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Studies on Egg Hatching in Bombyx Mori L., on Industrial Perspectives

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ABSTRACT

Bombyx mori L., as in other insects has four stages in its life cycle; egg, larva, pupa and moth. In the entire life cycle of silkworm, the active eating period is larval stage. The active eating stage of insects starts from egg hatching. The complex behaviour of egg hatching is the initial marker in the life an insect. Thus, egg hatching in silkworm is the most crucial step in its life cycle, because the larvae are freed from the constraints of life in tiny eggs through egg hatching so that they are ready for furthering their biological destiny outside world. Among several developmental events in B. mori, hatching has been referred to as the first developmental marker event in the life cycle of mulberry silkworm, Bombyx mori and a decisive aspect in commercial silkworm rearing. It is well established that the daily rhythm in hatching in many insects is a gating event, controlled by circadian system. As in the other insects, hatching, in Bombyx mori too is a controlled by a circadian oscillator. It is also reviewed that the influence of external signals which regulates insect egg hatching and opined that light and/or temperature cycles are major ones. When the B. mori I continued in constant temperature and relative humidity, hatching has been accounted for its dependency on the voltinism for certain economic aspects such as hatching duration, hatching magnitude etc. However, the essential aspects of hatching rhythmicity did not show any difference. Comparative studies of on the first developmental event between two contemporary commercial silkworm hybrids, multivoltine x bivoltine hybrid (PM x CSR2) and bivoltine x bivoltine hybrid (CSR2 x CSR4), such as expression of hatching (%) on day to day basis, hatching durations on day to day basis under all major possible photoperiodic regimes, LD 12 : 12, DD and LL are scanty and such important information from the basic input for commercial exploitation of these silkworm hybrids. Thus, studies on hatching rhythmicity and other related economic aspects in two contemporary popular mulberry silkworm hybrids, PM x CSR2 and CSR2 x CSR4 are reported in the present chapter under three photoperiodic schedules viz., LD 12 : 12 (normal day), DD (continuous dark) and LL (continuous light) situations, keeping the temperature and humidity conditions constant. All the above review/introductions are of mere academic value. The silkworm rearing is a commercial based avocation for the silkworm grower. The application of scientific knowledge on hatching in silkworm is aimed development or re-examining of already developed technologies.

Keywords: Bombyx Mori L., Egg Hatching, Circadian System, Voltinism

Introduction:

In the entire life cycle of silkworm, the active eating period is larval stage, starting from egg hatching. The complex behaviour of egg hatching is the initial marker event in the life an insect (Saunders, 2002). Thus, egg hatching in silkworm is the most crucial step in its life cycle, because the larvae are freed from the constraints of life in tiny eggs through egg hatching so that they are ready for furthering their biological destiny outside world. Among several developmental marker events in B. mori, hatching has been referred to as the first developmental marker event in the life cycle of mulberry silkworm, Bombyx mori (Shanthan Babu, 2014; Srinath, 2014) and a decisive stage in commercial silkworm rearing (Rajan et al., 1996; Kawakami and Yanagawa, 2003; Kanika Trivedi, 2015;). It is well established that the daily rhythm in hatching in many insects is a gating event, controlled by circadian system (Beck, 1980; Saunders, 2002). As in the other insects, hatching, in Bombyx mori too is a controlled by a circadian oscillator (Anantha Narayana, 1980; Ananta Narayana et al., 1978; Sivarami Reddy and Sasira Babu, 1990; Sivarami Reddy et al., 1984; Sivarami Reddy et al., 1998; Narasimhulu et al., 2020; Suvarna et al., 2020). Saunders (2002) reviewed the influence of external signals that regulates insect egg hatching and opined that light and/or temperature cycles are major ones. When the B. mori L continued in constant temperature and relative humidity, hatching has been accounted for its dependency on the voltinism for certain economic aspects such as hatching duration, hatching magnitude (Shanthan Babu, 2014; Srinath, 2014; Suvarna et al., 2015). However, the essential aspects of hatching rhythmicity did not show any difference. Comparative studies of on the first developmental event between two contemporary commercial silkworm hybrids, multivoltine x bivoltine hybrid (PM x CSR2) and bivoltine x bivoltine hybrid (CSR2 x CSR4), such as expression of hatching (%) on day to day basis, hatching durations on day to day basis under all major possible photoperiodic regimes, LD 12 : 12, DD and LL are reported by Suvarna et al. (2015). The silkworm rearing is a commercial based avocation for the silkworm grower. The application of scientific knowledge on hatching in silkworm is aimed development or re-examining of already developed technologies.

