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# RESEARCH ARTICLE

# STUDIES ON HATCHING PATTERNS AND COMMERCIAL CHARACTERISTICS OF EGG HATCHING IN THE SILKWORM (*BOMBYX MORI* L.) HYBRID, CSR2 x CSR4 UNDER PHOTOPERIODIC AND BLACK-BOXING CONDITIONS

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## ABSTRACT

Comprehensives knowledge on a. hatching patterns and b. hatching parameters in the commercial mulberry silkworm, *Bombyx mori* L., are invariably essential for successful silkworm cocoon crop. Present study confirms the earlier reported hatching patterns under four photoperiodic conditions and further reports the untapped hatching parameters of bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4, more popular in contemporary Indian sericulture. Disease free layings (DFLs) of CSR2 x CSR4 were introduced into four photoperiodic conditions; LD 12 : 12 (natural day), DD (continuous dark), LL (continuous light) and black-box conditions on the third day of oviposition, under constant temperature (25 ± 1ºC) and relative humidity (80 ± 5%). Data on precise number of silkworm larvae hatched-out from the experimental eggs were recorded on hourly basis from five replications. Experiments were repeated for five times. Recorded macroscopic data were converted into percentage and represented as chronograms. Results on hatching irrevocably corroborated that the hatching rhythm in CSR2 x CSR4 under LD 12 : 12 conditions occurred for 2 consecutive days, with less hatching on day 1. Hatching rhythmicity under DD/LL obviously occurred for two consecutive days, with hatching phase advancing under DD and delaying with LL. Thus, rhythmicity was circadian, diurnal, taking 'lights-on' as signal and free-ran with DD/LL. Hatching with black-box condition confined to a single day, less hatching duration, in direct response to lights-on phase. From recorded macroscopic data on egg hatching and time of hatching, egg hatching parameters such as hatching duration, percentage of total hatching, brushing and early/late born larvae were extracted while percentage of unfertilized eggs and dead eggs were directly counted from experimental eggs and later converted into percentage. Total hatching and brushing percentage were significantly high under black-box system while early/late born larval percentage under DD. Imposed photoperiodic conditions did not show any impact on unfertilized eggs (%) while dead eggs percentage was more with LL condition. Data were discussed on the importance of hatching patterns and hatching parameters in commercial silkworm egg hatching.

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# INTRODUCTION

Animals display rhythmic oscillations (Solberger 1965) under light-dark schedules of natural day (≤ or≥24h; (Saunders 1978, 1982; 2002). Kogure (1933) first reported that embryonic diapause in *Bombyx* silkworm is the direct response to photoperiods characterizing short-day nature. Hirasaka and Koyama (1970, 1972), Sivarami Reddy *et al* (1984) showed that growth rates too exhibit cyclical fluctuations in *Bombyx mori* L. *B. mori* was reported as the short-day dependent insect (Kogure 1933; De Wilde 1962; Danilevskii 1965; Lees 1968; Beck 1980; Saunders 1982).

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According to Shimizu (1982) silkworm demonstrated long-day dependency reared on artificial diet, passed through embryonic diapause in the next generation. Later, researches were diverted onto silkworm hybrids as hybrids are only exploited for commercial purposes. Earlier report on Mysore race (PM, Pure Mysore) by Anantha narayana (1980) and Anantha Narayana *et al*. (1978) recited that silkworm hatching is a day-active one and phase-locked to lights-on signal. Sivarami Reddy (1993), Sivarami Reddy and Sasira Babu (1990) and Sivarami Reddy et al. (1998) reported that the silkworm hatching is under circadian control in PM x NB4D2. Later, the researches on silkworm egg hatching was further shifted to bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 (Shanthan Babu,2014; Srinath, 2014; Suvarna, 2021) who showed certain basic differences in circadian expression in egg hatching between multivoltine x bivoltine hybrids and bivoltine x bivoltine hybrids.

Further, the differences in hatching expressions between PM x CSR2 and CSR2 x CSR4 put forth a new hypothesis of mixed-age expression. However, all the above reports mainly focused on the overt phenomenon alone in *Bombyx mori* egg hatching. It is apparent that silkworm, an economic importance insect is broadly used for commercial exploitation and non-recollecting the important commercial hatching characters (which are otherwise not available in the context of egg hatching under different photoperiodic conditions) is of no utility. Therefore, the present investigation confirms the occurrence of circadian characteristics in the silkworm hybrid, CSR2 x CSR4 on one hand and further report afresh the commercial economic egg hatching characters under different photoperiodic combinations in *B. mori* (CSR2 x CSR4, a hybrid of CSR2 and CSR4) on the other.

## MATERIALS AND METHODS

Disease free layings (DFLs, each DFL consists of 450 to 500 eggs laid by a single silkmoth on a single day) of the silkworm, *B. mori*, CSR2 x CSR4(bivoltine x bivoltine silkworm hybrid) were procured on the third day of oviposition, from the Silkworm Seed Production Centre, (SSPC), National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Hindupur, Andhra Pradesh for the study. The DFLs were transported to the laboratory during evening hours and immediately spread in pre-disinfected rearing trays. The same day, DFLs were introduced, till completion of hatching, to normal day (LD 12: 12), DD (continuous dark condition), LL (continuous light) and Black-Box system (a modified method of DD, interrupting dark-phase at 06.00 h on the day of hatching).

