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ON THE EGG HATCHING IN SILKWORM (*BOMBYX MORI* L.) HYBRID, CSR2 x CSR4 AT FARMERS' LEVEL, A CASE OF CIRCADIAN ENTRAINMENT WITH THERMOPERIODIC AND HYGROPERIODIC CONDITIONS

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Abstract: **Understanding the hatching patterns in the commercial mulberry silkworm, *Bombyx mori* L., at laboratory level are amply available. Hatching phenomenon at farmers' level, however, is completely lacking and such knowledge is more crucial for successful commercial cocoon crop of *Bombyx* silkworm. Present study engendered certain important results, in contrast to the earlier reported hatching patterns under various photoperiodic conditions at laboratory level. Further, it reports strange 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 common photoperiodic conditions; LD 12 : 12 (natural day), DD (continuous dark), LL (continuous light) and Black-Box conditions on the third day of oviposition, at the rearing houses of five selected sericulture farmers of Mudigubba Mandal, Anantapur District, Andhra Pradesh. Other environmental conditions, temperature and relative humidity were not altered, keeping the farmers practice. Dial variations in both temperature and humidity were recorded to examine rhythmicity and their possible implications on the hatching rhythmicity in CSR2 x CSR4. Number of silkworm larvae hatched-out from experimental eggs was recorded on hourly basis from five replications each of five farmers. Experiments were repeated for five times. Recorded macroscopic data were converted into percentage and represented as chronograms for analysis. Results on hatching irrevocably revealed that CSR2 x CSR4 hatching rhythmicity under LD 12 : 12 conditions did not change, occurring for 2 consecutive days, with less hatching on day 1. Hatching rhythmicity under DD/LL also occurred for two consecutive days. Against the hypothesis, hatching phase neither advanced under DD nor delayed with LL conditions imposed. Hatching rhythmicity was circadian, diurnal, taking 'lights-on' as signal as synchronizing cue, however without expressing free-running nature under DD/LL. Egg hatching under DD occurred for two consecutive days, hatching peak expressing at 06.00 h of normal day condition, with high hatching on day-1 and less on day-2, as against that with LD 12 : 12. Egg hatching under LL also occurred for two consecutive days, hatching peak expressing at 06.00 h, with less hatching on day-1 and more on day-2. However, the only circadian hatching phenomenon of LL was a clear-cut appearance of broadening/widening of hatching activity, just reminding a near damping-out situation. Notably, hatching with black-box condition was its confinement to a single day. From recorded data on egg hatching patterns at farmers' level, it is amply evident that dial variations in temperature (thermoperiod) and humidity (hygroperiod), in DD/LL conditions are operating as subtle, but definite cues in maintaining the phenomenon of hatching, just like that under LD 12 : 12 photoperiodic condition. This implies that both thermoperiods and hygroperiods, in the absence of light dark signals, are entraining circadian rhythmicity, at least under DD and LL conditions. The present study is just a beginning of such strange observations and need further detailed probe. Data were discussed on the importance of hatching patterns in commercial silkworm egg hatching at farmers' level.**

Keywords: **Silkworm, *Bombyx mori*, CSR2 x CSR4, photoperiod, thermoperiod, hygroperiod, hatching, farmers' condition.**