Materials methods:

Two mulberry silkworm (B. mori) hybrids; PM x CSR2 (multivoltine x bivoltine hybrid) and CSR2 x CSR4 (bivoltine x bivoltine hybrid) that are well accepted and popularly exploited for commercial silkworm rearing in the contemporary Indian sericulture are selected.

Procurement of experimental material: Disease free layings (DFLs, commonly called) of two silkworm hybrids, PM x CSR2, a multivoltine x bivoltine hybrid and CSR2 x CSR4, a bivoltine x bivoltine hybrid were procured on the third day of oviposition, from the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Madanapalli, Chittoor District, Andhra Pradesh. The DFLs were transported to the Department of Sericulture, Sri Krishnadevaraya University, Anantapuramu; where the investigations were carried out. The DFLs were transported from procurement SSPC to the work spot during evening cool hours. The DFLs were immediately spread into the pre disinfected rearing trays.

Except hatching in PM x CSR2 under LD 12 : 12 condition, hatching with the other conditions for the silkworm hybrids, PM x CSR2 and CSR2 x CSR4 are reported to occurred for two consecutive days (Suvarna et al., 2015). Hatching for more than two consecutive days, was also reported for PM x NB4D2 (Sivarami Reddy and Sasira Babu,1990; Sivarami Reddy, 1993), but under extreme photoperiodic conditions. These results are of academic importance only and need to be considered in technology perspectives, finding specific points to help in improving the hatching activity to best suit to the requirements of sericulture farmers. Kanika Trivedi (2015) and Kawakami and Yanagawa (2003) viewed that egg hatching in silkworm should be confined to a single day with economic hatching (over 95%). Kawakami and Yanagawa (2003) have mostly pointed out the efforts towards development of bivoltine sericulture in India, indicating that the bivoltine sericulture needs more technologies. Regarding the multivoltine x bivoltine hybrid, PM x CSR2, no other technology(ies) may not be necessary (Suvarna et al., 2015) as the hybrid confined to hatch on a single day with shortest durations. On the contrary, the bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 resulted in two days hatching under LD 12 : 12 conditions (Suvarna et al., 2015) with less hatching durations. Observations on this aspect of hatching magnitude simply comparable in CSR2 x CSR4, but extended to occur for two consecutive days necessitated to probe further into development of a new technology(ies) are simply re-examining the already existing technology.

B. METHODS:

Temperature conditions: Temperature maintained in the laboratory was 25 ± 1 ºC throughout the experimentation as the temperature was reported to be ideal (Krishnaswami, 1986).

Humidity conditions: Relative humidity (RH) of 80 ± 5% was maintained in the laboratory throughout the experimentation.

Photoperiodic regimens: The same day when the DFLs were brought to the laboratory, they were introduced into light-dark schedules in two batches. Two batches for each hybrid were maintained. The photoperiodic schedules imposed were:

a. Natural solar day – LD 12:12: The 24 h natural solar day was divided into 12 h dark part (scotophase) and 12 h light part (photophase). The photophase was initiated from 06.00 h and lasted at 18.00 h local time. Similarly, the scotophase was imposed from 18.00 h and continued up to 06.00 h local time. A 60 W bulb, as light source for illuminating the experimental animals during photophase of rearing period was arranged above the rearing tray, its height from the rearing tray was so monitored that the light intensity at the surface where the experimental animals were exposed did not exceed 50 lux.

b. Continuous conditions:

i. Constant light (LL) condition: For illuminating the animals throughout the day (24 h) under constant conditions (around 50 lux) a 50 W bulb as light source was arranged.

ii. Constant dark (DD) condition: The experimental larvae were provided continuous dark. The animals were kept in dark room. However, a dim red light source (below 0.1 lux) was utilized for handling the animals and recording observations whenever needed.