The light phase (photophase) (around 50 Lux) for LD12 : 12 commenced from 0600h for continuous 12 hours and the scotophase spanned for 12 hours from 1800 to 0600 h. Optimum temperature (25± 1 °C) and relative humidity (RH,80 ± 5%) (Krishnaswami 1986) was maintained in the laboratory throughout experimentation. Five DFLs were kept separately under each photoperiodic condition (five replications). Rhythmic pattern in egg hatching and economic egg hatching parameters were studied under these photoperiodic schedules. The hatching experiments were repeated for five times. Precise timings, in hourly intervals, of hatching were determined and numbers of larvae hatched-out from experimental DFLs were recorded.

Data on number of larvae hatched-out from experimental DFLs on hourly basis were utilized for computing total hatching percentage as well as hour to hour hatching percentage. Hatching durations were determined from the initiation hour to the completion of hatching for all photoperiodic conditions. On the other hand, brushing percentage was calculated based on total hatched-out larvae and larvae that were continued further for silkworm rearing. Late/early hatched larval percentage was calculated taking total hatched-out larvae and brushed larvae into account. Each individual DFL was covered to observe, count and record number of a) un-fertilized eggs and b) dead eggs. These data were then utilized for arriving at percentage utilizing total number of eggs of that specific DFL.After attending simple statistical calculations of average, standard deviation and percentage, the resultant data were utilized to determine the statistical validity, through ANOVA.

## RESULTS

**Hatching patterns:** Precise time of the hatching was noted and thereon number of eggs hatched-out was counted on hourly basis. Hatched-out larvae were gently transferred into another rearing tray with the help of soft feather. Count of hatched larvae was then converted into percentage and plotted in chronogram to represent the rhythmicity in hatching in CSR2 x CSR4 (*Bombyx mori L.*). Hatching patterns were studied in four photoperiodic conditions; a. natural day photoperiodic (LD 12 : 12) condition, continuous dark (DD), continuous light (LL) and braking dark under DD (black-boxing) conditions.

***Hatching patterns in CSR2 x CSR4 under natural day; LD 12: 12 conditions*:** Results on egg hatching patterns in the bivoltine x bivoltine silkworm, *Bombyx mori* L. (CSR2 x CSR4) under natural day condition, LD 12 : 12 photoperiodic conditions at the Laboratory level are presented in Figure 1. The graph is chronogram, the best fitted of five replications. Cross-hatched area in the chronogram represents the dark phase imposed while open area is the light part of the day. Two 24 h cycles are given in the graph as the hatching occurred for two consecutive days. Hatching count of the experimental DFL sample were converted into the percentage hatching and suitably graphed in the chronogram. From the chronogram (Figure 1.) it can be observed that the hatching in CSR2 x CSR4 occurred for two consecutive days, day 1 and day 2. However, hatching magnitude on day 1 was very less while that on day 2 was high. The hatching occurred at or after the beginning of the photophase, 06.00 h. Thus, it is referred as light-on synchronized activity. As the hatching occurred in the day (light) part of the day, the hatching is referred to as diurnal. Since the distance between two hatching peaks are near to 24 hours, the hatching rhythmicity is coined as circadian (≈ 24 h). The peaks of hatching on both days (day 1 and Day 2) are very distinct and sharp. Thus, it is interesting to note that hatching is a. diurnal, b. phase-locked to 'lights-on', c. circadian, d. sharp hatching peaks and magnitude of hatching on day 1 was less than day 2.

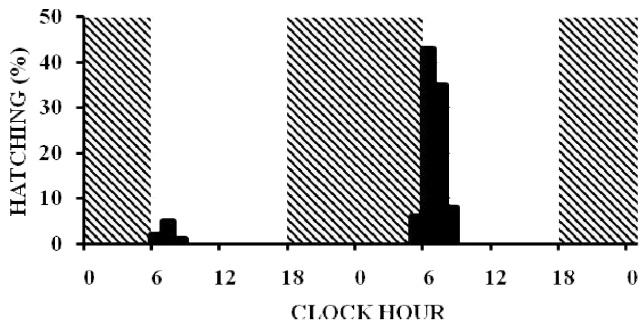


Figure 1: Chronogram representing distribution of hatching (%) under LD 12:12 conditions in the silkworm, *Bombyx mori* L. (CSR2 x CSR4). Note less hatching on the day 1 and maximum hatching on the day 2. Also note that the hatching occurred just after 'lights-on' phase of the LD cycle. Cross-hatched area in the histogram indicates the dark phase imposed and the open area, the light phase of the day.

***Hatching patterns in CSR2 x CSR4 under continuous dark (DD) conditions*:** The eggs of *B. mori* were kept under continuous dark (DD) condition and the hatching patterns were studied. Results on the chronological occurrence of hatching are presented in chronogram (Figure 2.). Hatching trend in CSR2 x CSR4 under DD was similar to that observed under LD 12 : 12 condition; occurring for two continuous days, distance between two peaks was around 24 h. However, notable differences are; a. hatching advanced into the dark phase (before 6.00 h) and b. hatching on day 1 was high than on day2. The expression means that the silkworm is short say insect, advancing into dark phase from lights-on phase (phase advancement), circadian and free running hatching behaviour.

