Cold shock response in biological and commercial traits of the silkworm, *Bombyxmori*

Abstract

In response to the ambient temperature, insects' physiology and behaviour change just like those of all other living things. For this investigation, the FC1 X FC2 double hybrid silkworm strain/~~breed~~ was used.The larvae were collected on the third day of their fifth instar and put in little paper trays for a one-hour cold shock treatment at 10, 15 and 20°C with a relative humidity of 75±5% in B. O. D. The CS-induced larvae were allowed to recuperate for an hour at room temperature.Compared to the control (2.16 g) of the FC1 X FC2 larvae, the average weight of the recorded larvae was 2.30, 2.31, and 2.2 ~~g~~, which correspond to 10, 15, and 20°C, respectively. When compared to the control group, the population created from 10°C demonstrated an ERR improvement of 70.83 ~~percent~~ (67.50 ~~percent~~). However, at 15°C and 20°C, respectively, an elevated ERR of 86.67 and 75.83 percent was noted. Mortality was 11.67% between 10 and 15~~o~~C, whereas it was 12.50% at 20oC. The ability to transition into the next instar, spin a cocoon, become a pupa and eventually become a moth was utilised to assess the cold's potential impact on larval, pupal, and adult mortality. Highest cocoon weight of 1.37 g was observed at 15~~oC~~. Weights of the cocoons were measured and compared to the control (1.30 g), which corresponds to temperatures of 10 and 20~~oC~~, respectively. The cocoons made by CS larvae at 10, 15, and 20~~oC~~ and the control had shell weigh~~ts~~ of 0.22, 0.24, and 0.19 g, compared to 0.30 g for the control.Temperature of 15°Cshowed higher efficiency in all the traits studied whereas other temperatures showed a slight decline in all the traits.As a result, we inferred that FC2 X FC2 had exhibited a profound response to CS temperature of 15°Cand can be used to develop CS silkworm strains for the temperate areas.

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Key words: Cold~~,~~ shock, biological~~,~~ commercial, traits, *Bombyxmori*

Comment [H8]: Cold shock

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Introduction:

Silkworms~~are~~ one of the most significant domesticated insects, producing luxurious silk thread in the form of cocoons by eating mulberry leaves during their larval stage. Environmental factors have a significant impact on silkworm growth and development. Ambient temperature, rearing seasons, quality mulberry leaf and silkworm strain genetic makeup all influence on biological and cocoon-related characteristics. Temperature fluctuation during different phases of larval development, on the other hand, was found to be better for larvae growth and development than constant temperature. Many studies have shown that good quality cocoons are created at temperatures between 22 and 27°C and that temperature above these values result in low-grade cocoons (Suresh Kumar and Harjeet Singh, 2011). Chill-sensitive insects die as a result of cold-induced damage before ice forms within their bodies. Cold-intolerance is a term used to describe this method. False codling moth larvae, *Thaumatotobialeucotreta* (Lepidoptera: ~~*Tortricidae*~~), for

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example, freeze between 13 and 22°C but are killed by brief exposures between 8 and 12°C. Datta*et al.*, (2001)discovered that lower temperatures are always better than higher temperatures.Coldshock is the stress caused by a quick and rapid exposure to cold temperature~~s~~ that are not below freezing. Cold shock, also known as "direct chilling injury," is caused by a rapid cooling rate. The temperature threshold that causes injury varies by species and strain, but in the absence of ice formation and at temperature~~s~~ considerably over the super cooling limit, this type of injury is regularly found. Insects are continually confronted with adverse environmental conditions such as pathogen infections, UV radiation, pesticide activity, oxidative stress and extreme temperature. Insects, on the other hand, can cold-harden in a significantly shorter time frame, a process known as fast cold-hardening (Lee *et al.*, 1987). As a result of these procedures, the influence of cold shock on larval biology and commercial features is unclear. The goal of the coordinated efforts to improve the cocoon characteristics of domesticated silkworms was to produce superior quality silk. As a result, developing bivoltine breeds/hybrids that can tolerate low temperature stress conditions, particularly in temperate zones, becomes crucial. So, the current study was designed to see how low temperatures affected several quantitative and qualitative features in bivoltine hybrids of *B. mori*.