PARAMETERS STUDIED:

Overt phenomena:

Hatching: For studying the first developmental marker event in the life cycle of the silkworm, the hatching patterns in the silkworm, Bombyx mori, five DFLs of PM x CSR2 and CSR2 x CSR4 were kept separately under each photoperiodic condition (LD 12:12, DD and LL) at the temperature and RH conditions described earlier. The silkworm hatch from their eggs, generally, after 10 days of oviposition. The eggs will turn into blue on or after 8^{th} day (blue egg stage, Narasimhanna, 1987; Datta, 1992); the silkworm larvae hatch from the eggs two days from this stage (Datta, 1992). Rhythmic patterns in the hatching of the silkworm, Bombyx mori were studied under the above photoperiodic schedules. Precise number of larvae that hatched out from eggs was counted. The counting was done at one hour interval and recorded. The larvae, thus counted were gently transferred into the rearing trays with a feather brush for further studies. Data on hatching thus recorded were represented as distribution diagrams (hourly histograms, resolved for 24 h, $\omega = 360^{\circ}$).

RESULTS:

Hatching in multivoltine x bivoltine hybrid, PM x CSR2 was mainly diurnal, occurring at the beginning of the photophase under natural LD cycle (LD 12:12; Figure I. 1). Hatching started at 0600 h and completed by 0900 h. Notably, hatching confined to a single day. Thus, hatching was very sharp, with peak of activity at 0700 h. In the case of the bivoltine x bivoltine hybrid, CSR2 x CSR4 also, hatching was diurnal, occurring at the initiation of photophase (Figure I. 2. 2). However, hatching in CSR2 x CSR4 occurred for 2 consecutive days, with less (stray) hatching on day 1 and maximum on day 2.

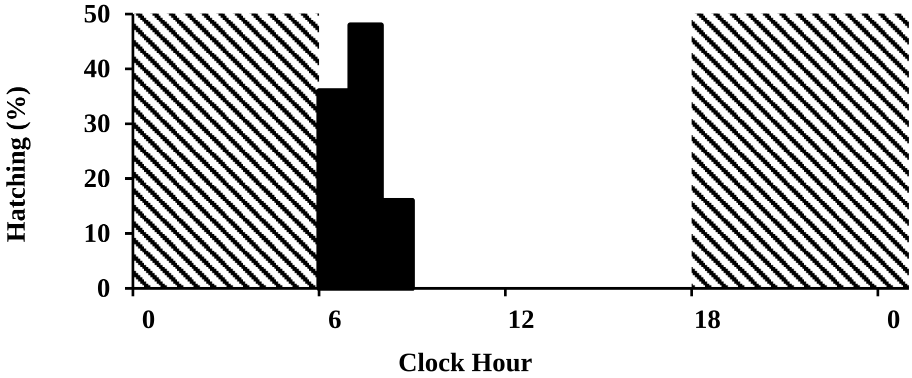

Figure I. 1: Chronogram representing distribution of hatching (%) in PM x CSR2 of Bombyx mori L. under LD 12 : 12 conditions. Note entire hatching on a single day. Also note that the hatching occurred just after 'lights-on' phase of the LD cycle. Cross-hatched area in the histogram indicates the dark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