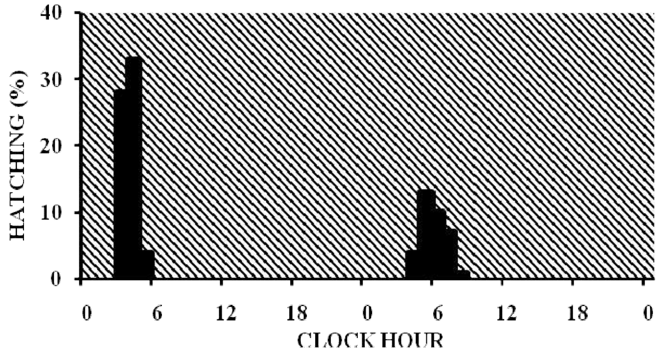


Figure 2: Hatching patterns in silkworm hybrid, CSR2 x CSR4 of *B. mori* under continuous dark (DD) conditions. Note more hatching on the day 1 and less hatching on the day 2.

Also note that the hatching phase advanced into dark phase of the LD cycle. Cross-hatched area in the histogram indicates the dark phase imposed and the open area, the light phase of the day.

***Hatching patterns in CSR2 x CSR4 under continuous light (LL) conditions*:** The silkworm hybrid, CSR2 x CSR2 behaved differently regarding hatching under continuous light (LL) condition. Hatching was continued to express for two continuous days. Hatching was less on day 1 and more on day 2.

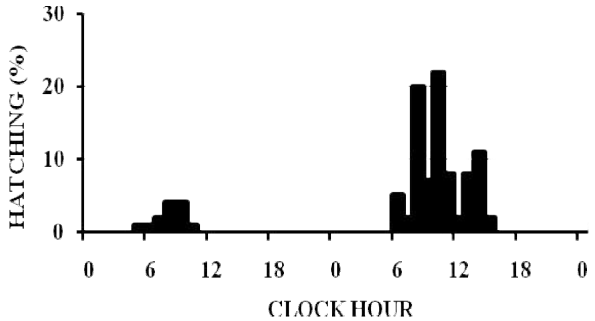


Figure 3: Hatching in CSR2 x CSR4 of *B. mori* under continuous light (LL) conditions. Note less hatching on the day 1 and more on the day 2. Also note that the hatching phase delayed into light phase of LD cycle. Hatching broadened to occur for more than 6 hours. The rhythmic character of hatching pattern of the hybrid was circadian, occurred after lights-on phase of LD schedule, free ran, hatching phase delayed into light phase of LD condition. Therefore, the rhythm can be said to be under circadian control. Notably, the hatching spread considerably, indicating that LL imposes a mixed age characteristics.

***Hatching patterns in CSR2 x CSR4 under black-boxing conditions*:** The eggs of B. mori (CSR2 x CSR4) were incubated under DD condition from the beginning and were exposed to light at 6.00 h on the day of hatching, the main feature of black-boxing system of egg incubation. Hatching under black-box system was very distinct, appearing for a single day only. Hatching occurred at or after lights-on phase. Hatching peak was very sharp with only 3 hours of hatching duration. Thus, hatching under black-box system was diurnal, occurring for a single day with short hatching durations.

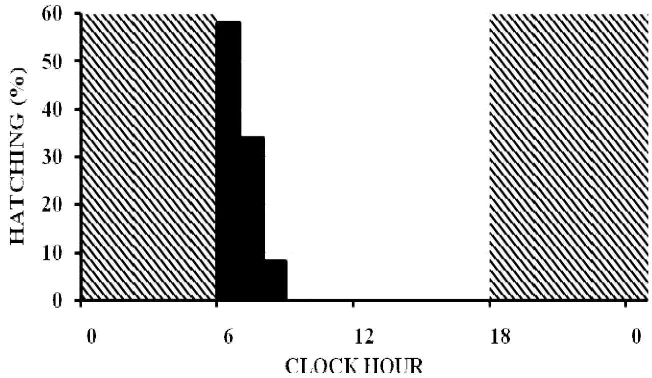


Figure 4: Distribution of hatching in CSR2 x CSR4 of *B. mori* under Black-Boxing conditions. Note hatching for a single day with sharp hatching peak and only 3 hours of hatching duration.

**Hatching parameters in CSR2 x CSR4:** Hatching parameters such as hatching duration, hatching magnitude on different days, total hatching percentage, brushing percentage, late/early born eggs percentage, unfertilized eggs percentage and dead eggs, which are crucial indicators to denote economical hatching, were studied in *B. mori* under the above four photoperiodic conditions.

***Hatching duration under different photoperiodic conditions*:** Under all the photoperiodic (LD 12 : 12, DD, LL and black-box) conditions, the hatching duration (period) was calculated and data presented in Table 1. Table 1: Data on hatching duration (hours) in CSR2 x CSR4 of *B. mor*i under different photoperiodic conditions. Values are mean of 5 replications ± SD, n = 5.

| S. No. | Total hatching (%) | | | |
|---|---|---|---|---|
| | LD 12 : 12 | DD | LL | Black-Box |
| 1 | 26.200± 0.837 | 28.200 ± 0.837 | 32.400 ± 2.074 | 32.400 ± 2.074 |

From the data, it is seen that under three photoperiodic conditions, LD 12 : 12, DD and LL, the hatching duration was more than 26 hours (for LD 12 : 12, it was 26.200 ± 0.837, for DD, it was 28.200 ± 0.837 and for LL, hatching duration was 32.400 ± 2.074). Interestingly, the hatching duration was below 3 hours (32.400 ± 2.074) with black-box photoperiodic condition.

