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RESEARCH ARTICLE

ON THE FIFTH INSTAR LARVAL POPULATION DENSITY REQUIREMENTS FOR LARVAL RIPENING IN THE MULBERRY SILKWORM, *BOMBYX MORI* L.

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ABSTRACT

In the commercial mulberry silkworm, *Bombyx mori* L., the completion of final larval instar stadium is recognized as larval ripening stage, leading to initiation and complete metamorphosis of larval into pupal stage. At this juncture, the silkworm larvae wander and select a suitable site to construct the more awaited economically important silk cocoon, from which continuous silk thread of 800 to 1000 meters length is extracted which is called the queen of textile. Silkworm larval population density, in the final instar stadium is one of the critical factors, influencing successful and uniform commercial cocoon crop. Three fifth instar 'larval population density zones' (LPDZs) were identified viz., a. uneconomical larval population density zone (ULPDZ), b. optimum larval population density zone (OLPDZ) and c. loss larval population density zone (LLPDZ) to describe the fifth instar larval population density requirements of *B. mori*. Experiments were conducted with two commercial silkworm hybrids; multivoltine x bivoltine (PM x CSR2) and bivoltine x bivoltine (CSR2 x CSR4), reared under natural day photoperiod, LD 12: 12 to probe into the implications of the designated three LPDZs on three important larval ripening characteristics. Three larval ripening characteristics; a. fifth instar larval ripening patterns, b. fifth instar larval ripening magnitude (percentage) and c. fifth instar larval ripening period (duration) were considered emphasizing their significance in final instar silkworm rearing. Results irrevocably connoted that the three LPDZs studied influenced two silkworm hybrids differently and decisively. Further, all the three parameters studied were diversely affected too. Ripening patterns of two silkworm hybrids under ULPDZ and OLPDZ did not vary much among themselves while that between these two LPDZs and LLPDZ varied with highly significance. PM x CSR2 took two consecutive peaks (days) to complete larval ripening process while CSR2 x CSR4 took three consecutive peaks (3 days). Ripening patterns in PM x CSR2 and CSR2 x CSR4 exhibited a diurnal circadian rhythmicity with phase-locked to dawn. Further, LLPDZ induced a near arrhythmicity in both of the hybrids studied, leading to appearance of a near damp-out expression. Larval ripening magnitude and larval ripening period were differently reacted to LPDZs. Larval ripening magnitude showed inverse relationship to increased LPDZs. Thus, ULPDZ and OLPDZ registered high ripening magnitude, of course comparable, while ripening magnitude significantly decreased according to increase in LLPDZ (statistically significant). Opposing the ripening magnitude, ripening duration was directly related to increase in LPDZ. Thus, low larval ripening duration was observed for the first two larval population density zones (LPDZs); uneconomical larval population density zone (ULPDZ) and optimum larval population density zone (OLPDZ). The larval ripening period was high for loss larval population density zone (LLPDZ) compared to ULPDZ and OLPDZ and the differences were statistically significant. Results were discussed on the importance of identifying the larval population density zones in to three LPDZs (ULPDZ, OLPDZ and LLPDZ) and their applicability in commercial mulberry silkworm rearing. Finally, it is suggested that optimum larval population density zone (OLPDZ) is the most economic LPDZ for profitable commercial mulberry silkworm rearing.

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INTRODUCTION

Silkworms are the larval or caterpillar form of domesticated mulberry silk moth, *Bombyx mori* L. that are of commercial value as these larvae are the producer of the queen of textile, the silk.

Native to northern China, the silk moth was domesticated from its wild progenitor species, *Bombyx mandarina*. Silkworm is greatly exploited for valuable silk thread. The most economic important stage in the silkworm rearing is the cocoon, spun by the larva themselves immediately after completion of fifth and final larval stadium, denoted by ripening stage. Sericulture is the science that deals with the production of silk through

rearing of silkworm. Silk is preferred over all the other fibers. India is ranked as second largest producer of silk in the world, next to China. Insect growth is discontinuous and is characterized by a series of moults. The uniformity in occurrence of these series of moults (four larval-to-larval moults, one larval-to-pupal moult and one pupal-to-moth eclosion) is the main important aspect in commercial sericulture. Many biotic and abiotic factors greatly affect the growth and development of the commercial silkworm, *B. mori* (Rahmathulla, 2012). Of these factors, photoperiod, temperature and humidity and population density are most important ones in the silkworm rearing. The earlier works on these aspects dealt with the impacts of photoperiod on the silkworm, *Bombyx mori* (Sivarami Reddy, 1993), temperature and humidity (Lakshminarayan Reddy, 2001), expression of mixed age characteristics (non-uniformity, Shanthan Babu, 2014; Srinath, 2014) with special reference to the photoperiod. However, one of the most important aspects of commercial silkworm rearing, the final larval instar density did not get adequate attraction.

Only limited references are available on the fifth instar larval density aspect in insects. Applebaum and Heifetz (1999) reviewed the density dependent physiological phases in insects. For the *B. mori*, Dar and Singh (1991) studied the influence of population density of silkworm on some economic traits. Saha et al. (2009) worked out on determination of larval critical weight in the *Bombyx* silkworm. Lakshminarayana Reddy et al., (2015) made certain initiations in this direction. Dar and Singh (1991) reported that population group of 300 worms per 6 feet² exhibited good results in spring whereas the population group of 200 worms behaved invariably well in summer and autumn. The commercial mulberry silkworm, *B. mori* enters a period of rapid growth after its fourth and final larval-to-larval ecdysis. Therefore, the entire studies of larval density effects on the silkworm, *Bombyx mori* were concentrated on the fifth instar larvae and cocooning. The larval period in the silkworm life cycle is the most crucial one as this stage is the only feeding stage for the species to continue further through pupa, moth and egg stage. Larvae feed on mulberry foliage and grow to their maximum size to pass onto the next stage. The growth in the fifth instar is rather visibly high than those of the earlier instars.