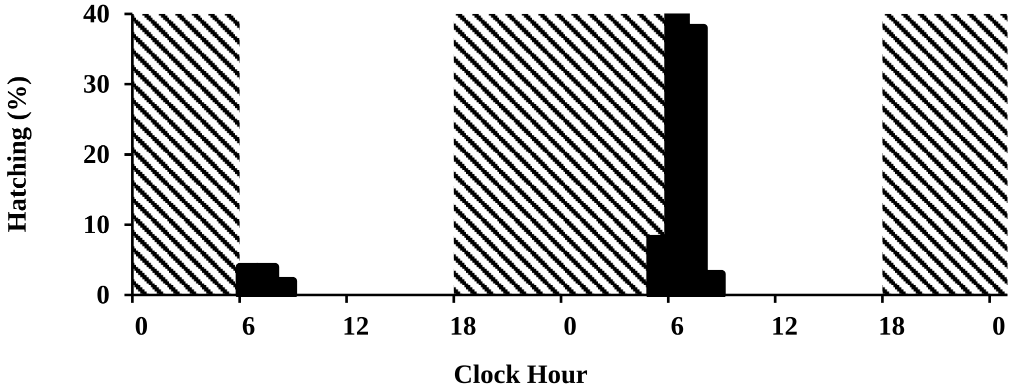

Figure I. 2: Chronogram representing distribution of hatching (%) in CSR2 x CSR4 of Bombyx mori L. under LD 12:12 conditions. Note stray hatching on the first day and maximum hatching on the second day (gating-phenomenon). Also note that the hatching occurred just after 'lights-on' phase of the LD cycle.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

Under continuous dark (DD), the hatching was observed for two consecutive days both for PM x CSR2 and CSR2 x CSR4, the hatching rhythm being almost identical (Figure I. 3 and I. 4). Interestingly, hatching occurred at or before 0600 h, indicating advancing of hatching phase. More hatching was recorded on day, 1 both for PM x CSR2 and CSR2 x CSR4 hybrids and less on day 2. The distance between two consecutive peaks of hatching (day 1 and day 2) was ≈ 24 h, indicating circadian nature and gating phenomenon in hatching rhythmicity of silkworm hybrids under DD condition. Further, hatching peak was sharp and restricted to limited duration each day of day 1 and day 2. Thus, the hatching was predominantly diurnal, occurring for two consecutive days, circadian, free-ran and expressed gating phenomenon.

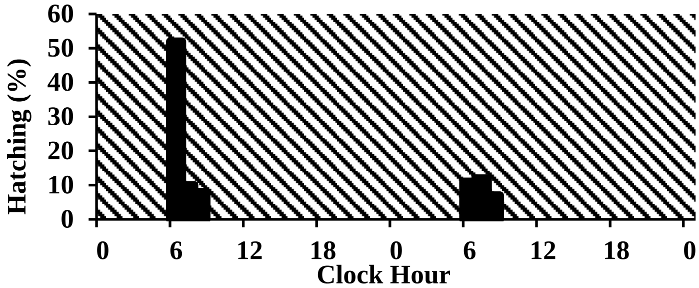

Figure I. 3: Chronogram representing distribution of hatching (%) in PM x CSR2 of Bombyx mori L. under continuous dark (DD) condition. Note, hatching occurring for two days (two gates) with more hatching on the first day. Also note that the hatching occurred at or before 06.00 h of local time.

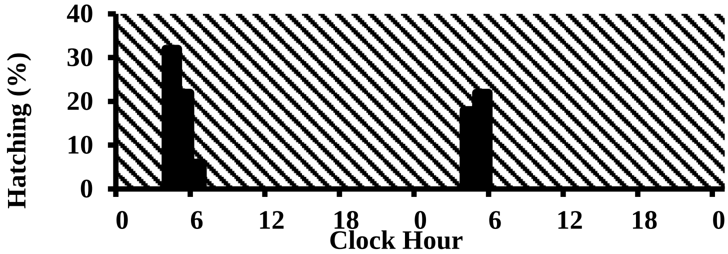

Figure I. 4: Chronogram representing distribution of hatching (%) in CSR2 x CSR4 of Bombyx mori L. under continuous dark (DD) condition. Note hatching for two consecutive days (gates) with more hatching on the first day. Also, hatching durations were narrowed. The hatching occurred before 06.00 h of the local time and therefore occurrence of hatching advanced. Hatching activity (peak) is very sharp.

Hatching under continuous light (LL) also occurred for two consecutive days both in PM x CSR2 and CSR2 xCSR4 hybrids (Figure I. 5 and I. 6). Opposing the hatching trends under DD condition, less hatching was recorded on day 1under LL and more on day 2. The distance between peak hatching on day 1 to that on day 2 was also ≈ 24 h, indicating circadian nature and gating phenomenon in hatching of both PM x CSR2 and CSR2 x CSR4. The hatching peaks in PM x CSR2 under LL conditions were sharp (Figure I. 5) on day 1 and 2. However, peak hatching expression broadened for CSR2 x CSR4 (Figure I 6), indicating a trend leading to arrhythmicity.