Comparison of hatching duration explained the uniqueness of black-box system in CSR2 x CSR4 silkworm hybrid hatching. Data on hatching durations under various photoperiodic conditions such as LD 12 :12, DD, LL and black-box system are also presented in Figure 5. Hatching durations were more than 26 hours for LD 12 :, DD and LL while it was below 3 hours.

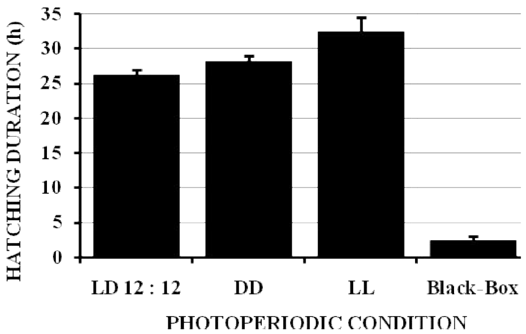


Figure 5: Hatching duration in CSR2 x CSR4 of *B. mori* under different photoperiodic conditions. Hatching duration was below 3 hours for black-box system of incubation.

***Hatching magnitude on different days*****:** In all the photoperiodic, except black-box method, hatching in CSR2 x CSR4 of *Bombyx* silkworm occurred for two continuous days. The data on hatching magnitude on different days in four photoperiodic conditions are reproduced in Table 2.

Table 2: Hatching magnitude in CSR2 x CSR4 of *Bombyx mori* in different days under four photoperiodic conditions; LD 12: 12, DD, LL and Black-Box conditions. Note hatching occurred for two consecutive days under LD 12 : 12, DD and LL conditions while it occurred for only one day. (n = 5).

| S. No. | Photoperiodic condition | Hatching magnitude(%) on | | Significance |
|---|---|---|---|---|
| | | Day 1 | Day 2 | |
| 1. | LD 12 : 12 | 4.600 ± 2.408 | 95.400 ± 2.408 | Highly significant |
| 2. | DD | 68.800 ± 8.136 | 31.200 ± 8.136 | Highly significant |
| 3. | LL | 11.600 ± 1.673 | 88.400 ± 1.673 | Highly significant |
| 4. | Black-Box | 99.400 ± 0.894 | 0.6000 ± 0.894 | Highly significant |

***Hatching magnitude on different days under LD 12 : 12 in CSR2 x CSR4*****:** Hatching quantity (magnitude) of hatching under natural day (LD 12 : 12) condition in the silkworm, *Bombyx mori* hybrid, CSR2 x CSR4 is presented in Figure 6.

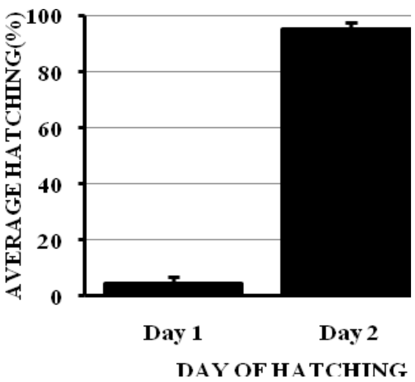


Figure 6: Hatching magnitude in CSR2 x CSR4 of *Bombyx mori* in different days under natural day condition, LD 12 : 12. Note high hatching on day 2 while the same on day 1 was a stray one. (n = 5).

As seen from the graph, hatching occurred for two consecutive days, day 1 and day 2. However, hatching magnitude was very low on day 1 (4.600 ± 2.408). It was very heavy (95.400 ± 2.408) on day 2. The differences in hatching magnitude between two days were statistically highly significant ($p < 0.01$). It is to be stressed that the hatching on day 2 only to be considered for experimental or commercial silkworm rearing leaving the hatching on day 1 as stray hatching.

***Hatching magnitude on different days under continuous dark (DD) in CSR2 x CSR4*****:** Hatching magnitude of CSR2 x CSR4 in different days under continuous dark (DD) is furnished in Figure 7. It is interesting that the hatching occurred for two consecutive days, with more hatching magnitude (68.800 ± 8.136) on day 1 and less (31.200 ± 8.136) on day 2. The differences in hatching magnitude was statistically highly significant ($p < 0.01$, n = 5). Important point to be noted is that the hatching on day 1 under LD 12 : 12 condition was low and under DD, the same was high, indicating the short day dependence of *Bombyx* silkworm.

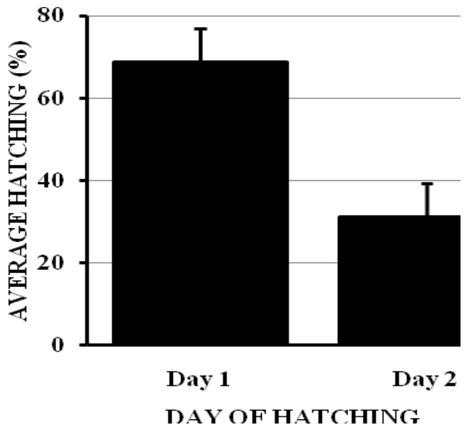


Figure 7: Hatching magnitude in CSR2 x CSR4 of *Bombyx mori* in different days under continuous dark (DD) condition. Note high hatching on day 1 while the same on day 2 was a low. The differences in hatching magnitude between two days are highly significant ($p < 0.01$, n = 5).

