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RESEARCH ARTICLE

INDICES STUDIES ON FIFTH INSTAR LARVAL POPULATION DENSITY REQUIREMENTS IN THE COMMERCIAL MULBERRY SILKWORM, *BOMBYX MORI* L.

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ABSTRACT

The final larval instar in the mulberry silkworm, *Bombyx mori* L. is very decisive period in commercial silkworm rearing. Silkworm larval population density, in the final instar stadium is one of the critical factors for successful commercial cocoon crop. Experiments were conducted with two commercial silkworm hybrids, PM x CSR2 and CSR2 x CSR4 to probe into the optimal larval population density requirements for commercial silkworm cocoon crop during fifth instar silkworm larval period. Four important and directly connected characteristics in the fifth instar larval period, fifth instar larval duration, fifth instar larval growth, fifth instar larval unequal percentage and ripening period after fifth instar larval period were considered in determining the larval population density requirements during fifth instar larval period. Results irrevocably signified that the fifth instar larval population density is directly related to three characteristics studied; fifth instar larval period, fifth instar larval unequal percentage and ripening period while the other character, the fifth instar larval growth (weight) is inversely related. The two commercial silkworm hybrids studied differ in their optimum larval population density requirements during the fifth larval instar period for maximum fifth instar larval growth and minimum fifth instar larval duration, minimal larval unequal percentage and minimal ripening period. For confirmation of the hypothesis, data were analyzed for indexing. Based on all the population density regimes studied and indices analysis as well, the results revealed three fifth instar larval density zones; a. un-economic larval density zone, b. optimum larval density zone and c. loss larval density zone. For PM x CSR2, the fifth instar larval density zone of 80 to 90 number of larvae/feet^2 was more suited and designated as optimal fifth instar larval population zone while that for CSR2 x CSR4 was 60 to 70 number of larvae/feet^2. Further, critical view revealed that the fifth instar larval density of 80 number of larvae/feet^2 and 70 number of larvae/feet^2 are to be considered as optimum fifth instar larval population densities for PM x CSR2 and CSR2 x CSR4 silkworm hybrids respectively. Results are discussed on the basis of contemporary Indian commercial silkworm rearing.

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INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. is a monophagous insect, as it takes only mulberry (*Morus* sp.) foliage. Silk, produced from silkworm is identified as the queen of textiles due to its glittering luster, softness, elegance, durability, and tensile properties. Therefore, sericulture is the science of production of silk through rearing of silkworm. Insect growth is discontinuous and is characterized by a series of moults. The uniformity in moults (four larval-to-larval moults and one larval-to-pupal moult) is the important aspect in the success of commercial silkworm. Many factors of biotic and abiotic greatly affect the growth and development of the commercial silkworm, *Bombyx mori* (Rahmathulla, 2009), the main factors being photoperiod, temperature, humidity and population density in rearing platform. From the authors' Laboratory, many findings were published. Impacts of photoperiod on the silkworm, *Bombyx mori* (Sivarami Reddy, 1993), implications of temperature and humidity (Lakshminarayan Reddy, 2001), expression of mixed age characteristics (non-uniformity) in silkworm (Shanthan Babu, 2014; Srinath, 2014) were studied. However, one of the most important aspects of commercial silkworm rearing, the larval density did not receive much attention. Only limited references are available on the larval density aspect in the insects. Applebaum and Heifetz (1999) reviewed the density dependent physiological phases in insects.

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For the *B. mori*, Dar and Singh (1991) studied the influence of population density of silkworm, *Bombyx mori* L. on some economic traits. Saha *et al.* (2009) studied on the 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 feet^{2}exhibited good results in spring whereas the population group of 200 worms behaved invariably well in summer and autumn. The commercial mulberry silkworm, *Bombyx 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 important one as this stage is the only feeding 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 feet^2 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 scanty. Therefore, it is felt to determine the optimum larval population density in the fifth instar period, considering four critical parameters *viz*., larval duration, larval weight, percentage of unequal larvae and ripening period through indices studies.

MATERIALS AND METHODS

Two silkworm 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 for the experimentation. The DFLs (disease free layings; each DFL is group of 400 to 500 silkworm eggs laid by a single silk moth on a single day) of two hybrids; PM x CSR2 and CSR2 x CSR4 were procured from the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Mysore, India. The silkworm larvae were reared on the foliage of popular mulberry variety, V1 (*Morus* sp.) according to Krishnaswami (1986). The larval density has been described as number of larvae/feet^2 (Rajan *et al*., 2003; Lakshminarayana Reddy et al., 2015). For determination of optimum larval density during fifth instar silkworm rearing period, 10 larval density regimes, starting from 40 number of larvae/feet^2 to 130 number of larvae/feet^2 were considered. For each larval density regime/silkworm hybrid combination, 5 replications were maintained. Till the completion of the fourth larval stadium, the silkworm larvae were reared according to standard rearing method (Krishnaswami, 1986). 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). Four parameters, that are important in determining larval density requirements; fifth instar larval duration, fifth instar larval growth, unequal larval percentage and larval ripening period were considered in the study. The fifth instar larval duration was recorded from the time of the larvae out of fourth larval-to-larval ecdysis to the time of initiation of larval ripening and expressed in hours. Similarly, the larval weight, indicating the larval growth in the contemporary Indian mulberry sericulture practices, has been recorded at 10.00 h on the fifth day for PM x CSR2 and on the sixth day for CSR2 x CSR4. One hundred numbers of larvae were selected at random from each treatment/replication and weighed individually on digital weighing balance. From the recorded weights of the larvae, the average larval weight was derived to denote fifth instar larval weight. The number of unequal larvae in the rearing treatments was determined through deducting the number of larvae remained at the end of fifth instar from that kept for experimentation at the beginning of fifth instar period. Further, the duration of ripening period was determined from the initiation of ripening in experimental silkworm batches to its completion. Indexing of the studied parameters against experimental larval density regimes was studied by determining the ratio of the difference between control and experimental over control in percentage was considered. For this purpose, the procedure of calculating the percentage of phytotoxicity (Chou and Lin, 1976) was employed. Thus, the index value is calculated as Index = (concerned data of control – concerned data of experimental)/concerned data of control x 100. The control larval density was taken from Lakshminarayana Reddy *et al*., (2015) as 80 larvae/feet^2 as control fifth instar larval density regime for PM x CSR2 and 70 larvae/feet^2 as control fifth instar larval density regime for CSR2 x CSR4. The data were treated statistically (ANOVA).