1. INTRODUCTION

The unique characteristics of the initial developmental marker event, the overt phenomenon of hatching in the mulberry silkworm, *Bombyx mori* L. has been studied by many researchers in the laboratory conditions (Anantha Narayana, 1980; Anantha Narayana *et al*., 1978; Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990; Sivarami Reddy *et al*., 1998; Shanthan Babu, 2014; Srinath, 2014; Suvarna, 2021; Suvarna *et al*., 2015; Lakshmi *et al*., 2021). The research on silkworm egg hatching followed that of Indian sericulture history (Lakshmi *et al*., 2021). It started with Mysore race (PM, Pure Mysore), reporting that silkworm hatching is a diurnal and phase-locked to lights-on signal (Anantha narayana, 1980; Anantha Narayana *et al*., 1978). It is generalized that silkworm hatching is under circadian control in PM x NB4D2 (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990; Sivarami Reddy *et al*., 1998). Further, researches were swigged to bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 (Shanthan Babu, 2014; Srinath, 2014; Suvarna, 2021; Lakshmi *et al*., 2021) pointing out certain basic diversities in circadian egg hatching phenomenon between multivoltine x bivoltine hybrids and bivoltine x bivoltine hybrids. Studies on the implications of temperature and humidity on silkworm egg hatching were also reported at laboratory level (Lakshminarayana Reddy, 2001; Lakshminarayana Reddy *et al*., 2005; Narasimhulu, 2020; Narasimhulu *et al*., 2020). They (Lakshminarayana Reddy, 2001; Lakshminarayana Reddy *et al*., 2005; Narasimhulu, 2020; Narasimhulu *et al*., 2020) reported that keeping the light dark cycles as programmed (LD 12 : 12, DD and LL), the egg hatching rhythmic pattern did not change by increase or decrease in temperature and humidity, but with implicating egg hatching economic characters greatly. It is apparent that egg hatching patterns are important in silkworm rearing since the crop pertains to commercial importance. Hitherto, studies were concentrated mainly at laboratory level (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990; Sivarami Reddy *et al*., 1998; Shanthan Babu, 2014; Srinath, 2014; Suvarna, 2021; Lakshmi *et al*., 2021). Such studies at farmers' level are completely lacking. Since the destination of all sericulture technologies is the farmer, an attempt has been made, in the present communication, to study the hatching patterns at farmers' level. It is apparent that silkworm, an economic importance insect is extensively used for commercial exploitation and non-experimenting at farmers rearing houses also is of no utility. The major environmental factors affecting silkworm hatching are light, temperature and humidity (Rahmathulla, 2012). Regulating light and dark cycles to our requirement are rather easy at farmers' level. But, maneuvering temperature and humidity are considerably difficult with high cost of applications and appliances. Therefore, as a first step, the present investigation is undertaken to study the occurrence of circadian characteristics in the silkworm hybrid, CSR2 x CSR4 at farmers' level through managing light conditions, keeping temperature and humidity conditions un-touched.

2. MATERIALS AND METHODS

Disease free layings (DFLs, each DFL consists of 450 to 500 eggs laid by a single silk moth on a single day) of the CSR2 x CSR4 (bivoltine x bivoltine silkworm hybrid, *B. mori*) 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 rearing houses of five selected sericulture farmers of Mudigubba Mandal, Anantapur District, Andhra Pradesh, India during evening hours and immediately spread in pre-disinfected plastic rearing trays (2' x 3', Neel Kamal, India). The same day, DFLs were introduced, till completion of hatching to four photoperiodic conditions; 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; light intensity around 50 Lux) for LD 12 : 12 commenced from 06.00 h for continuous 12 hours and the scotophase spanned for 12 hours from 18.00 to 06.00 h. However, temperature and relative humidity (RH) were not controlled, keeping farmers practice unchanged. To denote the dial variations in temperature and humidity in all five rearing houses of farmers' hourly variations were recorded to represent thermoperiod and hygroperiod subsisting in farmers rearing house conditions for further exploitation in explaining and reviewing results. Five DFLs were kept separately under each photoperiodic condition (five replications) with each of five farmers. Rhythmic patterns in egg hatching were studied under these photoperiodic schedules. The hatching experiments were repeated for five times. Data were analyzed for simple statistical calculations of average and standard deviation. The resultant data were further utilized to determine the statistical validity, through ANOVA.