***Hatching magnitude on different days under continuous dark (DD) in CSR2 x CSR4*****:** The trends in expression of hatching magnitude in CSR2 x CSR4 under continuous light (LL) condition (Figure 8) followed that under LD 12: 12. Thus, hatching on day 1 (11.600 ± 1.673) was less while the same on day 2 (88.400 ± 1.673) was more. In the case of hatching under LL also, the differences between two days was statistically highly significant at 1% level (n = 5).

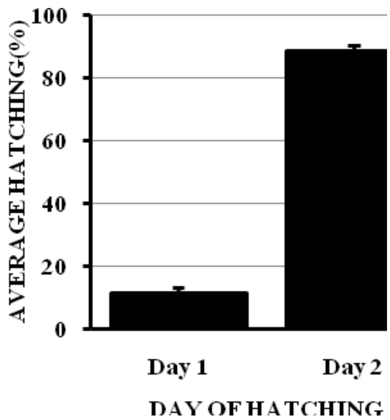


Figure 8: Hatching magnitude in CSR2 x CSR4 of *Bombyx mori* in different days under continuous light (LL) condition. Note low hatching on day 1 while the same on day 2 was a high one. The differences in hatching magnitude between two days are highly significant ($p < 0.01$, n = 5).

***Hatching magnitude on different days under black-box condition in CSR2 x CSR4*:** Data on hatching magnitude in CSR2 x CSR4 under black-boxing method is depicted in Figure 9). It can be observed that the trend in hatching magnitude was unique under black-box condition.

Thus, hatching on day 1 (99.400 ± 0.894) was more while the same on day 2 (0.6000 ± 0.894) was almost nil. The differences between two days were statistically highly significant at 1% level (n = 5). As against to the other photoperiodic conditions (LD 12 : 12, DD and LL) entire hatching was confined to day 1 only, indicating its best suited to black-boxing condition.

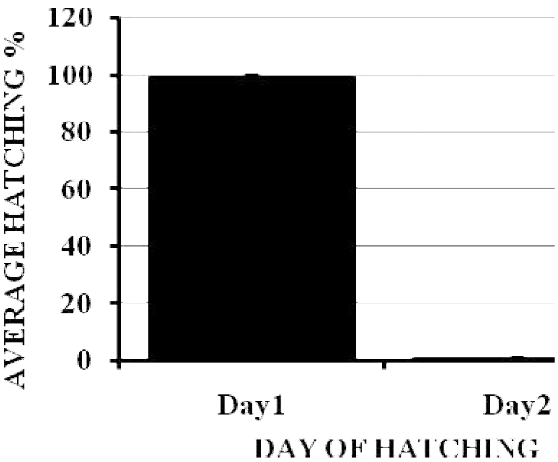


Figure 9: Hatching magnitude in CSR2 x CSR4 of *Bombyx mori* in different days under black-box condition. Note hatching only on day 1.

***Total Hatching in CSR2 x CSR4 under different photoperiodic condition*:** Hatching of *B. mori eggs* (CSR2 x CSR4) continued to occur for two consecutive days under all photoperiodic conditions, except under black-box condition. Recorded total hatching was converted into percentage and the data is furnished in Table 3.

**Table 3:** Data on total hatching (%) in CSR2 x CSR4 of *B. mori* under different photoperiodic conditions. Values are mean of 5 replications ± SD, n = 5.

| S. No. | Total hatching (%) | | | |
|---|---|---|---|---|
| | LD 12 : 12 | DD | LL | Black-Box |
| 1 | 94.800 ±1.924 | 96.800 ± 1.483 | 94.000 ± 2.915 | 98.400 ± 2.074 |

The data on total hatching (%) in CSR2 x CSR4 under different photoperiodic conditions studied are presented in graph (Figure 10.). From the graph, it is evident that total hatching percentage is very low in CSR2 x CSR4 under continuous light (LL) condition (94.000 ± 2.915), while the highest total hatching percentage was recorded with black-box condition (98.400 ± 2.074).

The next highest total hatching percentage observed was with DD (96.800 ± 1.483) followed by LD 12 : 12 (94.800 ±1.924). The differences between the total hatching (%) under black-box condition and LL was highly significant ($p < 0.01$).

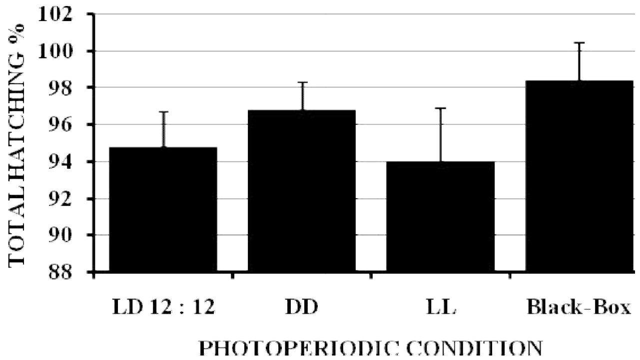


Figure 10: Total hatching (%) in CSR2 x CSR4 under different photoperiodic conditions studied. Note highest total hatching percentage with black-box condition followed by DD and LD 12: 12 photoperiodic conditions. Least total hatching percentage was observed under LL photoperiodic condition.