RESULTS

Studies on the implications of fifth instar larval population density in multivoltine x bivoltine hybrid, PM x CSR2: Data on the implications of fifth instar silkworm larval population density on four important parameters, fifth instar larval duration, fifth instar larval growth (weight), unequal larval percentage and larval ripening period in the multivoltine x bivoltine silkworm hybrid, PM x CSR2 are presented in Table 1. It is generally observed that all the data registered low values for three characters studied, fifth instar larval duration, unequal fifth instar larval percentage and ripening period, up to larval population density of 80 – 90 larvae/feet^2 from where they started increasing. Opposing the trend observed for the above three parameters, evidently high values were recorded for larval weight of fifth instar silkworm larvae on the fifth day of fifth instar period up to 80 – 90 larvae/feet^2 larval density regimes. From these regimes, the larval weight declined steadily. Therefore, the imposed fifth instar larval population density imposed a direct impact on three fifth instar characters, fifth instar larval duration, unequal fifth instar larval percentage and ripening period. It is obvious that the imposed fifth instar larval population density had an inverse pressure on fifth instar silkworm larval growth/weight. It is also notable that the data points are on higher side, at the larval population density of 80 larvae/feet^2 for the three characters while the same was low for larval weight (Table 1.).

Implications of fifth instar larval population density on fifth instar larval period in multivoltine x bivoltine hybrid, PM x CSR2: The results on the implications of fifth instar larval population density in multivoltine x bivoltine hybrid, PM x CSR2 are depicted in Figure 1. Data in the figure 1 indicated that the fifth instar larval period is directly and positively related to the fifth instar silkworm larval population density (y = 0.382x + 101.2, R² = 0.745). Thus, initial 8 larval population densities resulted in low larval period. From the larval population density of 100 number of larvae/feet^2, the larval duration registered increasing trend, thus reaching a high fifth instar larval duration of 159 hours. The lower larval period ranged from 123 to 126 hours for the initial larval density of 40 to 90 number of larvae/feet^2. The differences in larval period for these 6 initial population density regimes were not statistically significant while the other larval period for the other density regimes (100 to 130 number of larvae/feet^2) were statistically highly significant compared to the initial data on larval period.

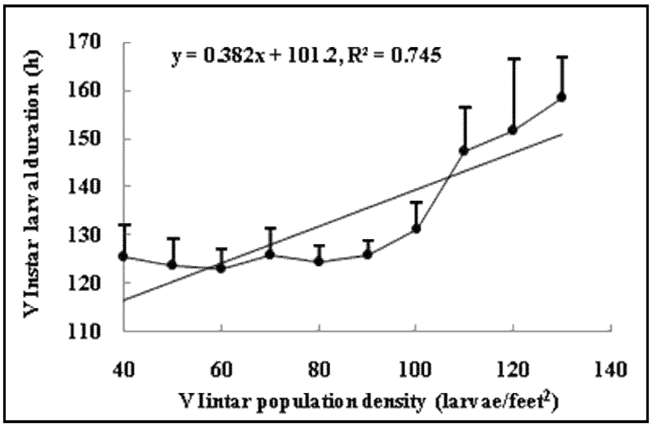
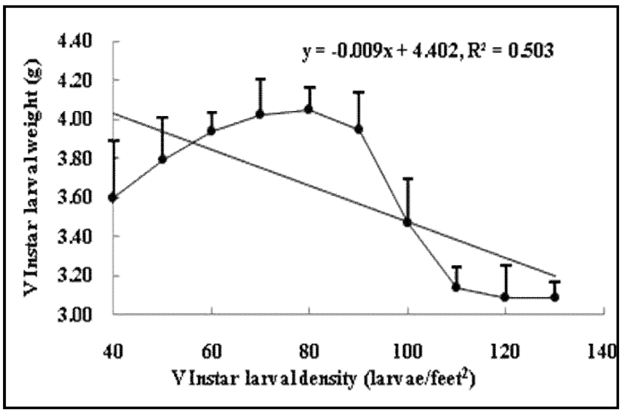


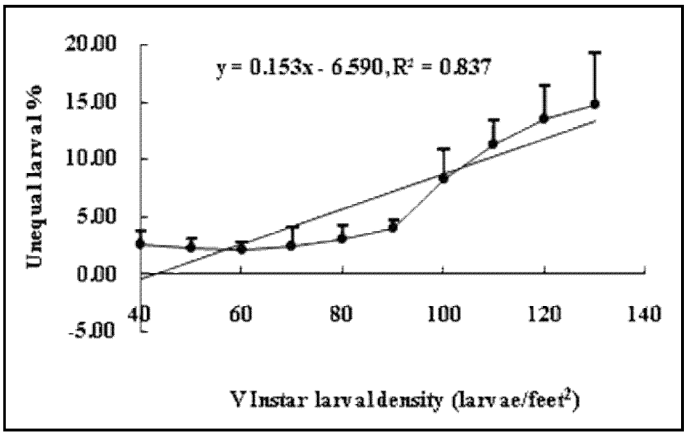
Figure 1. Impact of fifth instar larval population density (number of larvae/feet^2) on the fifth instar larval duration (in hours) of PM x CSR2. Values are mean of 5 replications ± SD. Differences in larval duration at larval population density range of 40 to 90 number of larvae/feet^2 are not significant among themselves while that for the other larval density (100 to 130) are significantly different over the initial 6 density regimes.



Implications of fifth instar larval population density on fifth instar larval weight in multivoltine x bivoltine hybrid, PM x CSR2: Figure 2: Impact of fifth instar larval population density (number of larvae/feet^2) on the fifth instar larval weight (taken at 10 am on 5th day of fifth instar) of PM x CSR2.

Values are mean of 5 replications ± SD. Values at larval population density range of 40 to 90 number of larvae/feet^2 are not significantly different among themselves while the other larval weights are significantly different (*p < 0.01*). The larval growth is indicated by the maximum weight of larvae on the fifth day of fifth instar silkworm larval period for multivoltine x bivoltine hybrid, PM x CSR2. The data on the impact of fifth instar larval population density in multivoltine x bivoltine hybrid, PM x CSR2 are depicted in Figure 2. The data clearly indicated that fifth instar larval population density had profound and inversely related to larval weight, indicated through measuring on the fifth day of fifth instar larval period at 10 am. Thus, larval weight was 3.6 g. at lowest imposed larval density of 40 number of larvae/feet^2, reached its peak at 80 larval density of 80 larvae and there after it sloppily decreased to reach larval weight of 3.1 g at 130 maximum imposed larval population density (y = -0.009x + 4.402, R² = 0.503). Differences in larval weight for larval population density range of 60 to 90 were not significant, while those for the other larval population density regimes are statistically highly significant.