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Materials and methods

The FC1 X FC2 double hybrid silkworm strain/~~breed~~ was chosen for this study. The Department of Sericulture, Jammu and Kashmir Union Territory, provided first instar (first moulting) larvae.In the laboratory, the first instar (first moulting) larvae were incubated at a temperature of 25±1°C and a relative humidity of 75±5%. The larvae were reared on mulberry leaves until they spun cocoons, as per conventional protocol (Jolly, 1987).The larvae were collected during the 5th instar (3rd day) and placed in small paper trays for cold shock treatment at 10, 15 and 20°C with a relative humidity of 75% in B. O. D for 1 hour. The CS-induced larvae were kept at room temperature for 1 hour to recover.

Comment [H14]: How many insect sample (B. mori larvae)?
How many replication was performed?

Comment [H15]: Temperature at 24 degree is too low for egg incubation. Normally the temperature for incubation silkworm egg is around 26-28 degree celcius and the optimum temperature for silkworm egg incubation is 27 degree celcius. Otherwise the author should provide the reference?

Comment [H16]: Why did author pick them up at the day 3 of the 5th instar?

Comment [H17]: What is the room temperature (what degree)?

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Analysis of biological and commercial traits:

Determination of cold sensitivity:

The capacity to enter the next instar or spin cocoon, transform into pupae and moth was used to determine cold sensitivity in terms of larval, pupal and adult mortality.

Effective rate of rearing (ERR):

The following formula was used to determine ERR,

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

Larval,cocoon, pupal and shellweight:

On day 6 of the fifth instar, approximately 6 larvae were randomly selected from each replication and their weight was recorded. Similarly, from each replication on day 6 after spinning, cocoon weight was determined.The pupae retrieved from randomly selected cocoons (6) of each replication were used to calculate pupal weight and after removing pupae, shells were weighed for cocoon shell weight.

Comment [H19]: From how many insect?

Shell ratio:

The following formula was used to calculate the shell ratio,

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

Emergence of moth and fecundity:

All of the cocoons derived from the various CS and control batches were kept in normal environmental conditions until eclosion. The pupal and adult mortality rates as impacted by the CS during different treatments were calculated using the moth that had emerged.The average number of eggs laid by 6 moths from each treatment was used to calculate fecundity. The eggs were counted and documented separately.

The data generated was put to statistical tool, one way ANOVA for analysis and interpretation of data by using SPSS (Statistical Package Software for Social Sciences) version 20.

Results

Changes in the larval growth due to cold shock

The weight of the larvae on day 3 of the fifth instar was used to determine how CS affected their growth at different temperatures. As a result, the average weight of the larvae recorded was 2.30, 2.31, and 2.2 g which corresponds to 10, 15 and 20°C, respectively, compared to the control (2.16 g) of the FC1 X FC2 larvae (Table 1), which was statistically significant at P<0.01. At all temperatures, however, there was a modest increase in larval weight. Intrestingly, the larvae produced from 15°C CS weighed 2.31 g more than the control larvae (2.31 g) (Figure 1).

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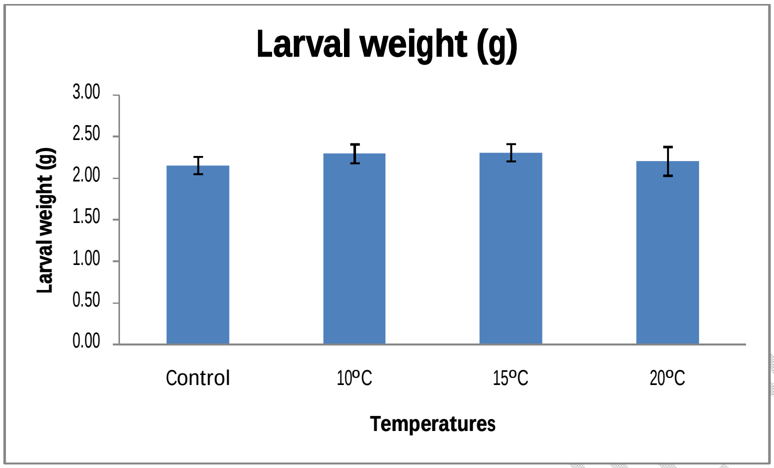