Therefore, the entire investigations, pertaining to larval population density, were concentrated in the fifth instar period only. Though the fifth instar larval period recorded high growth, studies on their rearing spacing requirement are scanty. Limited publications on the larval rearing spacing are available. Rajan et al. (2003) recommended a spacing of 50 to 70 larvae per feet² for fifth instar bivoltine silkworm to reduce secondary contamination, to support enough growth, to obtain better cocoon yield and to improve silk quality. Except Lakshminarayana Reddy et al., (2015) who made certain initiations in this direction, such studies are rare. Lakshminarayana Reddy et al., (communicated) further categorized these larval population density requirements in to three distinct zones; a. uneconomical larval population density zone, b. optimum larval population density zone and c. loss larval population density zone. In the present communication, the implications of these larval population density zones are studied on three important larval ripening characteristics; a. larval ripening patterns, b. larval ripening magnitude and c. larval ripening period.

MATERIALS AND METHODS

Procurement of silkworm hybrids: For the present experimentation, two mulberry silkworm (*B. mori*) hybrids; one from multivoltine x bivoltine hybrid, PM x CSR2 and the other from bivoltine x bivoltine hybrid, CSR2 x CSR4 that are popularly exploited for commercial silkworm rearing in the contemporary Indian sericulture are selected. The eggs of silkworm, commonly called as DFLs (disease free layings; each DFL is group of 400 to 500 silkworm eggs laid by a single silk moth on a single day on specific paper) of the two hybrids were procured from the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Mysore, India. The DFLs were transported to the Department of Sericulture, Sri Krishnadevaraya University, Anantapur; where the investigations were carried out, during evening cool hours. The DFLs, at the work spot were immediately spread into the pre disinfected plastic rearing trays (Nilkamal, India).

Silkworm rearing method: The silkworm rearing method followed was that as advocated by Krishnaswami (1986). The chawki (young age; I & II instar silkworm larvae) rearing was not conducted to maintain uniformity in climatic conditions all through the experimentation. Hatched out larvae from the egg sheet, collected into pre-disinfected rearing trays were daily fed three times (06.00, 14.00 and 22.00 h) of the day on fresh mulberry (*Morus* sp., V1 variety) leaves except during larval-to-larval moulting. While cleaning the unspent leaves 2 times during first and second instar periods and once in every day after second moult, the larvae were transferred into separate pre-disinfected rearing trays. The larvae under moult were not disturbed. Temperature maintained in the laboratory was 25 ± 1°C all through the experimentation. Relative humidity (RH) of 80 ± 5% was maintained in the laboratory. The same day when the DFLs were brought to the laboratory, they were introduced, till adult eclosion in five replications, into 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 are exposed, did not exceed 50 lux.

Fixing fifth instar larval density: The fifth instar larval density has been described as number of larvae/feet² (Rajan et al., 2003; Lakshminarayana Reddy et al., 2015). Lakshminarayana Reddy et al. (2015) categorized all the fifth instar larval population density regimes into three groups; a. uneconomical larval population density zone, b. optimum larval population density zone and c. loss larval population density zone.

Identification of representative larval population density zones (LPDZs): For better understanding convenience, these three larval population density zones (LPDZs) were designated and abbreviated as ULPDZ, OLPDZ and LLPDZ respectively. In the present study all LPDZs are symbolized by taking one representative larval density from each LPDZs and accordingly studies are planned. For PM x CSR2, larval population of 40 number of larvae/ft² was taken as the representative of ULPDZ. Similarly, larval population density

of 80 number of larvae/feet² and 130 number of larvae/ft² were regard as representatives of OLPDZ and LLPDZ respectively. For CSR2 x CSR4, the representative of ULPDZ and LLPDZ were as that of PM x CSR2. However, the representative larval population density of 70 number of larvae/ft² was taken for OLPDZ. For each LPDZ representative/silkworm hybrid combination, 5 replications were maintained. Till the completion of the fourth larval-to-larval ecdysis, the silkworm larvae were reared commonly. The larvae were shifted to plastic rearing trays of 2' x 3' dimension immediately after completion of fourth larval-to-larval ecdysis (one tray each for each treatment, each silkworm hybrid and each replication).

Recording of data: Three parameters, denoting the importance of silkworm larval ripening activity *viz.*, a. larval ripening pattern (distribution of larval ripening through solar day time), b. larval ripening magnitude (percentage, the ratio of number of successful larvae completed ripening in the population kept for experimentation over that of actual larvae assigned for ripening) and c. larval ripening period (duration, time lapse from the initiation of ripening by the first larva in the designated population and completion of ripening by the last individual larva) for two silkworm hybrids (PM x CSR2 and CSR2 x CSR4) and for each of five replications. Analysis of data: The data were treated for average and standard deviation. Further, data were analyzed statistically (ANOVA).