Implications of fifth instar larval population density on unequal larval percentage in fifth instar larvae in multivoltine x bivoltine hybrid, PM x CSR2: The percentage of unequal larvae in the fifth instar larvae of commercial silkworm is indicative of the uniformity of larval growth population. Data on the unequal larvae (%) in the population of fifth instar larvae of multivoltine x bivoltine silkworm hybrid, PM x CSR2 are graphically represented in Figure 3.



From the figure 3, it is seen that the unequal larval % is more directly dependent on larval density regimes studied (y = 0.153x - 6.590, R² = 0.837). Thus, larval unequal percentage was low at low larval density and it increased as the larval population density increased, revealing a case of proportionate increase. However, the differences in larval unequal percentage for larval density range from 40 to 90 larvae/feet^2 are not significant indicating much suitability of these larval population density zone is much suited for minimal larval unequal percentage. From the larval population density of 100 number of larvae/feet2, the differences are highly significant indicating that these high population densities of larvae are not suited as these regimes induced high unequal percentage.

Implications of fifth instar larval population density on ripening period in multivoltine x bivoltine hybrid, PM x CSR2: After a long fifth instar eating period, silkworm larvae enter into the stage of ripening from which point, the larvae stop feeding and start wandering in search of a suitable site for initiation of cocoon spinning. In a population, the ripening period is counted from the beginning of initial individual or batch entering into ripening to the end of last larva or batch of larvae initiating ripening. Data on the ripening period or duration for the multivoltine x bivoltine hybrid, PM x CSR2 are graphed in Figure 4. The trend in larval ripening duration in the silkworm larvae just resembled that of instar length and unequal larval percentage as the initial 6 larval population density range (40 to 90 number of larvae/feet^2) and the differences are not significant ($y = 0.120x + 16.41$, $R^2 = 0.881$). However, the larval ripening duration increased for silkworm larvae from larval population density of 100 number of larvae/feet^2 onwards and these larval ripening periods are highly significantly different from those of earlier larval population density zones.

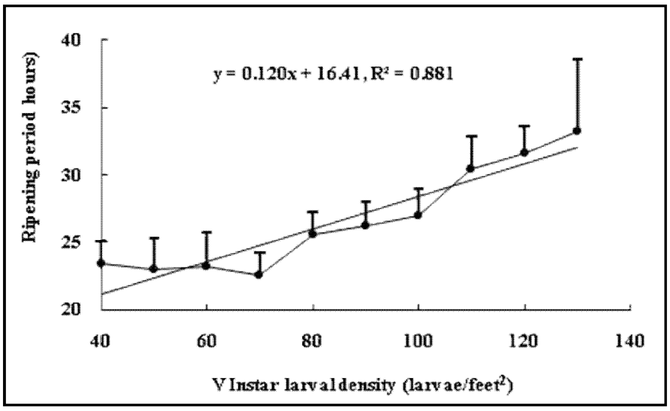


Figure 4: Graphic representation of the impact of larval population density (number of larvae/feet^2) on larval ripening period in PM x CSR2. Values are mean of 5 replications ± SD. Values at larval population density range of 40 to 90 number of larvae/feet^2 are not significantly different among themselves while the other larval density treatments are significantly (*$p < 0.01$*) different over the initial 6 density regimes.

Studies on the implications of fifth instar larval population density in bivoltine x bivoltine hybrid, CSR2 x CSR4: Data on the impact of fifth instar silkworm larval population density on four important parameters, fifth instar larval duration, fifth instar larval growth (weight), unequal larval percentage and larval ripening period in the bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 are given in Table 2. In the case of CSR2 x CSR4 also, it is generally followed the trends as recorded for PM x CSR2. However, the optimum larval population density is changed in case of CSR2 x CSR5.Larval population density of 70 number of larvae/feet^2 recorded the best commercial requirements for silkworm rearing. Thus, from density of 40 to 70 number of larvae/feet^2, the characters were on-par and the significant differences were noticed after this point of larval population density (Table 2.).

Implications of fifth instar larval population density on fifth instar larval period in bivoltine x bivoltine hybrid, CSR2 x CSR4: Results on the effect of fifth instar larval population density in bivoltine x bivoltine hybrid, CSR2 X CSR4 are graphed in Figure 5. Data indicated that the fifth instar larval period is directly and positively related to the fifth instar silkworm larval population density. Thus, initial 4 larval population densities resulted in low larval period. From the larval population density of 80 number of larvae/feet^2, the larval duration increased, thus reaching a high fifth instar larval duration of 182 hours. The lower larval period ranged from 152 to 154 hours for the initial larval density of 40 to 70 number of larvae/feet^2. The differences in larval period for these 4 initial population density regimes were not statistically significant, and on-par, while the other larval period for the other density regimes (80 to 130 number of larvae/feet^2) were statistically highly significant compared to the initial data on larval period ($y = 0.375x + 131.0$, $R^2 = 0.943$).

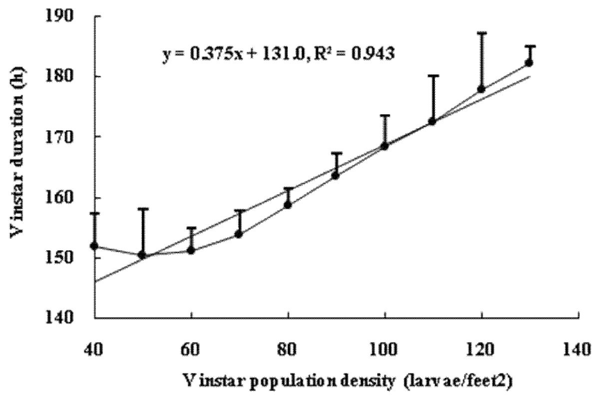


Figure 5: Impact of fifth instar larval population density (number of larvae/feet^2) on the fifth instar larval duration (in hours) of CSR2 x CSR4. Values are mean of 5 replications ± SD. Note that the differences in larval duration at larval population density range of 40 to 70 number of larvae/feet^2 are not significant among themselves while that for the other larval density (80 to 130) are significantly different over the initial 4 density regimes.