Figure 1. Larval weight FC1 X FC2 larvae at different temperatures

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Changes in the ERR due to cold shock

The ERR stands for the larvae that successfully spin cocoons. In the end, silkworm larvae derived from various CS induced were grown in the rearing house under natural environmental circumstances. Surprisingly, the population formed from 10°C showed a 70.83 %improvement in ERR when compared to the control group (67.50 %). However, at 15°C, an increased ERR of 86.67 percent was seen, and at 20°C, an increased ERR of 75.83 %was observed (Table 1, Figure 2), which is significant at P <0.01.

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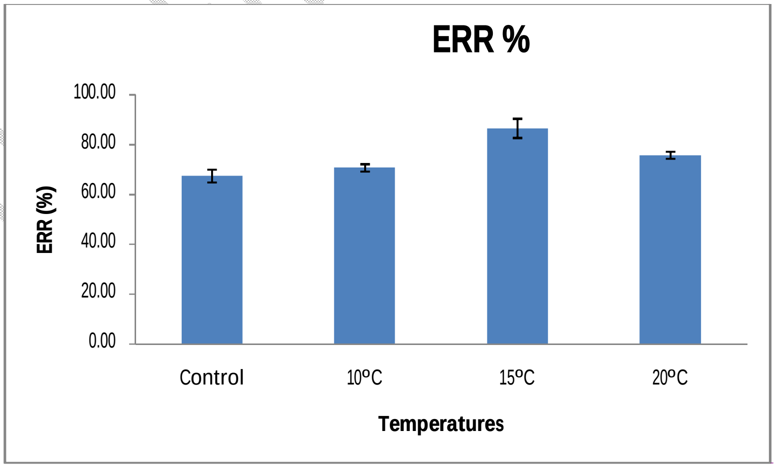

Figure 2.ERR of FC1 X FC2 larvae at different temperatures

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Changes in the larval mortality due to cold shock

FC1 X FC2 larvae are extremely sensitive to different cold shock (CS) temperatures (Figure 3). The FC1 X FC2 larvae were found to be sensitive to the CS temperatures of 10, 15, and 20ºC. At 10 and 15ºC, mortality was 11.67 percent, but at 20ºC, it was 12.50 percent (Table 1), which is significant at P< 0.01.

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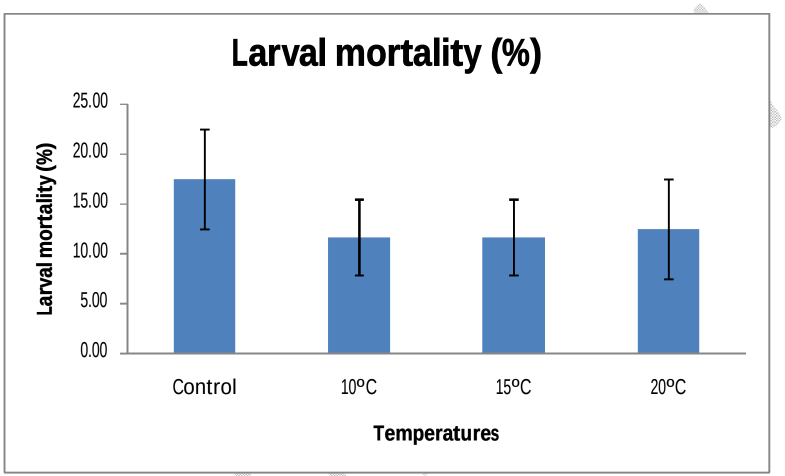

Figure 3. Larval mortality of FC1X FC2 at different temperatures

Table 1: Effect of cold shock on biological traits of the silkworm, *Bombyxmori*bivoltine hybrid FC1 X FC2