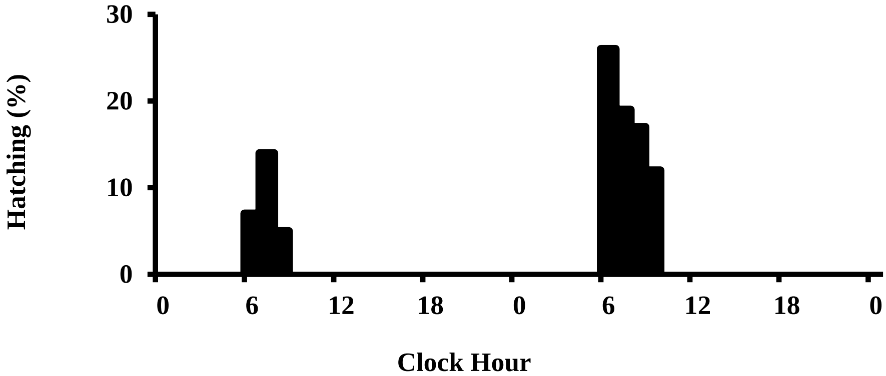

Figure I. 5: Chronogram representing distribution of hatching (%) in PM x CSR2 of Bombyx mori L. under continuous light (LL) condition. Note hatching occurring for two days, with more hatching on the second day. Also note that the hatching occurred after 06.00 h of local time.

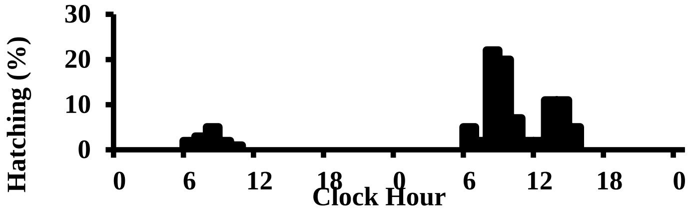

Figure I. 6: Chronogram representing distribution of hatching (%) in CSR2 x CSR4 of Bombyx mori L. under continuous light (LL) condition. Note hatching occurring for two days, with more hatching on the second day. Also note that the hatching occurred after 06.00 h of local time indicating a delay and hatching activity broadened.

Hatching occurred for one day for PM x CSR2 under LD 12 : 12 condition only and it was observed for two consecutive days under both DD and LL conditions. On the other hand, hatching was observed for 2 consecutive days for all the conditions (LD 12 : 12, DD and LL) for CSR2 x CSR4. Day to day average for hatching in PM x CSR2 under the imposed photoperiodic conditions (LD 12 : 12, DD and LL) is depicted in Figure I. 7. Thus, hatching in PM x CSR2 under LD 12 : 12 condition is restricted to day 1 only, with no or negligible hatching on day 2 (Figure I. 7a). With the other 2 photoperiodic conditions (DD and LL), hatching in PM x CSR2 occurred for two consecutive days. However, hatching on day 1 was more compared to that on day 2 (Figure I. 7b) under DD condition. Opposing this, hatching was more on day 2 under LL condition for PM x CSR2 (Figure I. 7c). Average hatching in CSR2 x CSR4 under three imposed photoperiodic conditions (LD 12 : 12, DD and LL; Figure I. 8) also indicated two consecutive days hatching. However, hatching was less on day 1 under LD 12 : 12 and LL (**Figure I. 8a and c) whereas it was more on day 1 under DD (Figure I. 8b)**