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3. RESULTS

The Farmers:

The hatching patterns of silkworm, *Bombyx mori* (CSR2 x CSR4) were studied at farmers' level. Five farmers were purposefully selected in Roddam Mandal of Anantapur District. Details farmers selected are given below (Table 1). Main criteria of such farmers selection was that the farmer should have at least: 1. one acre of V1 mulberry garden with wider spacing (2' x 2'), 2. a separate rearing shed and 3. a separate chawki (young larvae) rearing room.

Table 1: Details of five farmers selected for the study of hatching patterns in *Bombyx mori* (CSR2 x CSR4) in Mudigubba Mandal, Anantapur District, Andhra Pradesh.

S. No.	Name of farmer	Name of Village	Mulberry acreage	Rearing shed	Capacity
1.	R. Lakshminarayana Reddy, S/O Narayana Reddy	Motukupalli	2	Separate	200 DFLs
2.	V. Somasekhara Reddy, S/O Siva Reddy	Motukupalli	2	Separate	200 DFLs
3.	B. Rupa, W/O Suryanarayana Reddy	Martadu	2	Separate	200 DFLs
4.	D. Govardhan Reddy, S/O Vijaya Bhaskara Reddy	Kalasamudram	2	Separate	200 DFLs
5.	B. Somasekhara Reddy, S/O Bali Reddy	Kalasamudram	2	Separate	200 DFLs

The dial fluctuations in temperature:

From the review of literature, it is noticed that circadian biological rhythms are mainly regulated by cyclical fluctuations in light and dark. Other environmental fluctuations such as temperature and humidity are also involved in a subtle, but effective manner. Therefore, an attempt was made to record the oscillations in temperature and humidity in the rearing houses of selected farmers. The farmers did not attempt regulating these (temperature and humidity) scientifically, but through locally available means. HTC-1 High Precision Large Screen Electronic Indoor Temperature, Humidity Thermometer with Clock Alarm was used for the purpose. Data on clock variations of temperature (average of five farmers) are presented in Figure 1.

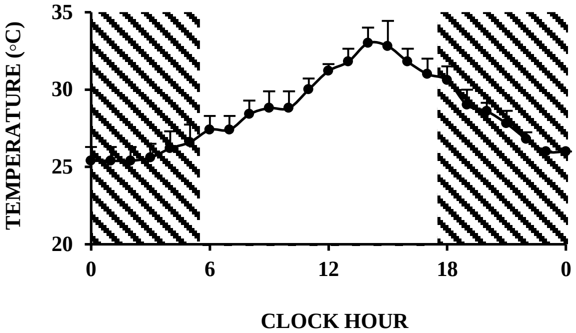

Figure 1: Clock variations of temperature (°C) recorded in the farmers' rearing house (average ± SD, n = 5 farmers).

As seen from the Figure 1, a clear-cut circadian variations and thus, thermo-rhythmicity was evident. Temperature during dark (night) phase of the day was low and it started rising from lights-on phase (06.00 h). Maximum temperature was observed after 12.00 h of the day. The rhythmicity is definitely is of circadian nature.

The dial fluctuations in humidity:

Similarly, the recorded relative humidity (RH %, average of data of five farmers' rearing houses) is also examined for any clue in implicating as cue to the hatching rhythmicity of CSR2 x CSR4 at farmers' rearing house conditions. The data on cyclical fluctuations in RH are presented in Figure 2. As seen from the RH graph, it is clear that RH also fluctuating cyclically denoting hygroperiodic condition, confined to a circadian clock. Temperature and RH are inversely related. Highest RH was recorded at lights-on phase while lowest at dusk phase.

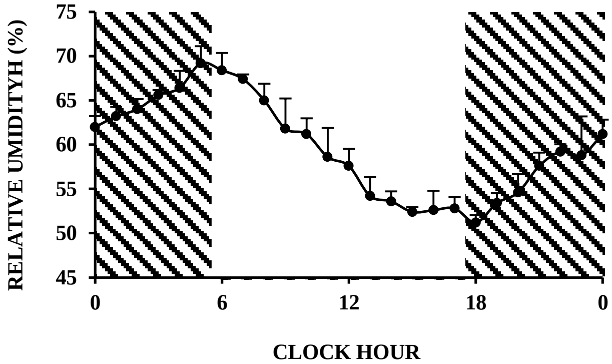

Figure 2: Cyclical variations of Relative Humidity (RH %) recorded in the farmers' rearing house (average ± SD, n = 5 farmers).

