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STUDIES ON THE PATTERNS OF THE FIRST DEVELOPMENTAL MARKER EVENT, HATCHING IN THE COMMERCIAL MULBERRY SILKWORM, *BOMBYX MORI* L.

Suvarna, G., Raghavendra, K., [a]Shanthan Babu, M.A., [a]Srinath, B.,*Lakshminarayana Reddy, P. & Sankar Naik, S.
Department of Sericulture, Sri Krishnadevaraya University, Anantapur - 515 003, Andhra Pradesh, India.
[a]Regional Sericultural Research Station, CSB, PB No. 50, Anantapur - 515 001, Andhra Pradesh, India.
*Corresponding author's email: lakshminarayanareddyp@yahoo.in

ABSTRACT

Knowledge on the patterns of the first developmental marker event in the life cycle of the commercial mulberry silkworm, *Bombyx mori* L., the hatching provides an important input for successful economic and commercial hatching. Disease free layings (DFLs) of two extensively exploited commercial mulberry silkworm hybrids; a multivoltine x bivoltine hybrid, PM x CSR_2 and a bivoltine x bivoltine hybrid, CSR_2 x CSR_4 were introduced into three photoperiodic conditions *viz*., natural day (LD 12 : 12), continuous dark (DD) and continuous light (LL) conditions on the third day of oviposition under constant temperature, 25 ºC and relative humidity, RH; 80%. Data on precise number of silkworm larvae hatched out from the eggs were recorded on hour-to-hour basis from five replications in five repetitions. Macroscopic data were converted into percentage and represented in chronograms. The results on hatching irrevocably proved that the hatching in PM x CSR_2 (multivoltine x bivoltine) hybrid is simple and restricted to a single day under LD 12 : 12 conditions. With LL and DD, however, the hatching rhythm continued its expression for 2 consecutive days. Further, hatching on the day 1 with LL was less than that on the day 2. Opposite trends in hatching percentage were observed under LD 12 : 12 and DD. With CSR_2 x CSR_4 (bivoltine x bivoltine) hybrid, the hatching rhythm under LD 12 : 12 conditions continued occurring for 2 consecutive days, with stray hatching on day 1. Similar results on hatching rhythmicity, as that of PM x CSR_2 hybrid, were observed for CSR_2 x CSR_4 hybrids under DD/LL. The hatching rhythm was circadian, diurnal, taking 'lights-on' as signal, free-ran with continuous light conditions (DD/LL) and expressed 'gating' phenomenon. Hatching duration was very short with PM x CSR_2 under LD 12 : 12 condition alone. With all the imposed photoperiodic conditions, the two silkworm hybrids recorded hatching duration of more than 24 h.

KEY WORDS: Silkworm, *Bombyx mori*, first developmental marker event, hatching, photoperiod.

INTRODUCTION

Egg hatching is the first complex behaviour manifest in the life of an insect (Saunders, 2002). Among several developmental marker events, egg hatching is the first and the most important one in the life cycle of lepidopteron as it frees the larva from the constraints of life in the egg to fulfilling its biological destiny in the outside world. 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 crucial aspect in commercial silkworm rearing (Rajan *et al.*, 1996). 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 gating event, regulated 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). Reviewing the external signals that regulate the hatching in insects, Saunders (2002) confirmed that major signals are light and/or temperature cycles. Continued in constant temperature and relative humidity, *Bombyx* hatching has been reported to be dependent on the voltinism for certain economic aspects such as hatching duration, hatching magnitude (Shanthan Babu, 2014; Srinath, 2014) etc., though the basic aspects of hatching rhythmicity did not show any discrepancy. However, comparative studies of these aspects of the first developmental marker event between two contemporary commercial silkworm hybrids, PM x CSR_2 (multivoltine x bivoltine hybrid) and CSR_2 x CSR_4 (bivoltine x bivoltine hybrid), the hatching are lacking. Such fundamental information from basic input for commercial exploitation of these silkworm hybrids. Therefore, hatching rhythmicity and other economic aspects of hatching in two contemporary popular silkworm hybrids of *Bombyx mori;* PM x CSR_2 and CSR_2 x CSR_4 are reported in the present communication under three photoperiodic schedules *viz*., normal day (LD 12 : 12), continuous dark (DD) and continuous light (LL) conditions, keeping the temperature and humidity conditions constant.