Implications of fifth instar larval population density on fifth instar larval weight in bivoltine x bivoltine hybrid, CSR2 x CSR4: Unlike the measurement of larval weight for PM x CSR2, the larval weight for CSR2 x CSR4 was taken at 10 am on sixth day of fifth instar larval period. Data on the larval weight as affected by different larval population densities are presented in Figure 6. The trend of larval weight was as observed for PM xCSR2, with decreasing in larval weight as the larval population density increased ($y = -0.018x + 5.737$, $R^2 = 0.904$). However, the optimum larval population density for maximum larval weight was 60 number of larvae/feet^2. The differenced in larval weight for the imposed initial population densities, up to 70 number of larvae/feer^2 were on-par, statistically not significant. When these differences of initial larval population densities are compared to that of later population densities are statistically highly significant. Maximum larval weight of 4.93 g was recorded at population density of 60 number of larvae/feet^2. On the other side, minimum larval weight (3.45 g) was observed for highest larval population density regime of 130 number of larvae/feet^2.

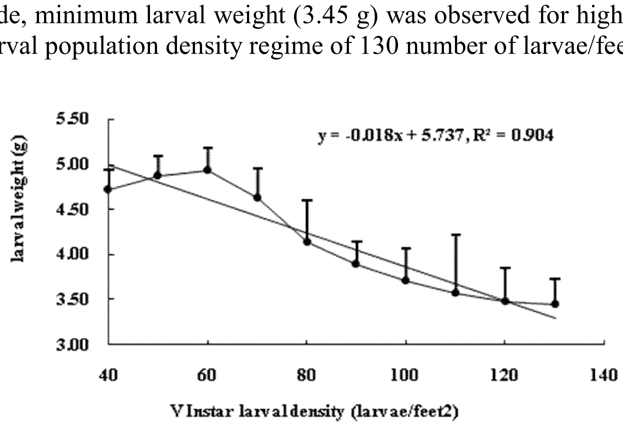


Figure 6: Effect of fifth instar larval population density (number of larvae/feet^2) on the fifth instar larval weight (taken

at 10 am on 6th day of fifth instar) of CSR2 x CSR4. Values are mean of 5 replications ± SD. Values at larval population density range of 40 to 70 number of larvae/feet^2 are not significantly different among themselves while the other larval weights are significantly different ($p < 0.01$).

Implications of fifth instar larval population density on unequal larval percentage in fifth instar larvae in bivoltine x bivoltine hybrid, CSR2 x CSR4: The percentage of unequal larvae in the fifth instar larvae of commercial silkworm is indicative of the uniformity of larval growth population. Data on the unequal larvae (%) in the population of fifth instar larvae of multivoltine x bivoltine silkworm hybrid, PM x CSR2 are graphically represented in Figure 7. From the figure 7, it is seen that the unequal larval % is directly related on larval density regimes studied (y = 0.181x - 7.707, R^2 = 0.898). Thus, larval unequal percentage was low at low larval density and it increased as the larval population density increased, revealing a case of proportionate increase. However, the differences in larval unequal percentage for larval density range from 40 to 70 number of larvae/feet^2 are not significant indicating much suitability of these larval population density zone is best suited for minimal larval unequal percentage. From the larval population density of 80 number of larvae/feet2, the differences are highly significant indicating that these high densities of larval population are not suited as these regimes induced high unequal percentage.

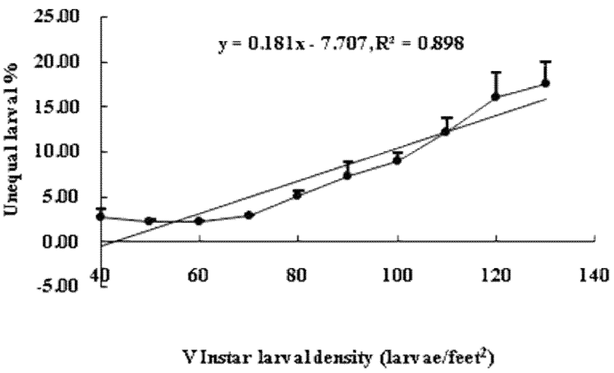


Figure 7: Impact of larval population density (number of larvae/feet^2) in the rearing bed on the unequal larval percentage of PM x CSR2. Values are mean of 5 replications ± SD. Values at larval population density range of 40 to 70 number of larvae/feet^2 are not significantly different among themselves while the other larval density treatments are significantly ($p < 0.01$) different over the initial 4 density regimes.

Implications of fifth instar larval population density on ripening period in bivoltine x bivoltine hybrid, CSR2 x CSR4: Data on the ripening period for the bivoltine x bivoltine hybrid, CSR2 x CSR4 are graphed in Figure 8. The trend in larval ripening duration in the silkworm larvae just resembled that of instar length and unequal larval percentage as the initial 4 larval population density range (40 to 70 number of larvae/feet^2) and the differences are not significant. However, the larval ripening duration increased for silkworm larvae from larval population density of 80 number of larvae/feet2 onwards and these larval ripening periods are highly significantly different from those of earlier larval population density zones (y = 0.163x + 21.37, R^2 = 0.558).

Studies on Index values of parameters against different imposed larval population densities in the multivoltine x bivoltine hybrid, PM x CSR2: Converting the observed data into a meaningful index values by proven and established equations will give a precise description of observed data. Therefore, indexing of observed data was considered by determining the ratio of the difference between control and experimental over control in percentage. The Index values of all four characters studied, fifth instar larval duration, fifth instar larval growth (weight), fifth instar unequal larval (%) and ripening period for multivoltine x bivoltine hybrid, PM x CSR2 are presented in Table 3. From the data on Index values, it is observed that all the designated control batched recorded '0' while the other treatments (larval population density regimes) have registered Index values either positive or negative values. For the initial 6 larval population density regimes, all the Index values were on the positive side, except for a few instances; Index value for larval duration at 40 number of larvae/feet^2 and the same for larval duration at density of 70 number of larvae/feet^2, however with no statistical differences. The situation with fifth instar larval weight was different. Thus, the Index value obtained was 0.00% for larval population density regime of 80 number of larvae/feet^2 (considered control) while that for all the other density regimes the Index values are positive indicating a special consideration, that larval weights are less in other experimental population density regimes compared to control (80 number of arvae/feet^2).

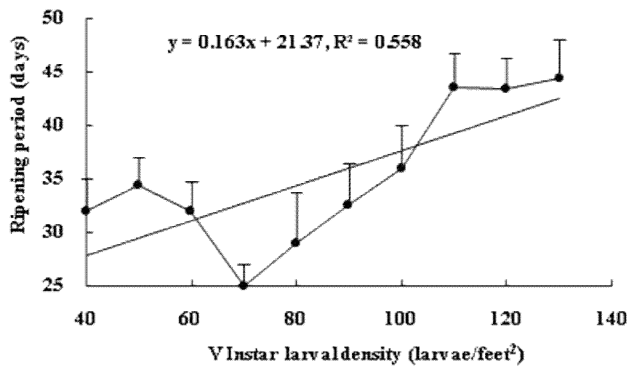


Figure 8. Graphic representation of the impact of larval population density (number of larvae/feet^2) on larval ripening period in CSR2 x CSR4. Values are mean of 5 replications ± SD. Values at larval population density range of 40 to 70 number of larvae/feet^2 are not significantly different among themselves while the other larval density treatments are significantly ($p < 0.01$) different over the initial 4 density regimes.