Thus, the cyclical fluctuations in both temperature and RH are presumed to be the cues in regulating the rhythmicity in CSR2 x CSR4 in line with LD 12 : 12. Light and dark cycles in the rearing houses were controlled while temperature and RH were not by the farmers.

***The hatching pattern under LD 12 : 12*:**

From the recorded data on hourly based hatching quantity, percentage hatching was computed and used to convert data into chronogram. Best fitted graph of the hatching patterns at farmers' level under LD 12 : 12 conditions is selected from five farmers and presented in Figure 3. Egg hatching in the bivoltine x bivoltine silkworm, *Bombyx mori* L. (CSR2 x CSR4) under natural day, LD 12 : 12 photoperiodic conditions at the farmers' level (chronogram) occurred for two consecutive days. Quantum of hatching was very low on day-1, symbolizing a stray-hatching stipulation. Hatching was high and complete on day-2. Hatching at farmers' conditions too occurred at or after the beginning of the photophase (06.00 h). Therefore, hatching is referred to as synchronized by light-on signal, and thus diurnal. The distance between two hatching peaks (day-1 and day-2) are ≈ 24 hours, and therefore, hatching rhythmicity is circadian. The peaks of hatching on both days (day-1 and Day-2) are very distinct and sharp. However, the duration of hatching on individual days (day-1 and day-2) was strangely broadened, 4 hours on day-1 and 7 hours on day-2. The circadian characteristics of hatching, however, did not alter, implying that hatching is: a. diurnal, b. phase-locked to 'lights-on', c. circadian, d. sharp hatching peaks and high magnitude of hatching on day-2. Notably, peculiarity recorded observation is that hatching durations on either day were broadened, 4 hours on day-1 to 7 hours on day-2, an observation point for further probing.

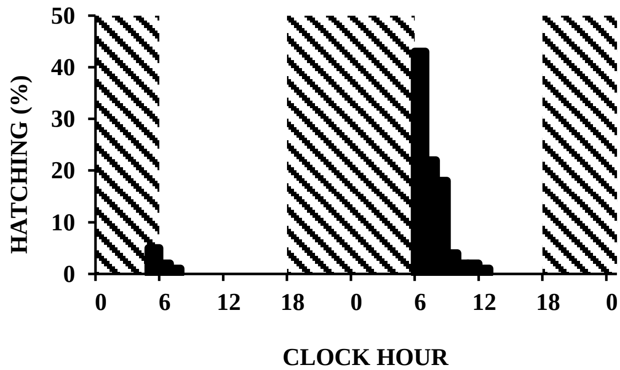

Figure 3: Chronogram representing distribution of hatching (%) under LD 12:12 conditions in the silkworm, *Bombyx mori* L. (CSR2 x CSR4) at farmers rearing house. Less hatching on day-1 and maximum on the day-2 is notable. Cross-hatched area in the histogram indicates the dark phase imposed and the open area, the light phase of the day.

***The Hatching pattern under DD*:**

The trends in hatching patterns of CSR2 x CSR4 under continuous dark (DD) condition at farmers' level did not, surprisingly, followed that at laboratory (Figure 4). The major observation was that the hatching peaks did not advance into dark phase of light dark cycles. As seen from the figure, hatching occurred for two consecutive days, day-1 and day-2. Hatching on day-1 was high, as against under LD 12 : 12 condition and it was low on day-2. Added, hatching peaks were very sharp with less hatching durations on both days (day-1 and day-2). The distance between two hatching peaks was invariably ≈ 24 hours, indicating circadian character of hatching activity. The other peculiar observation was that hatching did not show free running nature at farmers' level.