MATERIALS & METHODS

Disease free layings (DFLs, each DFL consists of 450-550 eggs laid by a single silkmoth on a single day) of two contemporary popular commercial silkworm hybrids, PM x CSR_2 (a hybrid of PM, Pure Mysore, a multivoltine breed and CSR_2, a bivoltine breed) and CSR_2 x CSR_4 (a hybrid of CSR_2, a bivoltine breed and CSR_4, another bivoltine breed) were procured, third day of oviposition, for the study from the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Hindupur, Anantapur District, India. The DFLs were transported to the laboratory during evening cool hours and immediately spread in the pre-arranged rearing trays (Neelkamal, plastic, 2' x 3' size) pre-lined with paraffin paper. The same day, DFLs were introduced till hatching, into three photoperiodic conditions; normal day (LD 12 : 12), continuous dark (DD) and continuous light (LL). For the normal day (LD 12 : 12) photoperiodic condition, the light phase (photophase, around 50 Lux, Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990) of the LD cycle spanned from 0600 h to 1800 h and the dark phase (scotophase) from 1800 h to 0600 h local time. The entire experimentation was conducted with constant temperature and humidity. Thus, optimum temperature (25 ± 1°C) and relative humidity (RH, 80 ± 5%, Krishnaswami, 1986; Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990) were maintained all through the experimentation. Five DFLs of each hybrid were kept separately under each photoperiodic condition (cellular system) to serve as replications. The experiment was repeated for five times. Hatching parameters like rhythmic attributes in hatching, hatching duration and its magnitude were studied under the above three photoperiodic schedules for the two popular commercial silkworm hybrids. Data on precise timings, in hours, and number of larvae hatched out from eggs were recorded for the study on hatching patterns. The hatched-out larvae were separated and the intact eggs were continued for further studies till completion of the experimentation. A dim red light source (below 0.1 Lux, Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990) was employed, enabling recording the data under DD and during scotophase of LD 12 : 12. From such recorded data, the other parameters like day to day hatching averages and hatching durations (time span in h from the initiation of hatching to its completion) were extracted. The hatching patterns were represented in chronograms with information on imposed photophase and scotophase. Macroscopic data were statistically (ANOVA) analyzed for microscopic data.

RESULTS

Hatching in multivoltine x bivoltine hybrid, PM x CSR_2 was predominantly diurnal, occurring at the beginning of the photophase under natural LD cycle (LD 12 : 12; Fig. 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, CSR_2 x CSR_4 also, hatching was diurnal, occurring at the initiation of photophase (Fig. 2). However, hatching in CSR_2 x CSR_4 occurred for 2 consecutive days, with less (stray) hatching on day 1 and maximum on day 2.

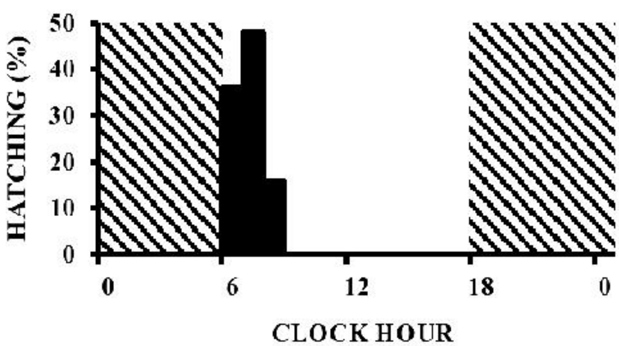

FIGURE 1: Chronogram representing distribution of hatching (%) in PM x CSR_2 of *Bombyx mori* L. under LD 12 : 12 conditions. Note entire hatching on a single day. Also note that the hatching occurred just after 'lights-on' phase of the LD cycle. Cross-hatched area in the histogram indicates the dark phase imposed and the open area, the light phase of the day.