***Early/late born larval percentage in CSR2 x CSR4 under different photoperiodic condition*:** As all the photoperiodic conditions, except black-box system resulted in two consecutive days hatching. With LD 12 :12 and LL conditions, the early (day 1) hatching was very meager while under DD condition, late born larvae (day 2) are considerable high. Such early/late born larval percentage was derived from recorded hatching data and presented in Table 4.

Table 4: Data on early/late born larval percentage in CSR2 x CSR4 of *B. mori* under different photoperiodic conditions. Values are mean of 5 replications ± SD, n = 5.

| S. No. | Early/late born larvae (%) | | | |
|---|---|---|---|---|
| | LD 12 : 12 | DD | LL | Black-Box |
| 1 | 4.600 ± 2.408 | 31.200± 8.136 | 11.600 ±1.673 | 1.080 ± 1.600 |

From Table 4, it is reflectively observed that late born larval percentage with continuous dark (DD) condition are very high (31.200± 8.136) while the other conditions resulted considerably low early born larval percentage. With black-box system, early/late born larval percentage is almost zero. The observations are statistically highly significant. The above data on early/late born larval percentage is also depicted as graph (Figure 11). Evidently, early/late born larval percentage was very high under DD condition followed by LL and LD 12: 12 conditions. With black-box method, the early/late born larval percentage was very negligible.

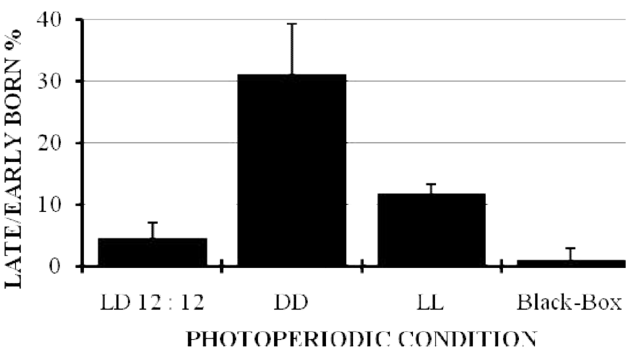


Figure 11: Early/late born larval percentage in CSR2 x CSR4 under different photoperiodic conditions studied. Note highest late born larval percentage with DD condition followed by LL and LD 12:12 photoperiodic conditions. With black-box condition it was almost zero.

***Brushing percentage in CSR2 x CSR4 under different photoperiodic condition*:** Brushing percentage is different from total hatching percentage. Brushing percentage is calculated based on actual brushed larvae from total hatched larvae. Brushing percentage excludes the percentage of early or late born larvae that are not considered for their continuing in the further commercial rearing. Such brushing percentage data of CSR2 x CSR4 under different photoperiodic (LD 12 : 12, DD, LL and black-box) conditions are presented in Table 5.

Table 5: Data on total brushing (%) in CSR2 x CSR4 of *B. mori* under different photoperiodic conditions. Values are mean of 5 replications ± SD, n = 5.

| S. No. | Brushing (%) | | | |
|---|---|---|---|---|
| | LD 12 : 12 | DD | LL | Black-Box |
| 1 | 95.400 ±2.408 | 68.800 ± 8.136 | 88.400 ± 1.673 | 97.800 ± 3.899 |

From the Table3, highest brushing percentage can be realized for CSR2 x CSR4 kept under black-box method of incubation. The data on brushing under black-box method was followed by LD 12 :12, LL and DD photoperiodic conditions. The low brushing percentage under DD photoperiodic condition is due to lot of late hatching on day 2. Differences in brushing percentages are statistically highly significant. Data on brushing percentage in CSR2 x CSR4 of *Bombyx mori* are also depicted as graph (Figure 12) for exact understanding of trend in brushing percentage under different photoperiodic conditions. With black-box condition, brushing percentage was highest than that recorded with other photoperiodic conditions (LD 12 : 12, DD and LL). Surprisingly brushing percentage in CSR2 x CSR4 was very low under DD conditions. The simple reason is that the late born larvae under DD are more and hence eliminated while calculating brushing percentage.

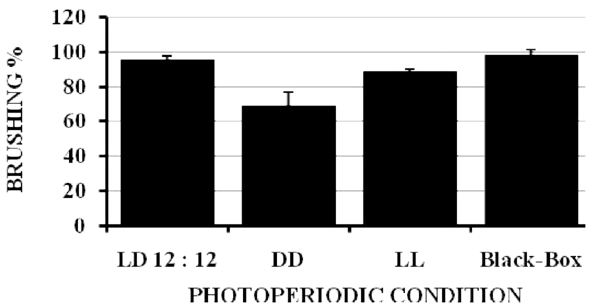


Figure12: Brushing (%) in CSR2 x CSR4 under different photoperiodic conditions studied. Note highest brushing percentage with black-box condition followed by LD 12 : 12, LL and DDphotoperiodic conditions. Least brushing percentage was observed under DD photoperiodic condition.

***Unfertilized eggs percentage in CSR2 x CSR4 under different photoperiodic condition*:** It is interesting to study the number and further the percentage of unfertilized eggs in CSR2 x CSR4 under different photoperiodic conditions. Data recorded for unfertilized eggs and consequently treated for percentage are given in Table 6. The unfertilized eggs percentage was very low, ranging from 1.6% (black box method) to 2.2% (LD 12 : 12). Thus, unfertilized eggs percentage seems not to be affected by imposed photoperiodic conditions, as they occur at the time of egg preparation itself. The differences in unfertilized egg percentage are not statistically significant.