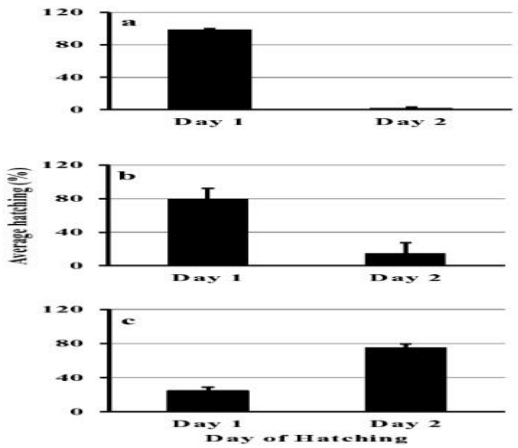

Figure I. 7: Average hatching (%) (± SD; n = 5) in the popular multivoltine x bivoltine silkworm hybrid, PM x CSR2 on day 1 and day 2 of hatching kept under natural day, LD 12 : 12 (a), continuous dark, DD (b) and continuous light, LL (c). The values are statistically significant at 1% level (p < 0.001).

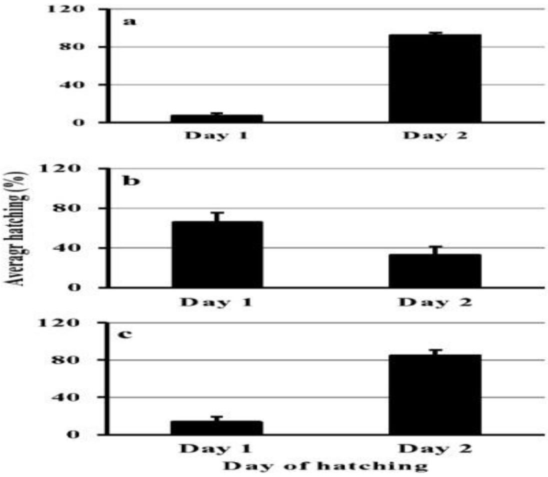

Figure I. 8: Average hatching (%) (± SD; n = 5) in the popular bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 on day 1 and day 2 of hatching kept under natural day, LD 12 : 12 (a), continuous dark, DD (b) and continuous light, LL (c). The values are statistically significant at 1% level ($p < 0.001$).

Hatching duration of the silkworm eggs (from initiation to the completion of hatching) (Figure I. 9) has giveninteresting trends for both PM x CSR2 and CSR2 x CSR4 under all photoperiodic combinations (LD 12 : 12, DD and LL) imposed. The only combination of silkworm hybrid and photoperiodic condition were PM x CSR2 and natural day condition, LD 12 : 12 (Figure I. 9a) that recorded hatching duration of 3 hours. The other two photoperiodic combinations (DD and LL) resulted in hatching durations more than 26 hours (28 h to be precise, Figure I. 9) for both the hybrids studied. Examining the hatching durations with CSR2 x CSR4 under the three photoperiodic combinations (LD 12 : 12, DD and LL) revealed hatching duration of more than 26 hours (28.4 h for LD 12 : 12, 27.2 hours for DD and 34 hours for LL; Figure I. 9).

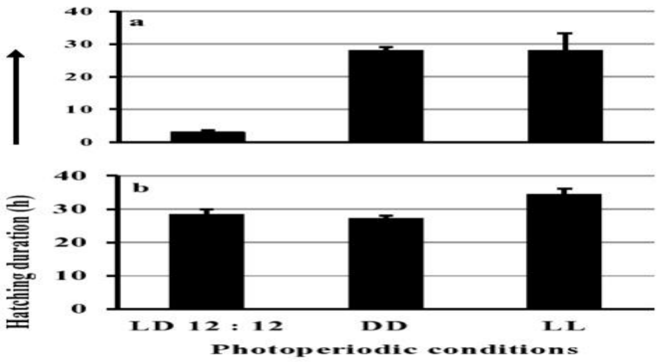

Figure I. 9 Average hatching durations (h; ± SD; n = 5) in the popular multivoltine x bivoltine silkworm hybrid, PM x CSR2 (a) and bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 under three photoperiodic conditions, normal day (LD 12 : 12), continuous dark (DD and continuous light (LL). The values are statistically significant at 1% level ($p < 0.001$).

Important observations made on hatching in PM x CSR2 and CSR2 x CSR4.