RESULTS

For better understanding, the results on the larval population density requirements for larval ripening in the mulberry silkworm, *B. mori* are explained in three sub-headings; 1. larval ripening patterns, 2. larval ripening magnitude (percentage) and 3. larval ripening period (duration) under three distinct larval population density zones *viz.*, a. uneconomical larval population density zone (ULPDZ), b. optimum larval population density zone (OLPDZ) and c. loss larval population density zone (LLPDZ) with two silkworm hybrids, PM x CSR2 and CSR2 x CSR4.

Larval ripening patterns of multivoltine x bivoltine hybrid, PM x CSR2: Larval ripening patterns of PM x CSR2 under ULPDZ: Results on the ripening patterns in PM x CSR2 under ULPDZ, larval population density zone represented by larval population density of 40 number of larvae/ft² are presented as chronogram in Figure 1.

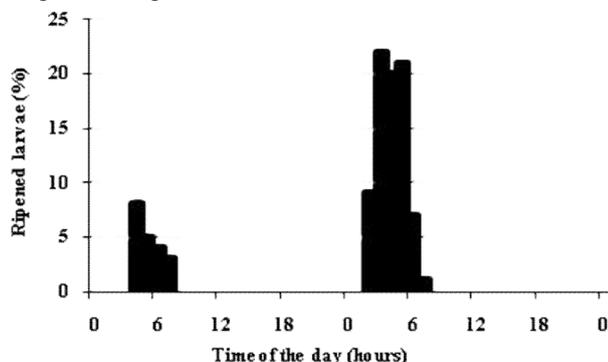


Figure 1: Chronographic representation of larval ripening pattern in the multivoltine x bivoltine silkworm hybrid (*Bombyx mori* L., PM x CSR2) under uneconomic larval population density zone (ULPDZ) represented by larval population density of 40 number of larvae/ft². Note the ripening pattern represented by two distinct chronograms occurring for two consecutive days, 24 h apart from each other. Also, note the peak of ripening on day-one is low over that of day-two and peak expression of ripening is very sharp.

Ripening pattern in PM x CSR2 under ULPDZ, represented by a larval population density of 40 number of larvae/ft² occurred for two consecutive days, revealing a circadian rhythmic expression as evidenced from the 24 h interval between two peaks (Figure 1). The first peak of the ripening activity in larvae of PM x CSR2 occurred at or around 06.00 h of the day, thus phase-locked to dawn, therefore should be termed as diurnal activity. The second phase also occurred at or around 06.00 h, apart from 24 h of first peak of ripening pattern indicating the circadian nature. The magnitude of ripening on day-one is less than that of day-two. The peak expression of ripening on both days is rather sharp, with no broadening of ripening activity.

Larval ripening patterns of PM x CSR2 under OLPDZ: Figure 2 represents the results on ripening patterns in PM x CSR2 under optimum larval population density zone (OLPDZ), represented by larval population density of 80 number of larvae/ft². It is recorded that the ripening pattern in PM x CSR2 under OLPDZ, represented by a larval population density of 80 number of larvae/ft² occurred for two consecutive days, representing a circadian rhythmicity, as clear from 24 h interval between two peaks (Figure 2). The first peak of ripening activity in larvae of PM x CSR2 occurred at or around 06.00 h of the day, thus phase-locked to dawn, thus, a diurnal representation. The second phase also occurred at or around 06.00 h of local time, apart from 24 h of the first peak expression of ripening pattern, indicating circadian rhythmicity in ripening pattern. The magnitude of ripening on day-one is less than that of day-two. The peak expression of ripening on both days is very sharp, without broadening of ripening activity.

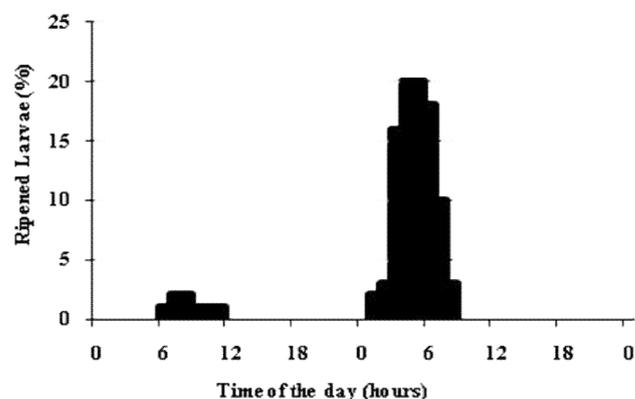


Figure 2: Chronographic representation of larval ripening pattern in the multivoltine x bivoltine silkworm hybrid (*Bombyx mori* L., PM x CSR2) under optimum larval population density zone (OLPDZ) represented by larval population density of 80 number of larvae/ft². Note the ripening pattern represented by two distinct chronograms occurring for two consecutive days, 24 h apart from one other. Also, note that the magnitude of ripening on day-two is high over that of day-one and peak ripening activity is sharp

Larval ripening patterns of PM x CSR2 under LLPDZ: Figure 3 depicts the results on ripening patterns in PM x CSR2 under loss larval population density zone (LLPDZ), represented by larval population density of 130 number of larvae/ft². It is observed that the ripening pattern in PM x CSR2 under LLPDZ, represented by a larval population density of 130 number of larvae/ft² expressed in two consecutive days, representing a circadian rhythmicity, as evidenced from 24 h interval between two peaks (Figure 3).