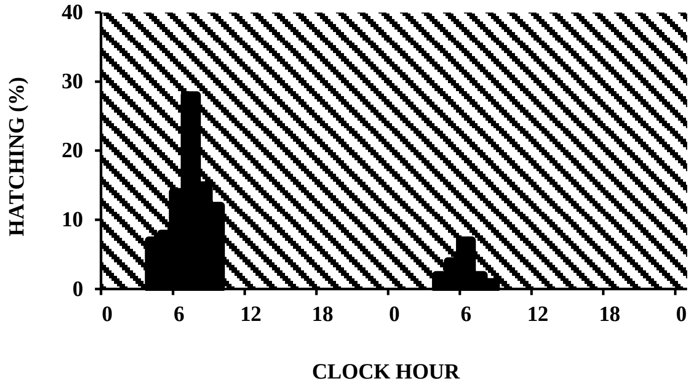

Figure 4: Hatching patterns in silkworm hybrid, CSR2 x CSR4 of *B. mori* at farmers' rearing house under DD conditions. Note, the hatching did not advance into dark phase of LD cycles and hence no free running behaviour.

***The hatching pattern under LL*:**

Data on hatching in CSR2 x CSR4 under continuous light (LL) condition at farmers' level are converted into hourly based percentages and presented in Figure 5. The trend in hatching at farmers' level under LL was identical to that under DD condition. Hatching occurred for two consecutive days, with less hatching on day-1 and more on day-2. With LL at farmers' level, hatching peak did not delayed to occur after lights-on phase (06.00 h) of light dark cycle. Peak hatching occurred very near to 06.00 h, both on day-1 and day-2. Hatching flow on day-1 was uninterrupted while it was definitely interrupted on day-2, indicating the beginning of damping-out situation under LL. Hatching rhythmicity followed the circadian rules as hatching peaks stood ≈ 24 hours apart. The notable feature of hatching under LL, as opposing that observed at laboratory condition, was that it did not show free-running nature and exhibited any delay in expression of hatching peaks.

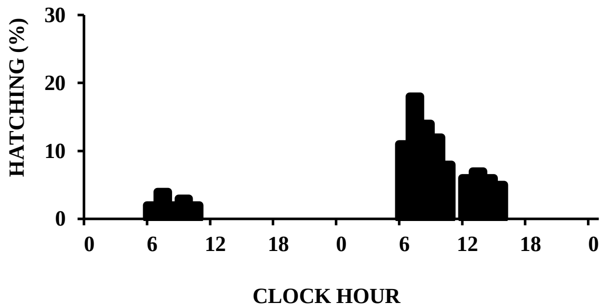

Figure 5: Hatching in CSR2 x CSR4 of *B. mori* under continuous light (LL) conditions at farmers' level.

***The hatching pattern under black-boxing conditions*:**

The egg hatching patterns of CSR2 x CSR4 with Black-Box system of incubation at farmers' followed that observed under laboratory condition alone (Figure 6). However, hatching duration is slightly increased with reduction in hatching peak. Hatching confined to a single day only, as against that under LD 12 : 12, DD and LL. Hatching initiated just before 06.00 h of the day, with peak hatching expression at 06.00 h and completed at 08.00 h of the day.

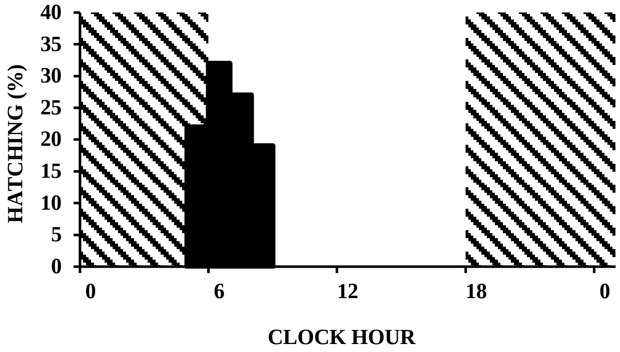

Figure 6: Distribution of hatching in CSR2 x CSR4 of *B. mori* under Black-Box system of incubation. Note hatching for a single day with well defined hatching peak. Hatching duration was just 4 hours.