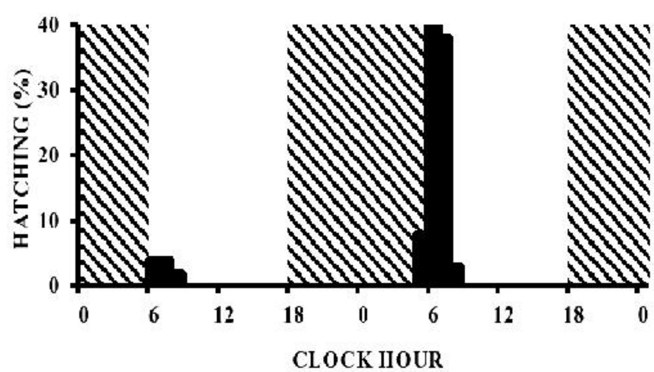

FIGURE 2: Chronogram representing distribution of hatching (%) in CSR_2 x CSR_4 of *Bombyx mori* L. under LD 12 : 12 conditions. Note stray hatching on the first day and maximum hatching on the second day (gating-phenomenon). Also note that the hatching occurred just after 'lights-on' phase of the LD 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 CSR_2 and CSR_2 x CSR_4, the hatching rhythm being almost identical (Fig. 3 and 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 CSR_2 and CSR_2 x CSR_4 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. Hatching under continuous light (LL) also occurred for two consecutive days both in PM x CSR_2 and CSR_2 x CSR_4 hybrids (Fig. 5 and 6). Opposing the hatching trends under DD condition, less hatching was recorded on day 1 under 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 CSR_2 and CSR_2 x CSR_4. The hatching peaks in PM x CSR_2 under LL conditions were sharp (Fig. 5) on day 1 and 2. However, peak hatching expression broadened for CSR_2 x CSR_4 (Fig. 6), indicating a trend leading to arrhythmicity.

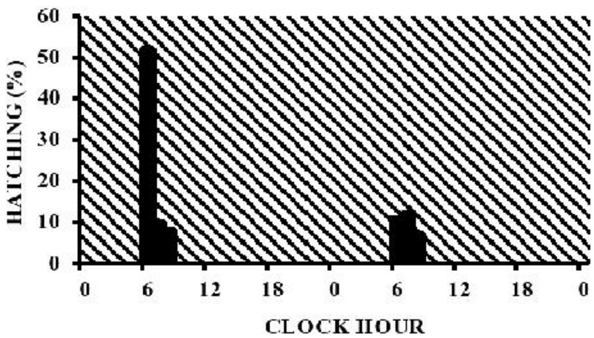

FIGURE 3: Chronogram representing distribution of hatching (%) in PM x CSR_2 of *Bombyx mori* L. under continuous dark (DD) condition. Note, hatching occurring for two days (two gates) with more hatching on the first day. Also note that the hatching occurred at or before 06.00 h of local time.

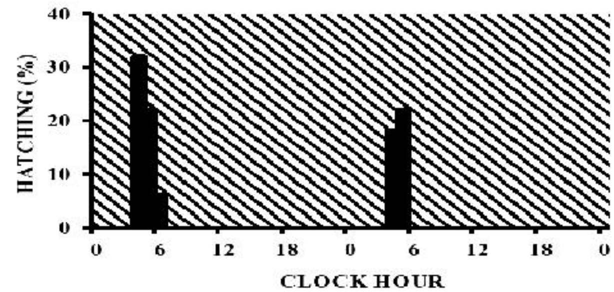

FIGURE 4: Chronogram representing distribution of hatching (%) in CSR_2 x CSR_4 of *Bombyx mori* L. under continuous dark (DD) condition. Note hatching for two consecutive days (gates) with more hatching on the first day. Also, hatching durations were narrowed. The hatching occurred before 06.00 h of the local time and therefore occurrence of hatching advanced. Hatching activity (peak) is very sharp.

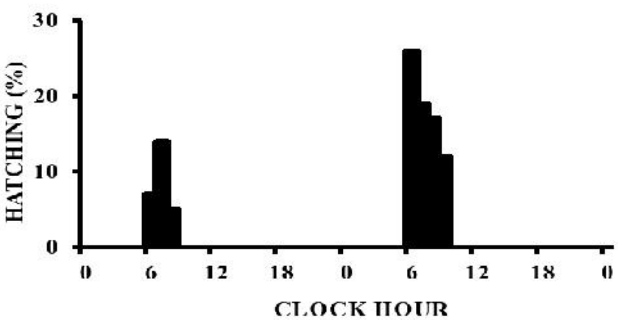

FIGURE 5: Chronogram representing distribution of hatching (%) in PM x CSR_2 of *Bombyx mori* L. under continuous light (LL) condition. Note hatching occurring for two days, with more hatching on day 2. Also note that the hatching occurred after 06.00 h of local time.