Index values for fifth instar larval duration under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for multivoltine x bivoltine silkworm hybrid, PM x CSR2: Derived Larval duration Index values for fifth instar larval duration under different larval population densities for multivoltine x bivoltine hybrid, PM x CSR2 are graphically represented in Figure 9. From the graph, it is observed that Index value for control (80 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000%. Index values after larval density regime of 90 number of larvae/feet^2 are scattered away from control Index and that too towards negative side of the graph. Figure 9: Derived larval duration Index values for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the multivoltine x bivoltine hybrid, PM x CSR2. Values are mean of 5 replications ± SD. Note the Index value for control (80 number of larvae/feet^2) is 0.000% while that for the initial population densities are very close to 0.000%. Index values after larval density of 90 number of larvae/feet^2 are scattered towards negative side of graph.

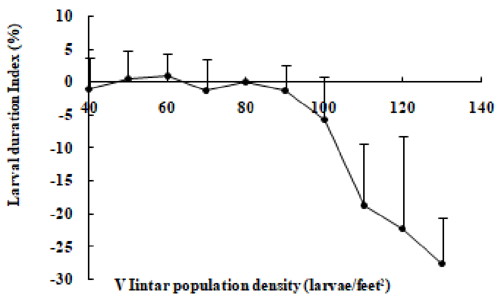


Figure 9. Derived larval duration Index values for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the multivoltine x bivoltine hybrid, PM x CSR2. Values are mean of 5 replications ± SD. Note the Index value for control (80 number of larvae/feet^2) is 0.000% while that for the initial population densities are very close to 0.000%. Index values after larval density of 90 number of larvae/feet2 are scattered towards negative side of graph.

Index values for fifth instar larval weight under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for multivoltine x bivoltine silkworm hybrid, PM x CSR2: Derived fifth instar larval weight Index under different larval population densities for multivoltine x bivoltine hybrid, PM x CSR2 are graphically represented in Figure 10. From the graph, it is observed that Index value for control (80 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000%. Index values after larval density regime of 90 number of larvae/feet^2 are scattered away from control Index and that too towards positive side of the graph, with statistical significance

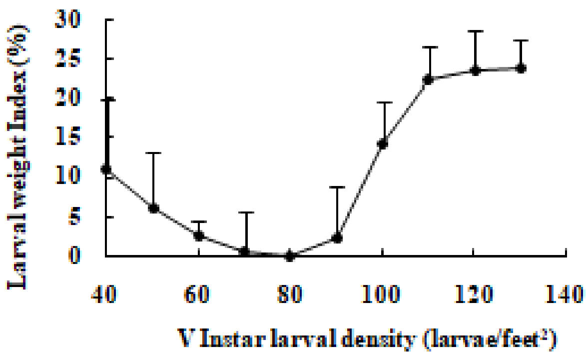


Figure 10. Derived larval weight Index values under different larval population densities (number of larvae/feet^2) for the multivoltine x bivoltine hybrid, PM x CSR2. Values are mean of 5 replications ± SD. Note the Index value for control (80 number of larvae/feet^2) is 0.000% while tat for all the larval population regimes recorded positive values. The Indices of initial population densities are very close to 0.000%. Index values after larval density of 90 number of larvae/feet^2 are scattered towards positive side of graph.

Index values for fifth instar unequal larval (%) under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for multivoltine x bivoltine silkworm hybrid, PM x CSR2: Fifth instar unequal larval percentage Index under different larval population densities for multivoltine x bivoltine hybrid, PM x CSR2 are graphically represented in Figure 11. From the graph, it is observed that Index value for control (80 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000%. Index values after larval density regime of 90 number of larvae/feet^2 are scattered away from control Index and that too towards negative side of the graph.

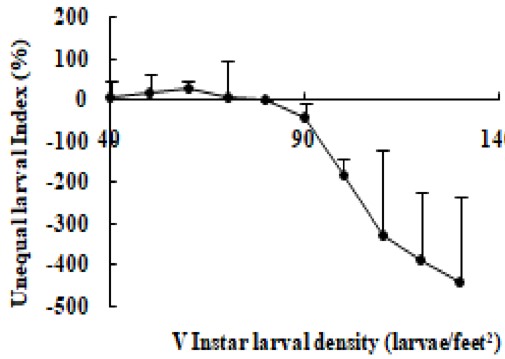


Figure 11. Derived unequal larval percentage Index values for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the multivoltine x bivoltine hybrid, PM x CSR2. Values are mean of 5 replications ± SD. Note the Index value for control (80 number of larvae/feet^2) is 0.000% while tat for the initial population densities are very close to 0.000%. Index values after larval density of 90 number of larvae/feet2 are scattered towards negative side of graph.

Index values for ripening under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for multivoltine x bivoltine silkworm hybrid, PM x CSR2: Larval ripening period Index under different larval population densities for multivoltine x bivoltine hybrid, PM x CSR2 are graphically represented in Figure 12. From the graph, it is observed that Index value for control (80 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000%. Index values after larval density regime of 90 number of larvae/feet^2 are scattered away from control Index and that too towards negative side of the graph.

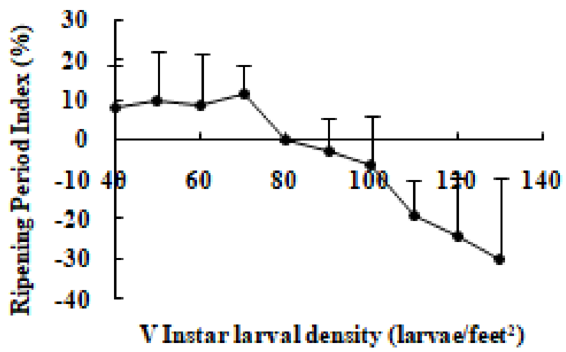


Figure 12. Derived larval ripening period Index values for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the multivoltine x bivoltine hybrid, PM x CSR2. Values are mean of 5 replications ± SD. Note the Index value for control (80 number of larvae/feet^2) is 0.000% while tat for the initial population

densities are very close to 0.000%. Index values after larval density of 90 number of larvae/feet2 are scattered towards negative side of graph.