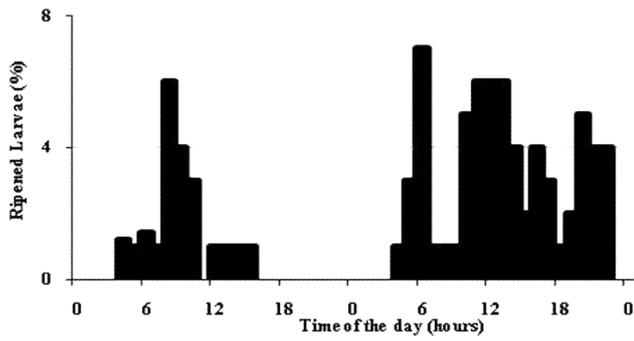


Figure 3. Chronographic representation of larval ripening pattern in the multivoltine x bivoltine silkworm hybrid (*Bombyx mori L.*, PM x CSR2) under loss larval population density zone (LLPDZ) represented by larval population density of 130 number of larvae/ft². Note the ripening pattern represented by two chronograms occurring for two consecutive days, 24 h apart from each other and day-two ripening activity extended to 00.00 h. Also, note that the magnitude of ripening on day-one is less over that on day-two and the peak activity is broadened mimicking near damped-out rhythmicity.

The first peak of ripening activity in PM x CSR2 occurred at or around 06.00 h of the day, thus phase-locked to dawn, thus, a diurnal representation. The second phase also occurred at or around 06.00 h of local time, apart from 24 h of the first peak expression of ripening pattern, indicating circadian rhythmicity in ripening pattern. However, the ripening pattern on day-two extended to 00.00 h and thus broadened. The magnitude of ripening on day-one is less than that of day-two. The peak expression of ripening on both days is not so sharp, with broadening of ripening activity on day-two, thus revealing a near damped-out situation.

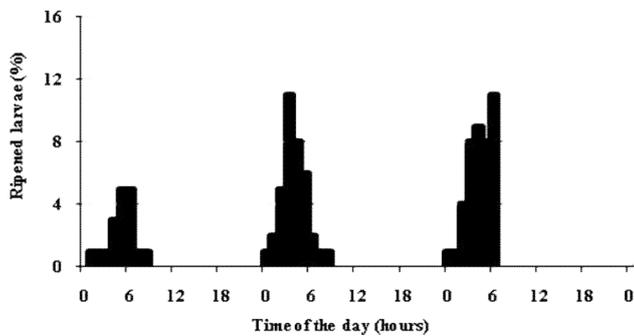


Figure 4. Chronographic representation of larval ripening pattern in the bivoltine x bivoltine silkworm hybrid (*Bombyx mori L.*, CSR2x CSR4) under uneconomic larval population density zone (ULPDZ) represented by larval population density of 40 number of larvae/ft². Note the ripening pattern represented by three distinct chronograms occurring for three consecutive days, 24 h apart from each consecutive peaks. Also, note the magnitude of ripening on day-one is low over that of day-two and day-three. Also, the peak expression of ripening on all three days is very sharp

Larval ripening patterns of bivoltine x bivoltine hybrid, CSR2 x CSR4: Larval ripening patterns of CSR2 x CSR4 under ULPDZ: Results on the ripening patterns in CSR2 x CSR4 under ULPDZ represented by larval population density of 40 number of larvae/ft² are presented as chronogram in Figure 4. The ripening pattern in CSR2 x CSR4 under ULPDZ, represented by a larval population density of 40 number of larvae/ft² occurred for three consecutive days, representing a circadian rhythmic expression as evidenced from 24 h interval between two consecutive peaks (Figure 4).

The first peak of the ripening activity in larvae of CSR2 x CSR4 occurred at or around 06.00 h of the day, thus phase-locked to dawn, therefore should be termed as diurnal activity. The second and third peaks also occurred at or around 06.00 h, apart from 24 h of consecutive peak occurrence of ripening pattern indicating a circadian nature. The magnitude of ripening on day-one is less than that of day-two and day-three. The peak expression of ripening on all the three days is rather sharp, without any broadening of ripening activity.

Larval ripening patterns of CSR2 x CSR4 under OLPDZ: Figure 5 represents the results on ripening patterns in CSR2 x CSR4 under optimum larval population density zone (OLPDZ), represented by larval population density of 70 number of larvae/ft². It is recorded that ripening pattern in CSR2 x CSR4 under OLPDZ, represented by a larval population density of 70 number of larvae/ft² occurred for three consecutive days, representing a circadian rhythmicity, as is clear from 24 h interval between two consecutive peaks (Figure 5). The first peak of the ripening activity in CSR2 x CSR4 occurred at or around 06.00 h of the day, thus phase-locked to dawn and expressing a diurnal pattern. The second and third phases also occurred at or around 06.00 h of local time, apart from 24 h of the two consecutive peak expression of ripening pattern, indicating circadian rhythmicity in ripening pattern. The magnitude of ripening on day-two is high than that of day-one and day-three. The peak expression of ripening on all the three days is very sharp, without broadening of ripening activity.

Larval ripening patterns of CSR2 x CSR4 under LLPDZ: Figure 6 depicts the results on the ripening patterns in CSR2 x CSR4 under loss larval population density zone (LLPDZ), represented by larval population density of 130 number of larvae/ft².