Table 6: Data on unfertilized eggs (%) in CSR2 x CSR4 of *B. mori* under different photoperiodic conditions. Values are mean of 5 replications ± SD, n = 5.

| S. No. | Unfertilized eggs (%) | | | |
|---|---|---|---|---|
| | LD 12 : 12 | DD | LL | Black-Box |
| 1 | 2.200 ± 1.304 | 1.800 ± 0.837 | 1.800 ± 0.837 | 1.600 ± 0.894 |

Data on unfertilized eggs (%) in CSR2 x CSR4 of B. mori under different photoperiodic conditions (Figure 13) also reflects that the unfertilized eggs under black-box system are very low.

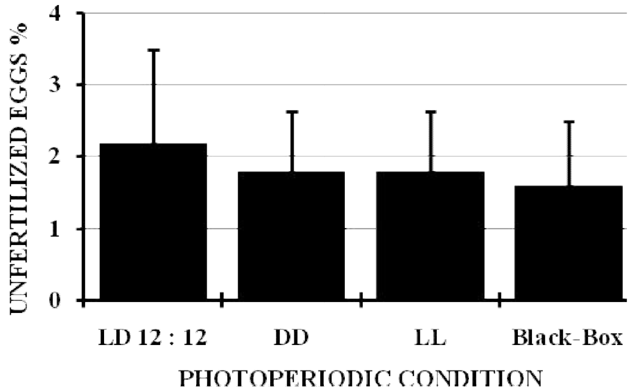


Figure 13: Unfertilized eggs percentage in CSR2 x CSR4 under different photoperiodic conditions studied. Note non-significant differences in unfertilized egg percentage.

***Dead eggs percentage in CSR2 x CSR4 under different photoperiodic condition*:** While the data on unfertilized eggs percentage registered on-par with trend among photoperiodic conditions studied in CSR2 x CSR4, the recorded data on appearance of dead eggs (%) implicated that it is definitely affected by photoperiodic conditions studied.

Data on appearance of dead eggs in CSR2 x CSR4 under photoperiodic conditions studied are presented in Table 7. The percentage of dead eggs was highest under LL (3.200 ± 0.837) condition followed by LD 12 : 12 (1.800 ± 1.483) condition and DD (0.800 ± 0.839). Dead eggs percentage was very low (0.600 ± 0.548) in CSR2 x CSR4 incubated under black-box system.

Table 7: Data on dead eggs (%) in CSR2 x CSR4 of *B. mori* under different photoperiodic conditions. Values are mean of 5 replications ± SD, n = 5.

| S. No. | Unfertilized eggs (%) | | | |
|---|---|---|---|---|
| | LD 12 : 12 | DD | LL | Black-Box |
| 1 | 1.800 ± 1.483 | 0.800 ± 0.839 | 3.200 ± 0.837 | 0.600 ± 0.548 |

The trends in appearance of dead eggs in CSR2 x CSR4 under studied photoperiodic conditions are still better seen when data is graphed (Figure 14). It is clear that dead eggs percentage is very high under LL condition, followed by LD 12 : 12 and DD photoperiodic conditions. The least dead eggs percentage is seen with black-box system of egg incubation

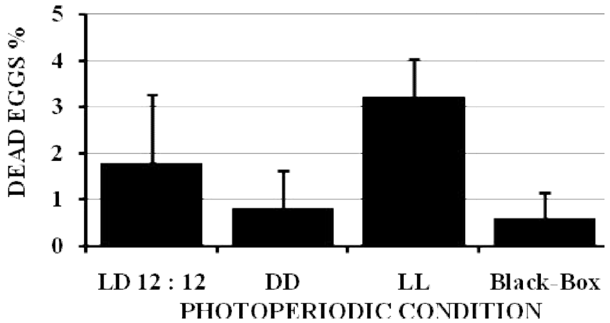


Figure 14: Dead eggs percentage in CSR2 x CSR4 under different photoperiodic conditions studied. Note non-significant differences in unfertilized egg percentage.

## DISCUSSION

Rhythmicity in egg hatching in *B. mori* received considerably good attention, because the insect is economically important as it is reared by large number of sericulture farmers in large scale throughout the year in various sericultural belts of India. The rhythmic hatching patterns were first initiated with Mysore race (Pure Mysore, PM) strain of *Bombyx* silkworm (Anantha Narayana, 1980; Anantha Narayana *et al*., 1978) in the mid-part of Indian Sericulture era. Later, Sivarami Reddy (1993) and Sivarami Reddy and Sasira Babu (1990) expanded these studies to silkworm hybrids with PM x NB4D2. At present, the Indian mulberry sericulture is mainly ruled bytwo silkworm hybrids, PM x CSR2 (multivoltine x bivoltine hybrid) and CSR2 x CSR4 (bivoltine x bivoltine hybrid). A study on these hybrids, thus, was felt important, which is completely lacking. Rhythmic patterns in hatching, as affected by photoperiods in silkworm were reported (Yamaoka and Hirao, 1975; Yamaoka *et al*., 1976; AnanthaNarayana *et al*., 1978; Sivarami Reddy *et al*., 1984; SivaramiReddy, 1993; Sivarami Reddy and Sasira Babu, 1990, Sivarami Reddy *et al*., 1998). Occurrence of hatching at or after lights-on phase under LD 12 : 12 condition for silkworm hybrid, CSR2 x CSR4 (Fig. I. 1) and its persistence under continuous dark (DD; Fig. I. 2) and light (LL; Fig. I. 3) conditions strongly suggest diurnal nature of the hatching rhythmicity, supported by earlier reports (Anantha Narayana *et al*., 1978; Sivarami Reddy *et al*.,1984; Sivarami Reddy, 1993; Sivarami Reddy and SasiraBabu, 1990, Sivarami Reddy *et al*., 1998; Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021). Hatching was confined for a single day under LD 12: 12 conditions in PM x NB4D2 (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Baby, 1989; Sivarami Reddy et al., 1998).