Studies on Index values of parameters against different imposed larval population densities in the bivoltine x bivoltine hybrid, CSR2 x CSR4: Index values of all four characters studied, fifth instar larval duration, fifth instar larval growth (weight), fifth instar unequal larval (%) and ripening period for bivoltine x bivoltine hybrid, CSR2 x CSR4 are presented in Table 4. From the data on Index values, the observed trends for PM x CSR2 are repeated. All the Index values were on the negative side. It is also surprised to observe all the Index values for fifth instar unequal larval percentage and ripening period registered negative values for the initial three larval population density regimes.

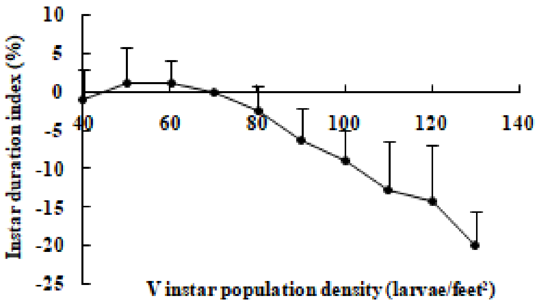


Figure 13. Larval duration Index values for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the bivoltine x bivoltine hybrid, CSR2 x CSR4. Values are mean of 5 replications ± SD. Note the Index value for control (70 number of larvae/feet^2) is 0.000% while that for the initial population densities are very close to 0.000%. Index values after larval density of 80 number of larvae/feet2 are scattered towards negative side of graph.

Fifth instar larval duration Index values under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4: Fifth instar larval duration Indies for fifth instar larval duration under different larval population density regimes for bivoltine x bivoltine hybrid, CSR2 x CSR4 are represented in Figure 13. From the graph, it is seen that Index value for control (70 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000% and differences in Index values among these larval density regimes are statistically non-significant. Index values after larval density regime of 80 number of larvae/feet^2 are scattered away from control Index in descending way. The differences in Index values between these larval population density regimes and that of early low larval population density regimes are statistically highly significant.

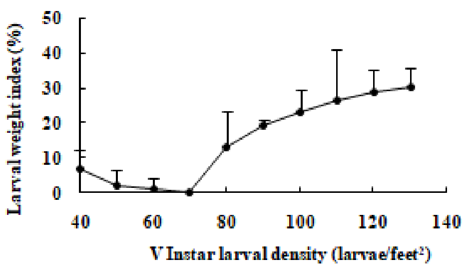


Figure 14. Larval weight Index values under different larval population densities (number of larvae/feet^2) for the bivoltine x bivoltine hybrid, CSR2 x CSR4. Values are mean of 5 replications ± SD. Note the Index value for control (70 number of larvae/feet^2) is 0.000% while tat for all the larval population regimes recorded positive values. The Indices of initial population densities are close to 0.000%. Index values after larval density of 80 number of larvae/feet^2 are scattered increasingly towards positive side.

Fifth instar larval weight Index under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4: Fifth instar larval weight Index under different larval population densities for bivoltine x bivoltine hybrid, CSR2 x CSR4 are depicted in Figure 14. It is observed that Index value for control (70 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000%. Index values after larval density regime of 80 number of larvae/feet^2 are scattered progressively, towards positive side of the graph, with statistical significance.

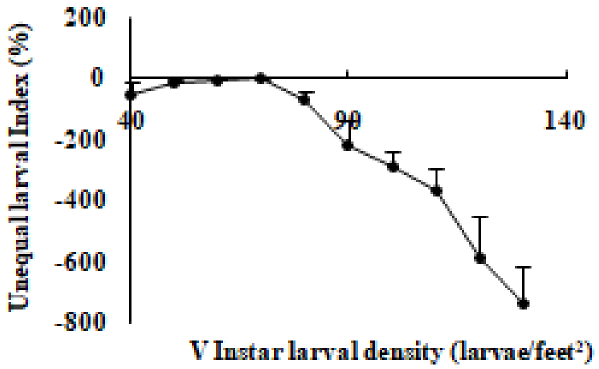


Figure 15. Unequal larval percentage Indices for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the bivoltine x bivoltine hybrid, CSR2 x CSR4. Values are mean of 5 replications ± SD. Note the Index value for control (70 number of larvae/feet^2) is 0.000% while tat for the initial population densities are very close to 0.000%. Index values after larval density of 80 number of larvae/feet^2 are scattered inversely.

Fifth instar unequal larval (%) Index under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4: Fifth instar unequal larval percentage Index under different larval population densities for multivoltine x bivoltine hybrid, PM x CSR2 are graphically represented in Figure 15. From the graph, it is clear that Index value for control (70 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000%. Index values after larval density regime of 80 number of larvae/feet^2 are scattered away from control Index inversely.

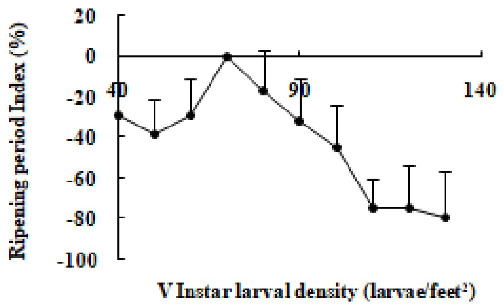


Figure 16. Derived larval ripening period Index values for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the bivoltine x bivoltine hybrid, CSR2 x CSR4. Values are mean of 5 replications ± SD. Note the Index value for control (70 number of larvae/feet^2) is 0.000% while tat for the initial population densities are very close to 0.000%. Index values after larval density of 80 number of larvae/feet^2 are scattered towards negative side of graph.

Table 1. Data on the implications of fifth instar silkworm (*Bombyx mori* L.) larval population density on four important parameters, fifth instar larval duration, fifth instar larval growth (weight taken on fifth day of fifth instar period), unequal larval percentage and larval ripening period in the multivoltine x bivoltine silkworm hybrid, PM x CSR2.