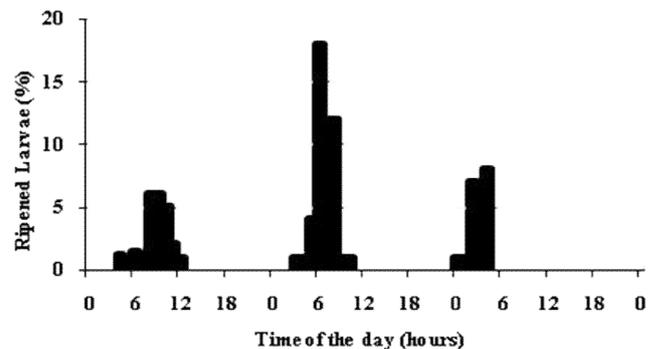


Figure 5. Chronographic representation of larval ripening pattern in the bivoltine x bivoltine silkworm hybrid (*Bombyx mori L.*, CSR2 x CSR4) under optimum larval population density zone (OLPDZ) represented by larval population density of 70 number of larvae/ft². Note the ripening pattern represented by three distinct chronograms occurring for three consecutive days, 24 h apart from each consecutive peak. Also, note that the magnitude of ripening on day-two is high over that of day-one and day-three and the peak activity is very sharp.

It is observed that the ripening pattern in CSR2 x CSR4 under LLPDZ, represented by a larval population density of 130 number of larvae/ft² occurred for three consecutive days, representing a circadian rhythmicity, as evidenced from 24 h interval between two consecutive peaks (Figure 6). The day-one peak of ripening activity in CSR2 x CSR4 occurred at or around 06.00 h of the day, phase-locked to dawn and thus a diurnal representation.

The day-two ripening phase delayed to occur at or around 12.00 h of local time and the day-three peak at or around 18.00 h, indicating circadian rhythmicity in ripening pattern. However, the ripening pattern on day-two extended to 00.00 h and thus broadened expressing a near damp-out condition. The magnitude of larval ripening on day-one is less than that of day-two. The peak expression of ripening on both days is not so sharp, with broadening of ripening activity on day-two.

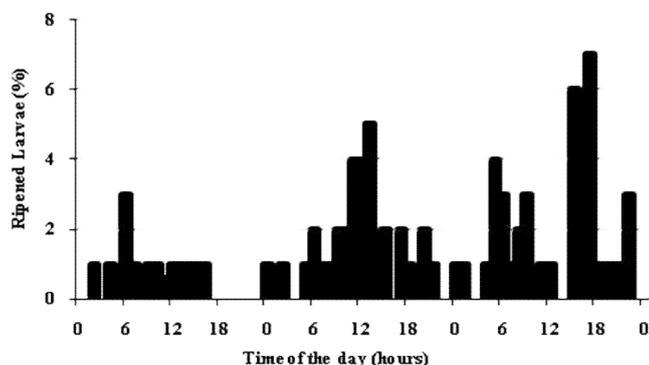


Figure 6. Chronographic representation of larval ripening pattern in the bivoltine x bivoltine silkworm hybrid (*Bombyx mori* L., CSR2 x CSR4) under loss larval population density zone (LLPDZ) represented by larval population density of 130 number of larvae/ft². Note the ripening pattern represented by three chronograms occurring for three consecutive days, 24 h apart from each consecutive peak. Also, note that the magnitude of ripening on day-three is high over that on day-one and day-two and the peak activity is seen more broadened, representing a near damped-out expression.

Larval ripening magnitude (percentage) of two silkworm hybrids, PM x CSR2 and CSR2 x CSR4: Data on the larval ripening magnitude (%) for the two mulberry silkworm hybrids, PM x CSR2 and CSR2 x CSR4 reared under three LPDZs are presented in Table 1. Larval ripening was inversely related to the increase in larval population density. Larval ripening (%) under ULPDZ and OLPDZ for both the hybrids was above 95%. Thus, larval ripening (%) for PM x CSR2 under ULPDZ was 96% and the same with OLPDZ was also 96% and therefore non significantly different. The larval ripening (%) for PM x CSR2 with LLPDZ was 91% and is significantly low from that of ULPDZ and OLPDZ conditions. Similar results, on larval ripening magnitude were noticed with CSR2 x CSR4 hybrid. Larval ripening for CSR2 x CSR4 with ULPDZ was 97% and the same with OLPDZ was almost equal (98%) to that with ULPDZ. Thus, the two conditions (ULPDZ and OLPDZ) recorded larval ripening magnitude with no statistical difference, and therefore, *on-par*. However, larval ripening percentage with LLPDZ condition was 89% and statistically low compared to that under ULPDZ and OLPDZ.

Table 1. Larval ripening magnitude (%) in larval population of the mulberry silkworm (*Bombyx mori* L.) hybrids, PM x CSR2 (multivoltine x bivoltine hybrid) and CSR2 x CSR4 (bivoltine x bivoltine hybrid) with three fifth instar larval population density zones (LPDZs); uneconomical larval population density zone (ULPDZ), optimum larval population density zone (OLPDZ) and loss larval population density zone (LLPDZ).