The experimentation in the present study was not made with mere intension of academic importance. It is conducted to know any specific points for further improvement in hatching of both the silkworm hybrids, PM x CSR2 and CSR2 x CSR4. Such points pf importance are tabulate in Table I. 1.

Table I. 1: Observations and specific point for further improvement in hatching of both the silkworm hybrids, PM x CSR2 and CSR2 x CSR4.

S. No.	Observations	Probable inference
I.	**General observations**	
a.	PM x CSR2 took a single day for complete hatching under LD 12 : 12 conditions	Multivoltine x bivoltine character
b.	CSR2 x CSR4 two 2 days for complete hatching under LD 12 : 12 condition.	Bivoltine character showing stray hatching on day 1.
c.	2 days hatching in DD for PM x CSR2 and CSR2 x CSR4	Free running hatching expression.
d.	2 days hatching in DD for CSR2 x CSR4 and CSR2 x CSR4	Free running hatching expression.
e.	2 days hatching in LL for PM x CSR2 and CSR2 x CSR4	Free running hatching expression.
f.	2 days hatching in LL for CSR2 x CSR4 and CSR2 x CSR4	Free running hatching expression.
g.	Hatching occurring just at lights-on phase, both in PM x CSR2 and CSR2 x CSR4.	Lights-on signal taken as synchronization factor for hatching.
h.	Hatching occurring during early cool and humid morning hours.	To avoid desiccation in tiny larvae of silkworm.
i.	Sharp hatching expression of hatching under DD and spread hatching expression under LL.	Short day dependent characteristics. DD induces sharp hatching. LL infuses broadening in hatching.
II	**Specific observations on hatching in PM x CSR2**	

a.	Only one day hatching under LD 12 : 12 condition.	Multivoltine x bivoltine character.
b.	2 days hatching under DD with more hatching on day 1 over day 2.	Short-day dependence and multivoltine characters.
c.	2 days hatching under LL with more hatching on day 2 over day 1	LL (light) induced broadening in hatching and shifting hatching to day 2.
III	**Specific observations on hatching in CSR2 x CSR4**	
a.	Only one day hatching under LD 12 : 12 condition.	Specifically, this point is true. Bivoltine x bivoltine character.
b.	2 days hatching under DD with more hatching on day 1 over day 2.	Short-day dependence.
c.	2 days hatching under LL with more hatching on day 2 over day 1	LL (light) induced broadening in hatching and more shifting of c hatching to day 2.
IV	**Specific observations on hatching in PM x CSR2 and CSR2 x CSR4**	
a.	Single day hatching in PM x CSR2.	Not necessary to go for any other technology.
b.	Two days hatching in CSR2 x CSR4 under LD 12 : 12.	Other technologies to restrict hatching for a single day.
c.	2 days of hatching in DD/LL for PM x CSR2 and CSR2 x CSR4	Its only of academic importance rather than economic/industrial.

DISCUSSIONS

Egg hatching in the silkworm, Bombyx mori has been described as the first and foremost developmental marker event (Shanthan Babu, 2014; Srinath, 2014). The rhythmic pattern in this developmental marker event in B. mori has received considerably good attention. However, the studies were not extended to available commercially exploited hybrids. At present, the Indian sericulture is mainly ruled by two 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 hatching, as affected by photoperiods in silkworm have been reported (Yamaoka and Hirao, 1975; Yamaoka et al., 1976; Anantha Narayana et al., 1978; Sivarami Reddy et al., 1984; Sivarami Reddy et al., 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 both the silkworm hybrids (Figure I. 1 and I. 2) and its persistence under continuous dark (DD; Figure I. 3 and 4) and light (LL; Figure I. 5 and 6) conditions strongly suggest diurnal nature of the hatching rhythmicity, supported by earlier reports (Anantha Narayana et al., 1978; Shanthan Babu, 2014; Sivarami Reddy et al., 1984; Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990, Sivarami Reddy et al., 1998; Srinath, 2014). Further, advancement of hatching under DD and its delay under LL for both the commercial silkworm hybrids indicate the free running nature of the hatching rhythmicity (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990).