Larval density (Larvae/ feet^2)	Larval duration (hours ± SD)	Larval weight (g ± SD)	Unequal larvae (% ± SD)	Ripening period (hours ± SD)
40	125.600 ± 6.542	3.5940 ± 0.294	2.640 ± 1.161	23.400 ± 1.673
50	123.800 ± 5.495	3.7980 ± 0.213	2.220 ± 0.861	23.000 ± 2.345
60	123.200 ± 3.899	3.9400 ± 0.097	2.120 ± 0.756	23.200 ± 2.588
70	126.000 ± 5.431	4.0260 ± 0.181	2.360 ± 1.658	22.600 ± 1.673
80	124.400 ± 3.578	4.0480 ± 0.113	2.998 ± 1.225	25.600 ± 1.673
90	125.800 ± 3.184	3.9500 ± 0.185	4.040 ± 0.713	26.200 ± 1.789
100	131.400 ± 5.459	3.4700 ± 0.225	8.244 ± 2.660	27.000 ± 2.000
110	147.400 ± 8.989	3.1420 ± 0.103	11.244 ± 2.204	30.400 ± 2.510
120	151.800 ± 14.755	3.0900 ± 0.161	13.424 ± 3.006	31.600 ± 2.075
130	158.600 ± 8.173	3.0840 ± 0.087	14.824 ± 4.469	33.200 ± 5.357

Table 2. Data on the implications of fifth instar silkworm (*Bombyx mori* L.) larval population density on four important parameters, fifth instar larval duration, fifth instar larval growth (weight taken on sixth day of fifth instar period), unequal larval percentage and larval ripening period in the bivoltine x bivoltine silkworm hybrid, CSR2 c CSR4.

Larval density (Larvae/ feet^2)	Larval duration (hours ± SD)	Larval weight (g ± SD)	Unequal larvae (% ± SD)	Ripening period (hours ± SD)
40	151.900 ± 5.320	4.722 ± 0.224	2.783 ± 0.783	32.000 ± 3.083
50	150.300 ± 7.629	4.874 ± 0.213	2.283 ± 0.199	34.400 ± 2.702
60	151.200 ± 3.647	4.929 ± 0.259	2.136 ± 0.122	32.000 ± 2.739
70	153.800 ± 4.000	4.629 ± 0.321	2.830 ± 0.071	25.000 ± 2.000
80	158.500 ± 2.966	4.145 ± 0.464	5.067 ± 0.650	29.000 ± 4.796
90	163.400 ± 3.847	3.898 ± 0.246	7.323 ± 1.522	32.600 ± 3.847
100	168.400 ± 5.177	3.711 ± 0.352	8.928 ± 0.929	36.000 ± 4.000
110	172.400 ± 7.668	3.565 ± 0.659	12.083 ± 1.645	43.600 ± 3.209
120	177.800 ± 9.450	3.483 ± 0.373	15.967 ± 2.810	43.400 ± 2.966
130	182.200 ± 2.864	3.452 ± 0.248	17.552 ± 2.517	44.400 ± 3.578

Table 3: Index values for four parameters under different imposed fifth instar larval population density (number of larvae/feet^{2}regimes for multivoltine x bivoltine silkworm hybrid, PM x CSR2.

Larval density (Larvae/ feet^2)	Larval duration (Index ± SD)	Larval weight (Index ± SD)	Unequal larvae (Index ± SD)	Ripening period (Index ± SD)
40	-0.973 ± 4.667	11.068 ± 8.807	5.929 ± 42.479	8.132 ± 10.721
50	0.456 ± 4.306	6.040 ± 7.124	17.931 ± 45.047	9.679 ± 12.381
60	0.922 ± 3.554	2.649 ± 1.733	27.275 ± 20.381	8.965 ± 12.775
70	-1.332 ± 4.776	0.490 ± 5.075	3.938 ± 90.103	11.529 ± 7.212
80	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
90	-1.188 ± 3.740	2.311 ± 6.345	-44.760 ± 36.164	-2.601 ± 8.197
100	-5.766 ± 6.705	14.258 ± 5.438	-182.961 ± 40.881	-6.007 ± 12.434
110	-18.661 ± 9.408	22.296 ± 4.218	-329.650 ± 206.939	-18.874 ± 8.930
120	-22.282 ± 14.097	23.590 ± 5.100	-389.003 ± 168.088	-24.167 ± 14.617
130	-27.552 ± 6.959	23.742 ± 3.759	-441.048 ± 204.590	-29.780 ± 20.178

Table 4. Index values for four parameters under different imposed fifth instar larval population density (number of larvae/feet^{2}regimes for bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4.

Larval density (Larvae/ feet^2)	Larval duration (Index ± SD)	Larval weight (Index ± SD)	Unequal larvae (Index ± SD)	Ripening period (Index ± SD)
40	-1.095 ± 3.924	7.103 ± 5.435	-51.792 ± 41.069	-28.668 ± 16.126
50	1.170 ± 4.657	2.038 ± 4.610	-14.099 ± 10.212	-38.567 ± 17.701
60	1.012 ± 3.023	1.053 ± 3.125	-3.505 ± 4.375	-28.951 ± 18.200
70	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
80	-2.419 ± 3.058	13.008 ± 10.513	-69.689 ± 31.336	-16.491 ± 19.906
90	-6.254 ± 4.120	19.495 ± 1.585	-215.143 ± 81.242	-31.424 ± 20.996
100	-8.856 ± 3.804	23.154 ± 6.322	-285.055 ± 49.882	-44.932 ± 21.624
110	-12.850 ± 6.315	26.736 ± 14.131	-366.008 ± 76.675	-74.927 ± 14.609
120	-14.164 ± 7.361	29.074 ± 6.501	-585.669 ± 140.360	-74.661 ± 20.766
130	-19.959 ± 4.530	30.270 ± 5.450	-737.225 ± 125.529	-78.838 ± 22.514

Index values for ripening period under different imposed fifth instar larval population density (number of larvae/feet^2) regimes for bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4: Larval ripening period Indices under different larval population densities for multivoltine x bivoltine hybrid, PM x CSR2 are graphically represented in Figure 16. From the graph, it is observed that Index value for control (70 number of larvae/feet^2) is 0.000%. Index values for the initial population densities are very closely scattered around control index value, 0.000%. Index values after larval density regime of 80 number of larvae/feet^2 are scattered away from control Index and that too towards negative side of the graph. Figure 16: Derived larval ripening period Index values for fifth instar larval duration under different larval population densities (number of larvae/feet^2) for the bivoltine x bivoltine hybrid, CSR2 x CSR4. Values are mean of 5 replications ± SD. Note the Index value for control (70 number of larvae/feet^2) is 0.000% while tat for the initial population densities are very close to 0.000%. Index values after larval density of 80 number of larvae/feet^2 are scattered towards negative side of graph.