Larval population density zones (LPDZs)	Larval ripening magnitude (%)	
	PM x CSR2	CSR2 x CSR4
ULPDZ	95.800 ± 2.588	97.000 ± 1.000
OLPDZ	96.200 ± 3.421	97.800 ± 1.483
LLPDZ	90.600 ± 4.393	89.200 ± 2.683

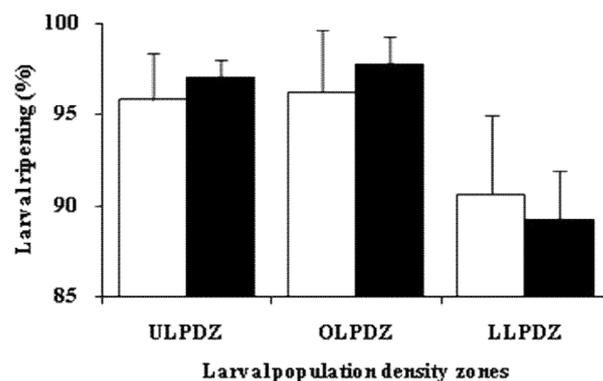


Figure 7. Graphic representation of larval ripening magnitude (%) in two silkworm hybrids, PM x CSR2 (open bars) and CSR2 x CSR4 (closed bars) of *Bombyx mori* L., under three population density zones (LPDZs). Note *on-par* ripening percentage among ULPDZ and OLPDZ while that of LLPDZ is statistically significantly low. Also note the inverse relation between LPDZ and larval ripening percentage.

Data on larval ripening percentage was also represented as histogram in Figure 7. The differences in ripening magnitude for two silkworm hybrids, PM x CSR2 and CSR2 x CSR4 with three LPDZs (ULPDZ, OLPDZ and LLPDZ) expressed clear-cut and distinct differences. The relationship between ripening percentage and LPDZs was inverse relation. With low LPDZ (ULPDZ and OLPDZ), ripening was high and it decreased as LPDZ increased. The initial two LPDZs (ULPDZ and OLPDZ) did not show any statistical difference among themselves. However, larval ripening magnitude was statistically ($p < 0.05$) different when compared between initial LPDZs and LLPDZ.

Larval ripening period (duration) of two silkworm hybrids, PM x CSR2 and CSR2 x CSR4: Data on the larval ripening period (duration) for the two mulberry silkworm hybrids, PM x CSR2 and CSR2 x CSR4 reared under three LPDZs are presented in Table 2. Larval ripening expressed a direct relation to the increase in larval population density. Larval ripening duration under ULPDZ and OLPDZ for both the hybrids was around 24 h. Thus, larval ripening duration for PM x CSR2 under ULPDZ was 23.4 h and the same with OLPDZ was 26 h and therefore non-significantly different. The larval ripening period for PM x CSR2 with LLPDZ was 33 h and is significantly high from that of ULPDZ and OLPDZ conditions. Similar results on larval ripening duration were noticed with CSR2 x CSR4 hybrid. Larval ripening for CSR2 x CSR4 with ULPDZ was 29 h and the same with OLPDZ was almost equal, recording 28 hours, to that with ULPDZ. Thus, the two conditions (ULPDZ and OLPDZ) recorded larval ripening duration with no statistical difference and therefore, *on-par*. However, larval ripening period with LLPDZ condition was 44 hours and statistically high compared to that under ULPDZ and OLPDZ.

Data on larval ripening duration was also represented as histogram in Figure 8. It is generally noted that the ripening durations in bivoltine x bivoltine (CSR2 x CSR4) are more compared to that of multivoltine x bivoltine (PM x CSR2) hybrid. The differences in ripening durations for two silkworm hybrids, PM x CSR2 and CSR2 x CSR4) with three LPDZs (ULPDZ, OLPDZ and LLPDZ) expressed clear-cut and distinct differences.

Table 1: Larval ripening period (duration) in larval population of the mulberry silkworm (*Bombyx mori* L.), hybrids, PM x CSR2 (multivoltine x bivoltine hybrid) and CSR2 x CSR4 (bivoltine x bivoltine hybrid) with three fifth instar larval population density zones (LPDZs); uneconomical larval population density zone (ULPDZ), optimum larval population density zone (OLPDZ) and loss larval population density zone (LLPDZ).

Larval population density zones (LPDZs)	Larval ripening period (duration) in hours	
	PM x CSR2	CSR2 x CSR4
ULPDZ	23.400 ± 1.673	28.600 ± 4.219
OLPDZ	25.600 ± 1.670	27.800 ± 2.864
LLPDZ	33.200 ± 5.357	44.400 ± 3.578

The relationship between ripening duration and LPDZs was direct. With low LPDZ (ULPDZ and OLPDZ), ripening period was low and it increased as LPDZ increased. The initial two LPDZs (ULPDZ and OLPDZ) did not show any statistical difference among themselves for ripening duration. However, larval ripening duration for LLPDZ was statistically ($p < 0.05$) difference from both the initial LPDZs (ULPDZ and OLPDZ).

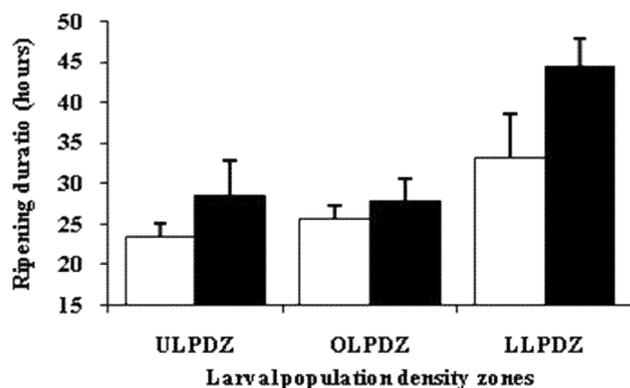


Figure 8. Graphic representation of larval ripening period (duration) in two silkworm hybrids, PM x CSR2 (open bars) and CSR2 x CSR4 (closed bars) of *Bombyx mori* L., under three population density zones (LPDZs). Note *on-par* ripening duration among ULPDZ and OLPDZ while that of LLPDZ is statistically significantly high. Also note the direct relation between LPDZ and larval ripening duration.