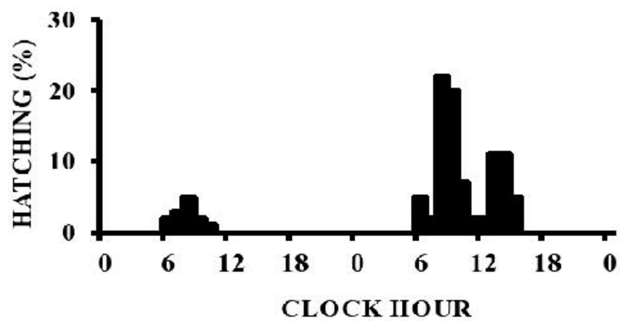

FIGURE 6: Chronogram representing distribution of hatching (%) in CSR_2 x CSR_4 of *Bombyx mori* L. under continuous light (LL) condition. Note hatching occurring for two days, with more hatching on day 2. Also note that the hatching occurred after 06.00 h of local time indicating a delay and hatching activity broadened.

Hatching occurred for one day for PM x CSR_2 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 CSR_2 x CSR_4. Day to day average for hatching in PM x CSR_2 under the imposed photoperiodic conditions (LD 12 : 12, DD and LL) is depicted in figure 7. Thus, hatching in PM x CSR_2 under LD 12 : 12 condition is restricted to day 1 only, with no or negligible hatching on day 2 (Fig. 7a). With the other 2 photoperiodic conditions (DD and LL), hatching in PM x CSR_2 occurred for two consecutive days. However, hatching on day 1 was more compared to that on day 2 (Fig. 7b) under DD condition. Opposing this, hatching was more on day 2 under LL condition for PM x CSR_2 (Fig. 7c). Average hatching in CSR_2 x CSR_4 under three imposed photoperiodic conditions (LD 12 : 12, DD and LL; Fig. 8) also indicated two consecutive days of hatching. However, hatching was less on day 1 under LD 12 : 12 and LL (Fig. 8a and c) whereas it was more on day 1 under DD (Fig. 8b).

Hatching duration of the silkworm eggs (from initiation to the completion of hatching) (Fig. 9) has given interesting trends for both PM x CSR_2 and CSR_2 x CSR_4 under all photoperiodic combinations (LD 12 : 12, DD and LL) imposed. The only combination of silkworm hybrid and photoperiodic condition were PM x CSR_2 and natural day condition, LD 12 : 12 (Fig. 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, Fig. 9) for both the hybrids studied. Examining the hatching durations with CSR_2 x CSR_4 under the three photoperiodic combinations

(LD 12 : 12, DD and LL) revealed hatching duration of more than 26 hours (28.4 hours for LD 12 : 12, 27.2 hours for DD and 34 hours for LL; Fig. 9).

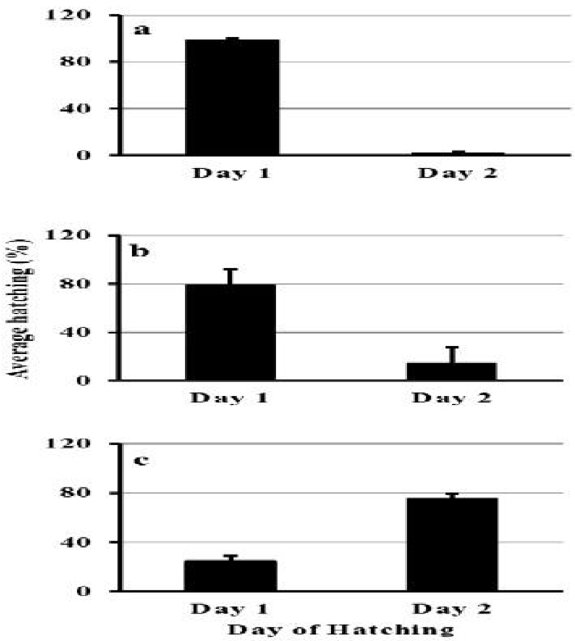

FIGURE 7: Average hatching (%) (± SD; n = 5) in the popular multivoltine x bivoltine silkworm hybrid, PM x CSR_2 on day 1 and day 2 of hatching kept under natural day, LD 12 : 12 (a), continuous dark, DD (b) and continuous light, LL (c). The values are statistically significant at 1% level ($p < 0.001$).