The occurrence of hatching for two consecutive days (Figure I. 2 to 6) indicates the periodicity of hatching rhythm to be circadian (Anantha Narayana et al., 1978; Sivarami Reddy et al., 1984; Sivarami Reddy et al., 1993; Sivarami Reddy and Sasira Babu, 1990, Sivarami Reddy et al., 1998). The observation also strongly suggests that the rhythm is gated one (Shanthan Babu, 2014; Srinath, 2014) and hints at mixed age population characteristics. Except the hatching for PM x CSR2 under LD 12 : 12 condition, hatching with the other conditions for both the silkworm hybrids (PM x CSR2 and CSR2 x CSR4) occurred for two consecutive days (Figure I. 2 to I. 6). However, no hybrid crossed two days in expression of their hatching. Hatching for more than two consecutive days, in fact, was reported for PM x NB4D2 by Sivarami Reddy and Sasira Babu (1990) and Sivarami Reddy (1993) under extreme photoperiodic conditions. Interestingly, the hatching on the first day (day 1) was more under LD 12 : 12 condition (Figure I. 7a) and DD as well (Figure I. 7b) for PM x CSR2. However, hatching magnitude was more on day 2 with LL condition (Figure I. 7c). In the case of CSR2 x CSR4, hatching magnitude was more on day 1 under DD alone (Figure I. 8b) and the same was more than under LD 12 : 12 condition (Figure I. 8a) and LL (Figure I. 8c) as well. This observation strongly suggest that the silkworm prefer LD 12 : 12 condition or/and DD to than LL condition. However, the occurrence of more hatching magnitude with CSR2 x CSR4 under LD 12 : 12 condition (Figure I. 8a) hints that the hatching is dependent on voltinism. Further, the results in the present study and those of Sivarami Reddy and Sasira Babu (1990) and Sivarami Reddy (1993) strongly suggest that extreme photoperiodic conditions are not suited to silkworm for commercial and economic hatching. For commercial and economic hatching, Shanthan Babu (2014 and Srinath (2014) suggested maintenance of incubating silkworm eggs under DD condition followed by interruption of DD early in the morning on the day of hatching. Results on total hatching duration have given interesting trends. Thus, hatching duration was very short (3 ± 0.71 h; Figure I. 9a) with PM x CSR2 under LD 12 : 12 condition alone. With all the imposed photoperiodic conditions the silkworm hybrids (PM x CSR2 and CSR2 x CSR4) resulted in more than 26 h of duration. The observed long hatching durations include two hatching peaks (gates) separated by non-hatching time (forbidden zone). Thus, photoperiodic approach offers useful tool in commercial silkworm hatching. Further, hatching duration of 34 hours for CSR2 x CSR4 under LL is attributed to fact that hatching occurred for two consecutive days as a result of broadening of hatching phenomenon.

Finally, the results documented in the present research are of academic importance only. What the researcher aimed at is finding specific point(s) that help in improving the hatching activity to best suit to the requirements of sericulture farmers. Kanika Trivedi (2015) and Kawakami and Yanagawa (2003) viewed that egg hatching in silkworm should be confined to a single day with economic hatching (over 95%). Kawakami and Yanagawa (2003) have mostly pointed out the efforts towards development of bivoltine sericulture in India, indicating that the bivoltine sericulture needs more technologies. Regarding the multivoltine x bivoltine hybrid, PM x CSR2, no other technology(ies) seems not necessary as the hybrid confined to hatch on a single day (Figure I.1, Figure I. 7a) with shortest durations (Figure I. 1.and Figure I. 8a). On the contrary, the bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 resulted in two days hatching under LD 12 : 12 conditions (Figure I. 2, Figure I. 8) with less hatching durations (Figure I. 9b). Interestingly, the hatching durations of CSR2 x CSR4 under LD 12 : 12 and DD are quite comparable. Observations on this aspect (hatching magnitude simply comparable in CSR2 x CSR4 under LD 12 : 12 and DD conditions) have necessitated to probe further into development of a new technology(ies) are simply re-examining the already existing technology, which is dealt in the Chapter 2.

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