DISCUSSION

The entire discussion in the present communication draws the basic support from attaining an optimum/maximum size/weight by fifth instar silkworm larvae and consequential hormonal control on the metamorphosis in *Bombyx mori*. It is apparent that the behavioral phases between eating period (larval) and that of pre-pupal are very distinct in *Bombyx mori*. The transitional phase between larval and pre-pupal period is distinguished by cessation of feeding, change in larval colour and larval wandering in search of a suitable place for spinning its pupal protective nest, the cocoon. This transitional phase was distinctly identified as 'wandering stage' by many researchers (Piepho *et al.*, 1960; Lounibos, 1976; Riddiford, 1980; Truman and Taghert, 1981; de Wilde *et al.*, 1980). In the present study, however, this classic stage is termed as 'ripening stage' as this terminology is broadly and extensively used in the contemporary Indian sericulture (Krishnaswami *et al.*, 1973; Krishnaswami, 1986; Sivarami Reddy, 1993; Sivarami Reddy *et al.*, 1993; Shanthan Babu, 2014; Srinath, 2014; Srinath *et al.*, 2018).

Insects are extremely diverse group of organisms. However, endocrine control of growth, eclosion and metamorphosis within this group are notably identical (Riddiford, 1980). Well established fact is that the major features of moulting cycles are controlled by a sequence of three hormones; PTTH, ecdysone and JH (Riddiford, 1980; Reynolds, 1980; Truman and Taghert, 1981; Happ, 1984). When JH is present at high levels in haemolymph, the moulting cycle proceed to produce additional larval or nymphal stage (Williams, 1961; Riddiford, 1980; Happ, 1984). On the opposite side, if the JH titer is very low or virtually absent, morphogenesis is initiated leading to the production of pupal stage. The JH which is responsible for the maintenance of larval characters (Riddiford, 1980) remains high, up to ultimate larval-to-larval moult, and suddenly decreases to plateau level when final instar larva attains its maximum size. At this point of time, brain releases PTTH during the next allowable gate and subsequently causes the secretion of first installment of ecdysone, 5 to 8 times less in quantity (as in *M. sexta*, Riddiford, 1980) than that necessary to elicit ecdysis which itself has, in the absence of JH, a profound effect on the insect larva, leading to the initiation of metamorphosial process.

The second release of PTTH, approximately 1.5 to 2 days before the larval-to-pupal ecdysis, as in *M. sexta* (Riddiford, 1980; Truman and Taghert, 1981) and in *Bombyx mori* (Shimada, 1989) in turn releases the second installment of ecdysone that is 5 to 8 times more than of the first installment (Riddiford, 1980). This hormone primarily executes the pupation process, starting with apolysis (Hinton, 1973). Truman (1972) and Truman and Taghert (1981) demonstrated that ecdysis itself is not gated but occurs after certain fixed hours of gated PTTH release (Beck, 1980; Truman, 1972; Truman and Taghert, 1981). The synchrony of ecdysis is solely dependent on the gated release of PTTH (Beck, 1980) and the release of PTTH dependent on attaining of optimum size/weight by larvae (Riddiford, 1980). The release of PTTH in the insect system immediately causes the release of ecdysone in turn, initiating ecdysial process. During the final instar larval period the ecdysone at low concentration, in the absence of JH, initiates metamorphosis behavior (ripening, in the present study).

The time between the first release of PTTH and appearance of wandering stage (as in *M. sexta*) or ripening (as in *B. mori*, in the present study) should be apparently more and this period is utilized for search of a suitable site and completion of cocooning at preferred and safe site. Thus, the explanation of the present investigation results mainly take support from hormonal control of ripening and from further attaining of optimum size/weight by fifth instar *Bombyx* larvae. Further the works on photoperiod connected to silkworm larval ripening also taken as secondary support, since the worms were reared under natural solar day photoperiodic conditions, LD 12 : 12. It is apparent that the release of PTTH in the population of PM x CSR2 in the final instar larval period is in two gates as supported by two peaks of ripening for two consecutive days (Figure 1 to 3). On the other hand, CSR2 x CSR4 exhibited three peaks of ripening in three consecutive gates (days, Figure 4 to 6)). Shanthan Babu (2014), Srinath (2014) and Srinath *et al.* (2018) also reported 2 days ripening for PM x CSR2 and 3 days ripening for CSR2 x CSR4 by Srinath (2014) and Srinath *et al.* (2018). They (Shanthan Babu, 2014; Srinath, 2014; Srinath *et al.*, 2018) further reported that this non-uniformity

in rhythmic expression of ripening should be due to its long larval period, starting from hatching to ripening through a series of larval-to-larval moults in *Bombyx* silkworm. Hatching, being the initial developmental marker event in *B. mori* was restricted to a single day (Shanthan Babu, 2014; Srinath, 2014) and at the end of final instar larval period, the ripening is considered to be mostly non-uniform. In the case of PM x CSR2, the uniformity is not such complicated (Figures 1 to 3). Truman (1972) reported that uniformity in development in a population will be dependent on instar duration and larval size. The larval size of PM x CSR2 is less compared to CSR2 x CSR4. Further, the fifth instar larval duration of PM x CSR2 is less compared to CSR2 x CSR4 (Shanthan Babu, 2014; Srinath, 2014). Hence, it is predicted that the synchronization in ripening of PM x CSR2 larvae would be considerably fine compared to CSR2 x CSR4. Thus, the two silkworm hybrids, PM x CSR2 and CSR2 x CSR4 are irrevocably under circadian clock control with a gating periodicity of around 24 h, of course, with the secretion of PTTH and further ecdysone.