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Treatments	Larval weight (g)	ERR (%)	Larval mortality (%)
	Mean±S.E.	Mean±S.E.	Mean±S.E.
Control	2.160±0.060	67.500±1.443	17.500±2.887
10ºC	2.300±0.066	70.833±0.833	11.667±2.205
15ºC	2.313±0.059	86.667±2.205	11.667±2.205
20ºC	2.210±0.100	75.833±0.833	12.500±2.887
C.D.	N/A	4.780	N/A
SE(m)	0.073	1.443	2.569
SE(d)	0.103	2.041	3.632
C.V.	5.642	3.324	33.366

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F-Value	1.005	33.639	1.193
Significance	0.43906	0.00007	0.37228

Changes due to cold shock in relation to cocoon characters

Cocoon weight

The weight of the cocoons spun by FC1X FC2 silkworm larvae produced from CS induced on day 6 at 10, 15 and 20ºC was significantly higher than the control (Table 2, Figure, 4). At 15ºC, the cocoon weighed more (1.37 g). In comparison to the control (1.30 g), cocoon weight~~s~~ of 1.29 and 1.23 g were measured, which correspond to 10 and 20ºC, respectively (Table, 2).

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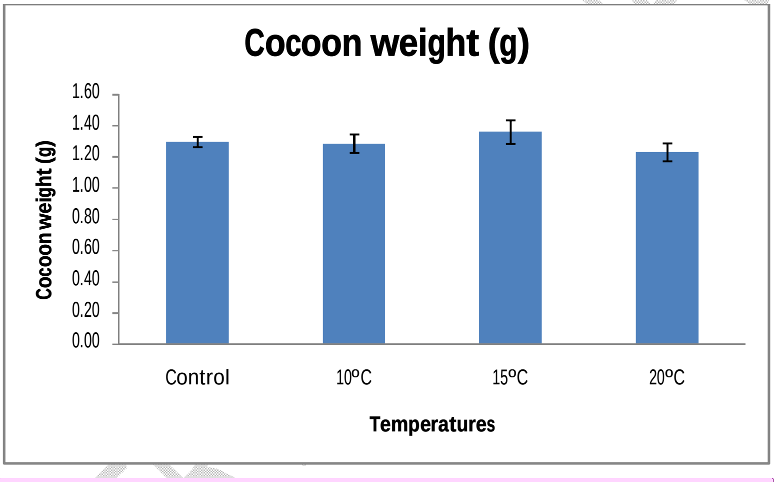

Figure 4. Cocoon weight of FC1 X FC2 larvae at different temperatures

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Shell weight

Due to the fluctuating environmental conditions in the rearing house, the cocoon shell weight was clearly altered, just like the cocoon weight in control. As a result, the weight of the cocoon shell in the control group was 0.33 g. However, cold shock induced larvae at 15ºC showed a considerable improvement in shell weight. The shell weight of the cocoons generated by CS larvae at 10, 15, 20ºC and control was 0.22, 0.24, and 0.19 g respectively (Table 2, Figure 5), compared to 0.30 g for the control.

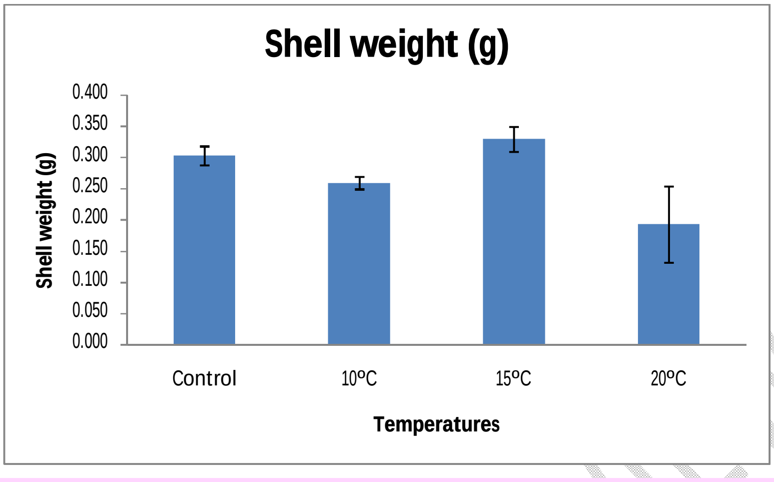

Figure 5. Cocoon shell weight of FC1 X FC2 larvae at different temperatures

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Pupal weight

Surprisingly, the weight of the pupa, as a measure of its growth, was highest in the population obtained from CS at 15 and 10°C on day-6. Pupal weight of 1.03 and 0.99 g of pupal weight were observed at 20 °C and control (Table, 2).