The identical reports on the occurrence of single day hatching were also reported for PM x CSR2 (Shanthan Babu, 2014; Srinath, 2014, Suvarna, 2021; Suvarna *et al*, 2015) suggests that in multivoltine x bivoltine silkworm hybrids alone. However, publications on bivoltine x bivoltine (CSR2 x CSR4) added a new concept that under LD 12 : 12 conditions, egg hatching rhythmicity occurred for two consecutive days (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021) reflects the appearance of mixed-age characteristics. Occurrence of magnitude of egg hatching was less in CSR2 x CSR4 under LD 12 : 12 conditions (Figure 1) on day 1 and more on day 2. In the present communication also such mixed-age prosperities are more visible, as supported by the other publications (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021). Under continuous light (LL), hatching on the day 1 was more (Figure 3) and that on day 2 was more under DD (Figure 2). With the black-box system, silkworm egg (Figure 4), however, entire hatching and its rhythmicity was restricted to a single day alone, with disappearance of hatching on day 2 and thus avoiding expression of mixed age characteristic hatching. Such observations were supported by earlier papers (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021). Hatching phase advancement under DD (Figure 2) and its delay under LL (Figure 3) for CSR2 x CSR4 silkworm hybrid indicate the free running nature of the hatching rhythmicity (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu,1990; Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021). The occurrence of hatching for two consecutive days indicated the periodicity of hatching rhythm to be circadian (Anantha Narayana *et al*., 1978; Sivarami Reddy *et al*., 1984; Sivarami Reddy, 1993; Sivarami Reddyand Sasira Babu, 1990, Sivarami Reddy *et al*., 1998).

With imposed photoperiodic conditions such as LD 12 : 12, DD and LL, the silkworm hybrid, CSR2 x CSR4 recorded more than 26 h of hatching duration (Table 1, Figures 5 to 7. The observed long hatching durations include two hatching peaks (gates) separated by non-hatching time (forbidden zone).Thus, more than 26 hours of hatching durations were justified with two consecutive days of hatching under LD 12 : 12, DD and LL. Hatching duration under black-box system was surprisingly below 3 hours, confined to a single day and hence, one can expect such low hatching durations as supported by Shanthan Babu (2014), Srinath (2014) Suvarna *et al*. (2015) and Suvarna (2021). Less hatching on day 1 and more hatching on day 2 were supported by Shanthan Babu (2014), Srinath (2014) Suvarna *et al*. (2015) and Suvarna (2021). Under DD conditions, however, more hatching was recorded on day 1 than on day 2. Interestingly, hatching magnitude was almost nil under black-box system. Based on the observed data on hatching magnitude under studied photoperiodic conditions, the total hatching percentage was very high with black-boxing system followed by DD, LD 12 : 12 and LL (Figure 10). The brushing percentage is entirely different to that of total hatching percentage. Only high hatching on high hatching day is considered as brushing percentage, for consequent consideration of silkworm rearing, eliminating less hatching on the opposite day, rejecting them for continuation. Therefore, brushing percentage under black-boxing system is the highest (Figure 12) and that for DD is very low as the hatching on day 2 was considerably high (31%, Table 5, Figure 12). Therefore, it is justified that brushing percentage is very low with DD (69%) eliminating day 2 hatching percentage (31%). With black-box system, the brushing percentage was highest (98%). Data pertaining to early/late born larval percentage in CSR2 x CSR4 under examined photoperiodic conditions (Table 4 and Figure 11) also confirm the observations on brushing percentage. Considering the data on unfertilized egg percentage (Table 6 and Figure 13), no statistical differences were observed between photoperiodic conditions, and hence, it (unfertilized egg percentage) is deemed as Grainage (silkworm egg laying) operation character than the attribute of photoperiodic conditions exposed. However, the data on percentage of dead eggs (Table 7 and Figure 14) indicated that photoperiod has definitely impact on dead eggs under incubation. Thus, dead eggs percentage was alarmingly high in the eggs of CSR2 x CSR4 under LL condition, as the LL condition implicated in low hatching, continued for 2 days and broadened hatching activity. Therefore, LL is felt influencing increasing dead eggs percentage. Dead eggs percentage was very negligible under black-box condition. Thus, the rhythmic patterns in the hatching of *Bombyx mori* are confirmed in the present study and the egg hatching economic characteristics are reported first time. While the three photoperiodic conditions, LD 12 : 12, DD and LL have implicated towards less economic level the black-box system solely upheld them to the maximization levels.

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