As observed for egg, phase locked hatching to dawn in *B. mori* by Sivarami Reddy and Sasira Babu (1990) the larval ripening also occurred immediately after or at dawn, referring to phase locked to dawn and thus diurnal rhythmicity. Further, the rhythmic peaks appeared 24 h apart and therefore should be treated as circadian. It could be inferred that the occurrence of larval ripening during early hours of the day might be due to the relatively high humidity in the micro/macro environment which minimizes 'the risk' of desiccation, as demonstrated for egg hatching in *B. mori* (Sivarami Reddy and Sasira Babu, 1990) and eclosion in *Drosophila* (Pittendrigh, 1966). It is obvious that with the increase in larval population density, the fifth instar larval duration increased with negative relation in larval optimum growth (weight, Lakshminarayana Reddy *et al.*, 2015). Therefore, the attaining of larval optimum weight is evidently delayed for larvae under LLPDZ and the resultant broadening of larval ripening phenomenon in peak appearance, compared to sharp ripening patterns under ULPDZ and OLPDZ as well. The broadening of larval ripening patterns has been demonstrated for *B. mori* larvae under continuous light (LL) conditions by many researchers (Sivarami Reddy, 1993; Sivarami Reddy *et al.*, 1990; Lakshminarayana Reddy *et al.*, 2001; Shanthan Babu, 2014; Srinath, 2014; Srinath *et al.*, 2018) indicating that *B. mori* larvae exhibit a near arrhythmicity under continuous light (LL) as the larvae enter into a stage of exhibiting mixed-age characteristics (Shanthan Babu, 2014; Srinath, 2014; Srinath *et al.*, 2018), not an uniformity issue for ripening. In the present study, the larval ripening pattern under LLPDZ expressed a broadening phenomenon, just like that under LL, as reported by Shanthan Babu (2014) and Srinath (2014). Therefore, it is safe to consider that ripening patterns in *B. mori* larvae under LLPDZ, in the present study, are not only broadening, expressing a near arrhythmicity (damp-out) as under LL conditions.

Therefore, high larval population leads to more damp-out situation (Sivarami Reddy, 1993) and mixed age characteristics (Shanthan Babu, 2014; Srinath, 2014). Hence, the larval ripening pattern should be considered as circadian, phase-locked to dawn, diurnal and gated. The next important *Bombyx* issue, in the present study is the ripening magnitude (percentage). As observed for ripening magnitude (Figure 7), it is inferred that ripening magnitude is inversely related to LPDZs.

Thus, under less LPDZs (both ULPDZ and OLPDZ), the ripening was high. The same was less in more LPDZ (LLPDZ). It is quite possible that under over crowded situations, larvae do not get sufficient food for their scheduled growth and further metamorphosis leading to increased unequal percentage (Lakshminarayana Reddy, communicated) and leading this unequal population percentage into infection (Rajan *et al.*, 2003; Mithilesh Kar *et al.*, 2009), further leading to mortality and failures in ripening. To explain the ripening duration issue, it is different (Figure 8) from that of ripening magnitude. It has evidenced a direct and positive response to the increased LPDZs. The less ripening duration is attributed to much sufficient available space to the larvae and further less competition among members in larval population for food (mulberry leaf). With ULPDZ and OLPDZ, the larvae got sufficient space and food leading to uniform growth and consequent attainment of optimum size/weight for PTTH release (Riddiford, 1980) followed by eclosion hormone secretion. This situation appears to be crucial for more ripening percentage under ULPDZ and OLPDZ rather than in LLPDZ. External source of phytoecdysteroid (ecdysone, moulting hormone mimic) were administered to silkworm larvae at 5% ripening stage by many researchers to increase ripening percentage/uniformity and decreasing ripening duration as well (Kanika Trivedi *et al.*, 2003; Sashindran Nair *et al.*, 2005; Nirmal Kumar *et al.*, 2006, 2007; Shanthan Babu, 2014; Srinath, 2014; Srinath *et al.*, 2009).

Necessity of categorizing fifth instar *Bombyx* larval population density into three different and specific zones, ULPDZ, OLPDZ and LLPDZ is a matter of serious concern. Though no statistical differences in either of larval ripening patterns, larval ripening and larval ripening magnitude are noticed between ULPDZ and OLPDZ, the issue of keeping them separate entities is in the interest of commercial silkworm rearing farmer. Farmers' attitude always will be intensive cropping with limiting space and resources. Therefore, providing huge space for limited larval population, in the views of farmer, will definitely be uneconomical and this larval population zone is termed as uneconomical larval population density zone (ULPDZ). Without opting for scarification on quantity and quality in cocoon crop, the optimum larval population density (OLPDZ) is coined. Further, the extreme larval population density zone has been named as loss larval population density zone (LLPDZ) where the question of getting economic silkworm crop itself is questioned and further at the adverse situations, the entire silkworm cocoon crop is lost.

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