As seen from the results on hatching patterns under LD 12 : 12, DD, LL and under Black-Box incubation method, there appeared certain characteristics that were not of circadian nature. For example, hatching duration was broadened, 4 to 7 hours at farmers' level under LD 12 : 12 condition of photoperiod. Similarly, hatching did not show free running nature under DD and LL as well. The non-expression of hatching phase advancement under DD and its delay under LL was very pertinent. Further, the hatching under Black-Box method was not very sharp at farmers' level compared to laboratory conditions. It is suspected that some other environmental cues, other than photoperiodic condition are influencing hatching at farmers rearing houses. And hence, the recorded circadian thermo- and hygroperiodic cycles (Figure 1 and 2) are considered in sufficiently explaining the peculiarities in hatching under farmers rearing house condition of controlled photoperiodic conditions, but non-controlled temperature and humidity conditions.

4. DISCUSSIONS

Silkworm egg hatching attracted extensive attention of many researchers at Laboratory level. The present unique attempt is to examine the hatching patterns at farmers' level, with controlling light dark cycles, while the next major environmental factors, such as temperature and humidity are untouched. For these factors, temperature and humidity, only farmers practices were followed. At laboratory level, experimenting on rhythmic phenomenon in egg hatching of *B. mori* were primarily initiated with Mysore race (Pure Mysore, PM) of *Bombyx* silkworm (Anantha Narayana, 1980; Anantha Narayana *et al.*, 1978) in the mid-part of Indian Sericulture Era. Later, such studies were drifted to and concentrated on hybrids, initially with PM x NB4D2 (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990) as PM x NB4D2 was the then ruling multivoltine x bivoltine *Bombyx* silkworm hybrid. Latest situation is that these studies are further extended to and concentrated on two silkworm hybrids, PM x CSR2 (multivoltine x bivoltine hybrid) and CSR2 x CSR4 (bivoltine x bivoltine hybrid) as these two hybrids are ruling the present silk industry in India.

Rhythmic patterns in hatching, as affected by photoperiods in silkworm were reported as early as 1975 (Yamaoka and Hirao, 1975; Yamaoka *et al.*, 1976). These studies were further stepped-up (Anantha Narayana *et al.*, 1978; Sivarami Reddy *et al.*, 1984; Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990, Sivarami Reddy *et al.*, 1998). Hatching in PM occurred at the beginning of Light part in LD cycle and took 'lights-on' as signal of hatching synchronization (Anantha Narayana *et al.*, 1978; Anantha Narayana, 1980). In PM x NB4D2, hatching occurred for a single day under alternative light dark cycles (Sivarami Reddy *et al.*, 1984; Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990, Sivarami Reddy *et al.*, 1998). Under continuous dark (DD) and light (LL) hatching expression was different. In both DD and LL, hatching occurred for two consecutive days. In addition, hatching peak phase advanced under DD and it delayed with LL. Hatching activity recorded spreading or widening under LL (Sivarami Reddy *et al.*, 1984; Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990, Sivarami Reddy *et al.*, 1998). With recent multivoltine x bivoltine (PM x CSR2), the rhythmic characters did not change (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al.*, 2015; Suvarna, 2021), but, hatching occurred for two consecutive days, as against to one day hatching of PM x NB4D2 (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990). With bivoltine x bivoltine (CSR2 x CSR4) hybrids, the rhythmic appearance of hatching was moderately revolutionized that, hatching in CSR2 x CSR4 occurred for two consecutive days, phase-locked to dawn, phase advanced under DD and phase delayed with LL (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al.*, 2015; Suvarna, 2021; Lakshmi *et al.*, 2021).

