recorded the highest shell ratio at all three temperatures *viz.*, 30±1ºC (19.99%), 35±1ºC (18.03%) and 25±1ºC (21.10%), whereas lowest single cocoon weight was observed in breed $PO_1$ *viz.*, 30±1ºC (8.72%), 35±1ºC (7.21%) and 25±1ºC (16.19%). The shell ratio among breeds at 30±1ºC ranged from 8.72 to 19.99% with the highest recorded in U-3 followed by U-4 and the lowest was observed in $PO_1$. However, the shell ratio decreases simultaneously with increase in temperature as at 35±1ºC breed shell ratio was ranged from 7.21 to 18.03% and the breed U-3 recorded maximum shell ratio followed by U-4 and breed $PO_1$ observed minimum shell ratio. On the other hand, at 25±1ºC the shell ratio ranges from 16.19 to 21.10% with the highest shell ratio in breed U-3 followed by U-4 and lowest in $PO_1$ breed (Table 3).

**Table 3:** Shell ratio (%) of silkworm breeds reared at three different temperatures

| Temperature \ Breeds | WM | $ND_5$ | U-4 | $PO_1$ | $ND_3$ | U-6 | SPO | NSP | U-3 | $SH_6$ | $NB_4D_2$ | $CSR_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30±1ºC | 13.03 ±0.87$^a$ | 17.92 ±0.18$^b$ | 19.03 ±0.63$^{ab}$ | 8.72 ±0.46$^a$ | 14.60 ±0.69$^{ab}$ | 16.07 ±0.67$^b$ | 9.99 ±0.36$^a$ | 10.27 ±0.43$^b$ | 19.99 ±0.61$^{ab}$ | 12.58 ±0.79$^a$ | 11.48 ±0.90$^b$ | 15.14 ±0.74$^b$ |
| 35±1ºC | 10.47 ±0.49$^a$ | 15.06 ±0.77$^a$ | 17.04 ±0.74$^a$ | 7.21 ±0.59$^a$ | 12.37 ±1.03$^a$ | 12.99 ±0.81$^a$ | 8.22 ±0.62$^a$ | 8.04 ±0.61$^a$ | 18.03 ±0.71$^a$ | 10.16 ±0.96$^a$ | 8.45 ±0.71$^a$ | 11.95 ±0.87$^a$ |
| 25±1ºC | 16.88 ±0.33$^b$ | 19.07 ±0.10$^b$ | 19.88 ±0.19$^b$ | 16.19 ±0.20$^b$ | 16.98 ±0.21$^b$ | 18.81 ±0.12$^c$ | 16.77 ±0.19$^b$ | 18.65 ±0.30$^c$ | 21.10 ±0.25$^b$ | 17.83 ±0.23$^b$ | 17.75 ±0.43$^c$ | 17.68 ±0.12$^b$ |

Each value is a mean±standard error of three replications.
Figures followed by same letters in column are non-significant by Tukey HSD test.

## 4. Discussion

Silkworm breeds that are reared over a series of environments exhibiting less variation are considered stable. One of the main aims of the breeders is to recommend to farmers that are stable under different environmental conditions. In India, indigenous races are well adapted to fluctuating climatic conditions characterized by high temperature, but having poor productivity. Keeping this in view, efforts have been made over a decade to improve the quality of raw silk which resulted in development of many productive and qualitatively superior bivoltine breeds (Basavaraja *et al.*, 1995) [1]. But, these breeds have been recommended to rear during favourable months and their unsuitability to rear during hot climatic conditions led to the development of robust hybrids tolerant to high temperature (Kumar *et al.*, 2002) [4].

Among many factors responsible for poor performance of the bivoltine breeds under fluctuating environmental conditions, the main reason is temperature. Indeed, many quantitative characters decline sharply as temperature rises higher than 28ºC. Sericultural breeders agree to the fact that it is a difficult to produce such bivoltine breeds that are suitable to high temperature conditions with productive traits. Under high temperature conditions, the productivity of bivoltine breeds decreases because of poor leaf quality. In order to achieve greater success in this regard, it is important to understand the level of temperature tolerance in silkworm bivoltine breeds. The main objective of this study was to identify bivoltine silkworm breeds tolerant to high temperature among twelve breeds evaluated for tolerance to thermal stress at 30±1 ºC and 35±1 ºC. This study shows that highest single cocoon weight was recorded in breed U-3 at all three temperatures followed by U-4, whereas the maximum single shell weight was observed in U-3 followed by U-4 but no significant variations were observed in WM and NB4D2 breeds at 30±1 ºC and 35±1 ºC. Also breed U-3 and U-4 recorded highest shell ratio among all breeds but the breeds WM, $PO_1$, SPO and $SH_6$ showed no significant variations at 30±1ºC and 35±1 ºC whereas the breed $CSR_2$ observed no significant variation at 25±1 ºC and 30±1 ºC. The breeds with no variations in cocoon parameters at high temperatures revealed their stability at higher temperatures whereas breeds U-3 and U-4 with highest single cocoon weight, single shell weight and shell ratio indicating their capacity for thermotolerance. Thus these breeds can be adjudicated as promising breeds for commercial exploitation under high temperature.

## 5. Conclusion

In the present study, twelve bivoltine silkworm breeds were used to identify the potential silkworm breeds for the development of thermotolerant breed and resulted in identification of two breeds *viz.*, U-3 and U-4 as temperature tolerant silkworm breed based on their cocoon weight, shell weight and shell ratio percentage.

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