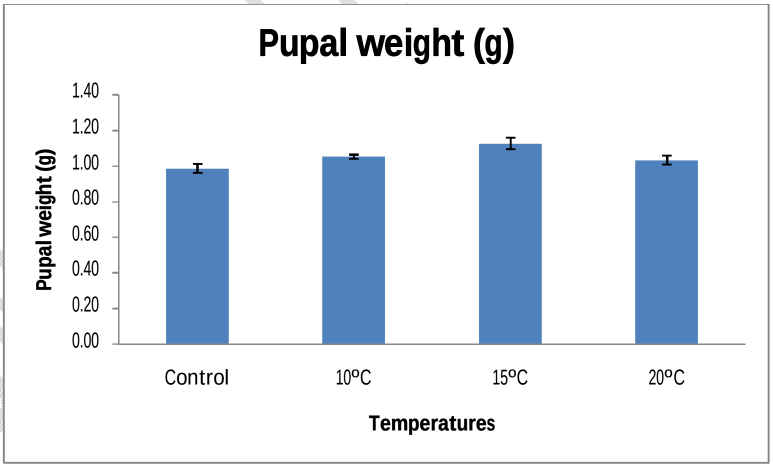

Figure 6. Pupal weight of FC1 X FC2 larvae at different temperatures

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Cocoon shell ratio

The cocoon shell weight ratio was similarly adjusted as the cocoon and shell weight in fifth instar larvae in FC1 X FC2. The control population had a cocoon shell ratio of 23.73 %, while the populations generated from FC1 X FC2 CS larvae at 10, 15, and 20°C had shell ratios of 17.67, 17.26 and 16.01 %, respectively (Table 2, Figure, 7).

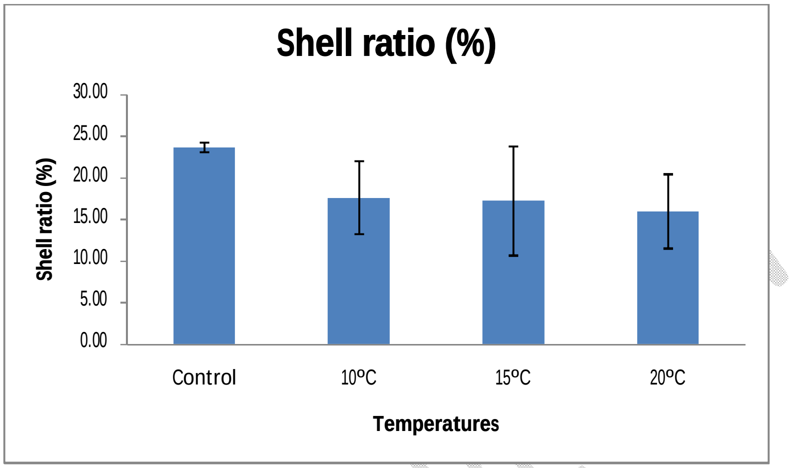

Figure 7. Shell ratio of FC1 X FC2 larvae at different temperatures

Table 2: Effect of cold shock on cocoon traits of the silkworm, *Bombyxmori*bivoltine hybrid FC1 X FC2

Treatments	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)
	Mean±S.E.	**Mean±S.E.**	**Mean±S.E.**	**Mean±S.E.**
Control	1.300±0.019	0.987±0.015	0.303±0.009	23.733±0.333
10°C	1.290±0.034	1.053±0.007	0.260±0.006	17.667±2.539
15°C	1.363±0.043	1.127±0.019	0.330±0.012	17.257±3.792
20°C	1.233±0.033	1.033±0.015	0.193±0.035	16.013±2.582
C.D.	N/A	0.047	0.064	N/A
SE(m)	0.034	0.014	0.019	2.627
SE(d)	0.047	0.020	0.027	3.715
C.V.	4.484	2.349	12.301	24.376
F-Value	2.517	16.731	9.562	1.724
Significance	0.13186	0.00083	0.00505	0.23905