A new concept of **Black-Box system** of incubation has been introduced recently, which a sudden disturbance or perturbation of DD in the early light phase under maintained DD, at 06.00 h on the day of egg hatching. The Black-Box system of photoperiodic condition resulted in only a single day hatching, highest hatching and further brushing percentage (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021; Lakshmi *et al*., 2021) and excellent hatching economic characters (Lakshmi *et al*., 2021). Anantha Narayana *et al*. (1978), Sivarami Reddy *et al*. (1984), Sivarami Reddy (1993), Sivarami Reddy and Sasira Babu (1990), Sivarami Reddy *et al*. (1998), Shanthan Babu (2014), Srinath (2014), Suvarna *et al*. (2015), Suvarna (2021) and Lakshmi *et al*. (2021) summarized the circadian hatching rhythmic characters; that hatching occurs at the beginning of light phase in LD cycles, phase-locked or taken lights-on as hatching synchronizing signal, circadian expression, free ran under DD and LL conditions, hatching peak phase advanced under DD, phase delayed under LL, hatching expressed a near damp-out condition under LL, more hatching on day-1 under DD and less hatching on day-1 under LL. It is to remind that these reports are at laboratory, with constant temperature and humidity conditions only.

In addition, experimentation with increase or decrease in temperature and humidity at constant level, keeping light dark cycles as scheduled, were also conducted/reported. Thus, Lakshminarayana Reddy (2001), Lakshminarayana Reddy *et al*. (2005), Narasimhulu (2020) and Narasimhulu *et al*. (2020) reported that increase or decrease in temperature and humidity, either separately or in combination did no way alter the rhythmic characteristics. But, these increase or decreased temperature and humidity conditions influenced much the hatching economic parameters. However, these studies too were conducted at laboratory level, but not at farmers rearing house conditions. This observation may be a ready matter for further probing.

The present experiments give sufficient information on hatching patterns in CSR2 x CSR4 at farmers' rearing house condition. CSR2 x CSR4 hatched for 2 consecutive days under all photoperiodic conditions imposed (Figures 3 to 5), except restricted to a single day hatching under Black-Box condition (Figure 6). With LD 12 : 12, photoperiodic condition, the hatching patterns in CSR2 x CSR4 (Figure 3) best matched to earlier reports (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021; Lakshmi *et al*., 2021). However, the hatching appearance at farmers' rearing house did not match to those at laboratory under DD and LL conditions (Figures 4 and 5). Hatching under both DD and LL conditions, invariably, occurred for two consecutive days. Hatching was more on day-1 with DD (Figure 4) and less under LL (Figure 5) condition. However, hatching peak neither phase advanced under DD (Figure 4) condition nor phase delayed with LL condition (Figure 5). Instead, hatching peaks appeared at lights-on phase of normal day LD cycle under both DD and LL (Figures 4 and 5). The only agreement in hatching expression at farmers' level with that under laboratory conditions was that under LL (Figure 5), hatching flow scattered and broadened to express a near 'damp-out' condition (Figure 5). Hatching in CSR2 x CSR4 under Black-Box condition (Figure 6) at farmers' level (present report) readily matched to that under laboratory condition (Shanthan Babu, 2014; Srinath, 2014; Suvarna *et al*., 2015; Suvarna, 2021; Lakshmi *et al*., 2021).