DISCUSSION

The uniformity in moults is the important aspect in the success of commercial silkworm. Many factors of biotic and abiotic greatly affect the growth and development of the commercial silkworm, *Bombyx mori* (Rahmathulla, 2009), the main factors being photoperiod (Sivarami Reddy, 1993), temperature and humidity (Lakshminarayan Reddy, 2001), and population density (Lakshminarayana Reddy *et al.*, 2015) in rearing platform that result in expression of mixed age characteristics (non-uniformity) in silkworm (Shanthan Babu, 2014; Srinath, 2014). Only limited references are available on the larval density aspect in the 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, *Bombyx mori* L. on some economic traits. Saha *et al.* (2009) studied on the 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 feet^{2}exhibited good results in spring whereas the population group of 200 worms behaved invariably well in summer and autumn. The commercial mulberry silkworm, *Bombyx 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 important one as this stage is the only feeding 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. Further, this is the stage from where the larvae enter into pupa through metamorphosis. Although insects are an extremely diverse group of organisms, the endocrine control of growth, moulting and metamorphosis, with in this group appears to be remarkably similar (Riddiford, 1980). It is well established that the major features of moulting cycles are regulated by a sequence of three hormones; PTTH, ecdysone and JH (Riddiford, 1980; Reynolds, 1980; Truman and Taghert, 1981; Happ, 1984). In the presence of high haemolymph titer of JH, the moulting cycle produces an additional larval or nymphal stage (Williams, 1961; Riddiford, 1980; Happ, 1984). On the other hand, if the JH titer is very low or virtually absent, morphogenesis is initiated leading to the production of pupal or adult stage. The JH, responsible for the maintenance of larval characters (Riddiford, 1980) remains high, up to ultimate larval-to-larval moult, and drastically decreases to plateau level when the final instar larva attains its absolute size. At this time, the brain releases PTTH during the next allowable gate and causes the secretion of first installment of ecdysone, of course, 5 to 8 times less in quantity (as in *Manduka sexta*, Riddiford, 1980) than that necessary to elicit ecdysis which itself has, in the absence of JH, a profound effect on the animal leading to the beginning of metamorphosis process. The second release of PTTH, approximately 1.5 to 2 days before the pupal ecdysis, as in *M. sexta* (Riddiford, 1980; Truman and Taghert, 1981) and in *Bombyx mori* (Shimada, 1989) in turn releases that second installment of ecdysone that is 5 to 8 times more than the first installment (Riddiford, 1980), and this hormone executes the pupal ecdysial process initiating apolysis (Hinton, 1973). The entire process is dependent on attaining of larvae a critical size during fifth instar period. Therefore, the entire investigations, in the present study, pertaining to larval population density, were concentrated in the fifth and final instar larval period.

From the results, it is clear that the larval density provided during rearing of fifth larval instar period in terms of number of larvae per feet^2 has definite relationship for traits studied in both the multivoltine x bivoltine (PM x CSR2) and bivoltine x bivoltine (CSR2 x CSR4). Four traits were studied; 1) fifth instar larval period, 2) growth (weight) of fifth instar larvae on fifth/sixth day, 3) unequal larval percentage during fifth instar and 4) ripening period as influenced by the larval density. It is observed that initial low fifth instar larval population densities have little impact on the traits studied. Thus, fifth instar larval densities of 40, 50, 60 and 70 larvae per feet^2 have recorded results having no statistical differences among themselves for CSR2 x CSR4. PM x CSR2 has also responded identically in terms of results of the above four traits against the first few larval population density. However, the initial 6 larval density treatments have recorded results with statistically no differences. This observation clearly indicate that the bivoltine hybrids require less fifth instar larval densities for better results on the above four traits studied while the multivoltine x bivoltine hybrid can withstand fifth instar larval densities up to 90 larvae per feet^2. Supporting the results, multivoltine growth is restricted over bivoltine silkworm. Therefore, multivoltine silkworm larvae are recommended more larval population density (80 number of larvae/feet^2) while 70 number of larvae/feet^2 for CSR2 x CSR4 Lakshminarayana Reddy *et al*., 2015) and are considered as control larval population density regime in the present study. Larval duration in mulberry silkworm depends on many factors. While photoperiod, temperature and humidity are kept constant in laboratory rearing conditions, the other obligatory factor that affect the fifth instar larval period seems to be the larval population density or the spacing as used in contemporary Indian silkworm commercial rearing. With less larval population densities or more spacing, the larvae get more food to eat, more spacing to wander and breathing, larvae tend to grow vigorously and healthy, waiting for ripening uniformly. With high larval population density or low spacing, the larvae suffer from competition from the other larvae in the population resulting in scanty availability of food to eat and less space to breathe and grow. Therefore, the initiation of ripening delayed resulting in prolonging in larval duration. Thus the inverse relation of fifth instar larval period to that of larval population density is justified. Similar justification is applicable for unequal larval percentage. As the larvae are growing healthy with less larval population densities, the chance of unequal and further infection leading to mortality is less. On the other hand, the occurrence of unequal larval percentage increased many folds under more larval population density or less spacing. The issue of ripening period is also dependent of spacing or larval population density. Under low population densities, larval growth is uniform to attain critical size at required time to get released moulting hormones (PTTH and ecdysone) and further finish ripening uniformly. Under high larval population densities or less spacing, the larval growth is not uniform leading to unsynchronized moulting hormones release and long larval population ripening period.

Recorded data itself may not provide sufficient conclusive information. Therefore, the Indices studies are more essential in biological studies. Indexing of the studied parameters against experimental larval density regimes was studied by determining the ratio of the difference between control and experimental over control in percentage was considered. For this purpose, the procedure of calculating the percentage of phytotoxicity (Chou and Lin, 1976) was employed. Thus, the index value is calculated as Index = (concerned data of control – concerned data of experimental)/concerned data of control x 100. In this Indexing, all the Index values connected to control will be recorded as zero and the others scattering around control to indicate their direct or inverse relation, their close or distant relation. Thus, the data on all the parameters studied indicated two types of response, clearly and decisively. The positive or direct related parameters are fifth instar larval duration, percentage of unequal fifth instar larvae and ripening period. Therefore, the Index values of experimental larval population densities scattered towards negative side with more scattering. The case with indirect and inverse related parameter, the fifth instar larval growth or weight, the trend is that all the experimental larval population data points are scattered towards positive side from control Index value and as the density increase, the distance or scattering increased. Though the initial few larval populations recorded in on-par results, It is not at all advised to have such low larval population densities in commercial silkworm rearing by farmers. Therefore, the larval populations prior to optimum larval population densities (70 number of larvae/feet^2 for CSR2 x CSR4 and 80 number of larvae/feet^2, Lakshminarayana Reddy *et al*., 2015) are designated as *Un-economic zones of larval densities*' for a commercial PM x CSR2 hybrid silkworm rearing farmer, those at optimum larval population densities as *desired or optimum fifth instar larval density zones* and those after optimum larval population densities as *loss zones of larval densities* (Lakshminarayana Reddy *et al*., 2015).

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