Moth emergence (%)

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In the FC1 X FC2 double hybrid silkworm strain, moth emergence was similarly adjusted as other parameters changed. Moth emergence was 89.04 percent in the control population, while moth emergence was 94.16, 96.18, and 95.28 percent in the FC1 X FC2 double hybrid silkworm strain CS populations grown at 10, 15, and 20ºC, respectively (Table 3, Figure, 8).

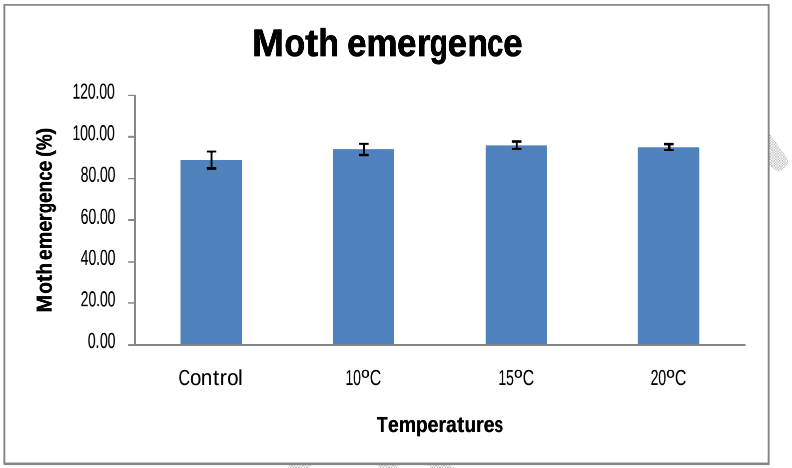

Figure 8. Moth emergence (%) of FC1 X FC2 double hybrid at different temperatures

Fecundity ~~***(No. of eggs laid)***~~

Fecundity altered in the FC1 X FC2 double hybrid silkworm strain as other parameters changed. The control moth laid 446.00 eggs, while the FC1 X FC2 double hybrid silkworm strain CS populations reared at 10, 15, and 20ºC laid 438.33, 559.00, and 460.00 eggs respectively (Table 3, Figure, 9).

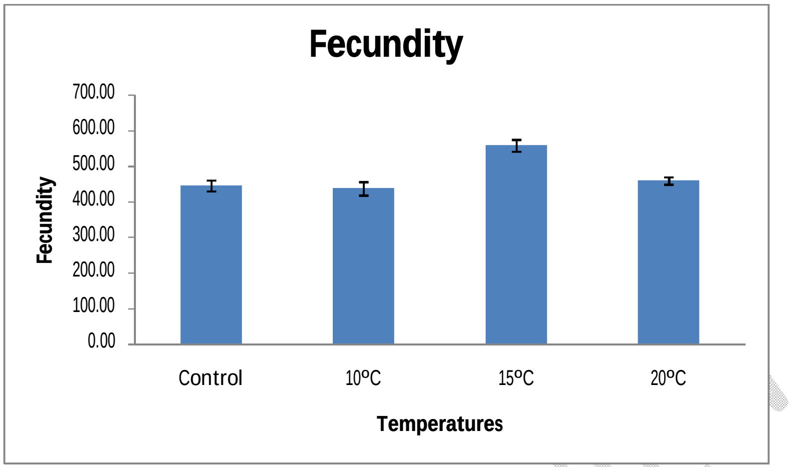

Figure 9. Number of eggs laid by female moth of FC1 X FC2 double hybrid at different temperatures

Table 3: Effect of cold shock on Moth emergence of the silkworm, ***Bombyxmori*bivoltine hybrid FC1 X FC2**