With all the above perplexing observation, especially with DD and LL, it is obvious that hatching in CSR2 x CSR4 is, definitely, a circadian controlled phenomenon. However, the other important circadian characters such as hatching peak phase advancement under DD and its delay under LL at laboratory level are completely missing at farmers' conditions. This uncharacteristic phenomenon hinted in probing into possible influencing factor that entirely or moderately affecting the circadian nature of hatching at farmers' level under certain photoperiodic conditions' DD and LL. Three important environmental factors are reported to be key aspects in controlling *Bombyx* silkworm hatching, light, temperature and humidity (Rahmathulla, 2012). Saunders (2002), reviewing biological clocks, stressed that not alone light dark cycles execute cues and direct the internal clock of insect (and *Bombyx mori*) but also certain other evidencing cyclical fluctuations like temperature, humidity etc., too exert considerable influence in driving the internal oscillations. The attempt that was made to examine such possible circadian fluctuating cycles of temperature and humidity, in the present study, at farmers' rearing houses provided good support to the predictions in unusual behaviour in expressing silkworm hatching circadian characters, especially those observed with DD and LL conditions. Thus, circadian fluctuations in both temperature and humidity at selected five farmers rearing houses were recorded and averaged (Figure 1 and 2 respectively). The cyclical fluctuations in temperature and humidity maintained an inverse relationship. Thus, temperature, which was low during night time, started increasing from 06.00 h and humidity initiated declining. Notably, temperature was low (cool, 27 °C, Figure 1) while humidity was high at 06.00 h of the day (moist, RH 68%, Figure 2). Such cool and humid conditions are more decisive for insect growth and metamorphosis. For example, Pittendrigh (1966) established that relatively high humidity and low temperature in the micro/macro environment during early hours of the day, no doubt, minimizes 'the risk' of desiccation for eclosion in *Drosophila.* Identically, Sivarami Reddy and Sasira Babu (1990) and Sivarami Reddy (1993) reported that hatching in *B. mori* occurred at the early dawn hours of day and attributed that low temperature and high humidity at this point of time minimized the risk of desiccation in both eggs and newly born silkworm larvae. Thus, the temperature and humidity cycles prevailing in rearing houses of farmers, which were otherwise not controlled as in laboratory have implicated manifestation of *Bombyx* hatching (CSR2 x CSR4) only to mimic that of LD 12 : 12 cycles, even under DD and LL. Continuous dark (DD) effected only the magnitude of hatching (reduction of hatching percentage) on day-2 (present study) under farmers' rearing house conditions compared to those under laboratory condition (Lakshmi *et al*., 2021). On the other hand, LL induced no change in hatching rhythm peak expression, appearing at 06.00 h, as in LD 12 : 12 condition. But, LL broadened hatching which is unchanged, leading to initiation of damp-out situation. With Black-Box system of photoperiodic condition, at farmers' level (Figure 6), no change either in hatching peak expression or hatching on a single day was observed, comparable to that at laboratory condition (Lakshmi *et al*., 2012). This situation implies that with LD 12 : 12 condition, DD and Black-Box condition, hatching rhythmicity did not change, hinting at the reports that *B. mori* is a short-day insect (Hirasaka and Koyama, 1970; 1972; Kogure, 1933; De Wilde, 1962; Danilevskii, 1965; Lees, 1968; Beck, 1980; Saunders, 1982; Saunders, 2002). But, LL resulted in inducing broadening of hatching, resulting in initiation of damping-out expression at farmers' level also as in laboratory condition which is not an economic point (Lakshmi *et al*., 2021). Therefore, LL is suspected to induce uneconomic hatching phenomenon with such broadening of hatching, initiation of damp-out situation at initial level of silkworm life cycle (hatching) itself, more hatching durations, less hatching percentage, unacceptably low brushing percentage and appearance of more dead egg percentage (Lakshmi *et al*., 2021). Thus, unnoticed temperature and humidity circadian cycles did affect the circadian nature of hatching rhythmicity in *B. mori* at farmers' level in a subtle but effective way. The present study definitely proved that thermoperiods and hygroperiods prevailed in farmers rearing houses entraining the circadian clocks in hatching at farmers' level, especially under DD and LL. However, it is the beginning of identifying such phenomenon and needs further deep and detailed probe.

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