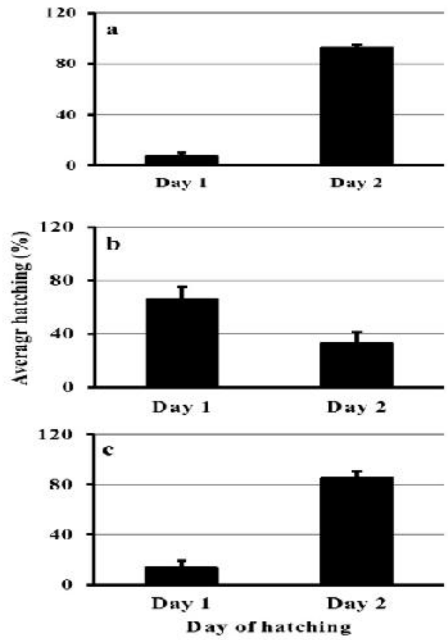

FIGURE 8: Average hatching (%) (± SD; n = 5) in the popular bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4 on day 1 and day 2 of hatching kept under natural day, LD 12 : 12 (a), continuous dark, DD (b) and continuous light, LL (c). The values are statistically significant at 1% level ($p < 0.001$).

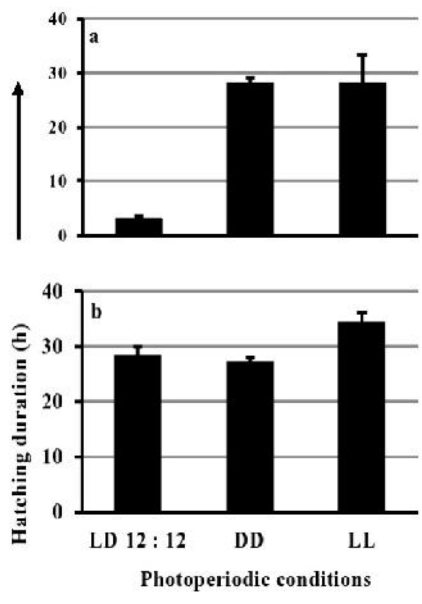

FIGURE 9: Average hatching durations (h; ± SD; n = 5) in the popular multivoltine x bivoltine silkworm hybrid, PM x CSR_2 (a) and bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 under three photoperiodic conditions, normal day (LD 12: 12), continuous dark (DD) and continuous light (LL). The values are statistically significant at 1% level ($p < 0.001$).

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 CSR_2 (multivoltine x bivoltine hybrid) and CSR_2 x CSR_4 (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 have been reported (Yamaoka and Hirao, 1975; Yamaoka *et al*., 1976; Anantha Narayana *et al*., 1978; Sivarami Reddy *et al*., 1984; Sivarami Reddy, 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 (Fig. 1 and 2) and its persistence under continuous dark (DD; Fig. 3 and 4) and light (LL; Fig. 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 (Fig. 2 to 6) indicates the periodicity of hatching rhythm to be circadian (Anantha Narayana *et al*., 1978; Sivarami Reddy *et al*., 1984; Sivarami Reddy, 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 CSR_2 under LD 12: 12 condition, hatching with the other conditions for both the silkworm hybrids (PM x CSR_2 and CSR_2 x CSR_4) occurred for two consecutive days (Fig. 2 to 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 NB_4D_2 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 (Fig. 7a) and DD as well (Fig. 7b) for PM x CSR_2. However, hatching magnitude was more on day 2 with LL condition (Fig. 7c). In the case of CSR_2 x CSR_4, hatching magnitude was more on day 1 under DD alone (Fig. 8b) and the same was more on day 2 under LD 12 : 12 condition (Fig. 8a) and LL (Fig. 8c) as well. This observation strongly suggest that the silkworm prefer LD 12 : 12 condition or/and DD to LL condition. However, the occurrence of more hatching magnitude with CSR_2 x CSR_4 under LD 12 : 12 condition (Fig. 8a) on day 2 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; Fig. 9a) with PM x CSR_2 under LD 12 : 12 condition alone. With all the imposed photoperiodic conditions the silkworm hybrids (PM x CSR_2 and CSR_2 x CSR_4) resulted in more than 26 h of hatching duration. The observed long hatching durations include two hatching peaks (gates) separated by non-hatching time (forbidden zone) indicating the gating phenomenon. Thus, photoperiodic approach offers useful tool in commercial silkworm hatching. Further, hatching duration of 34 hours for CSR_2 x CSR_4 under LL is attributed to the fact that hatching occurred for two consecutive days as a result of broadening of hatching phenomenon.

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