Treatments	Moth emergence (%)	Fecundity
	Mean±S.E.	Mean±S.E.
Control	89.040±2.335	446.000±8.718
10°C	94.157±1.545	438.333±10.929
15°C	96.180±1.036	559.000±9.539
20°C	95.277±0.846	460.000±5.774
C.D.	5.139	29.611
SE(m)	1.552	8.941
SE(d)	2.194	12.645
C.V.	2.869	3.255
F-Value	4.230	39.459
Significance	0.04566	0.00004

Discussion

Because the insects were rapidly cooled and spontaneous ice formation in extracellular fluids did not begin until around 15 °C lower, these data indicate that mortality was caused by cold shock (Chen *et al.*, 1987; Lee *et al.*, 1987). The average weight of the larvae observed was 2.30, 2.31 and 2.2 g, respectively, corresponding to 10, 15 and 20°C, as opposed to the control (2.16 g) of the FC1 X FC2 larvae (Table 1), which was statistically significant at P<0.01. The weight of the larvae increased somewhat at all temperatures. Surprisingly, the 15°C CS larvae weighed 2.31 g greater than the control larvae. FC1 X FC2 larvae are very sensitive to various cold shock (CS) temperatures. The CS temperatures of 10, 15, and 20ºC were found to be responsive to the FC1 X FC2 larvae. Mortality was 11.67 % at 10 and 15ºC, but 12.50 % at 20ºC, which is significant at $P< 0.01$. The cocoon weight spun by FC1 X FC2 silkworm larvae induced on day 6 at 10, 15, and 20ºC was significantly higher than the control. The cocoon weighed was highest at 15ºC (1.37 g).The cocoon shell weight, like the cocoon weight in control, was clearly affected by changing environmental conditions in the rearing house. The weight of the cocoon shell in the control group was 0.33 g as a result. Cold shock induced larvae at 15ºC, on the other hand, showed a significant improvement in shell weight. The shell weight of the cocoons produced by CS larvae at 10, 15, 20ºC and control was 0.22, 0.24 and 0.19 g respectively, compared to 0.30 g for the control.The pupal weight as a metric of growth was highest in the population derived from CS on day-6 at 15 and 10ºC. At 20ºC and control, pupal weights of 1.03 and 0.99 g were reported during the 6th day larval stage of FC1 X FC2. The cocoon shell weight ratio was similarly adjusted as the cocoon and shell weight in fifth instar larvae in FC1 X FC2. The control population had a cocoon shell ratio of 23.73 %, while the populations generated from FC1 X FC2 CS larvae at 10, 15, and 20ºC had shell ratios of 17.67, 17.26, and 16.01 %, respectively.The FC1 X FC2 double hybrid silkworm strain's fecundity fluctuated as other factors changed. The FC1 X FC2 double hybrid silkworm strain CS populations raised at 10, 15 and 20^{o}C deposited 438.33, 559.00, and 460.00 eggs, respectively.

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Conclusion

The recent decade has seen a huge rise in studies addressing the processes of cold hardening thanks to developments in molecular biology and the 'omics' revolution.Rapid cold hardening (RCH) normally occurs at temperatures below the developmental threshold of most species, whereas cold acclimation occurs at temperatures that allow for growth and reproduction.In contrast to cryoprotectant mobilisation, both seasonal cold-hardening and RCH are thought to include cell membrane changes.The function of gene expression is perhaps the most fundamental mechanistic difference between seasonal cold-hardening and RCH.Furthermore, further functional research is needed to determine which processes and pathways are required for cold hardening and which just correlate with increased cold tolerance.

Moth emergence was also altered as other parameters changed in the FC1 X FC2 double hybrid silkworm strain. The control population had an emergence rate of 89.04 %, while the FC1 X FC2 double hybrid silkworm strain CS populations reared at 10, 15, and 20ºC had emergence rates of 94.16, 96.18, and 95.28 %, respectively. As other variables changed, the fertility of the FC1 X FC2 double hybrid silkworm strain fluctuated. The CS populations of the FC1 X FC2 double hybrid silkworm strain layed 438.33, 559.00, and 460.00 eggs, respectively, when grown at 10, 15, and 20ºC.

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