Economics of rearing of bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 (*Bombyx mori* L.) with reference to avoiding mixed age characters.

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ABSTRACT

The utilization of phytoecdysteroid in synchronizing larval-to-pupal ecdysis is the recent technology. Considerably, commendable work has been conducted on utilization of phytoecdysteroid in synchronizing larval ripening, just before the beginning of pupal stage in *B. mori*. The application of phytoecdysteroid freed the silkworm population from the internal clock and forced the silkworm population to behave as an independent population to complete larval ripening and other components in larval-pupal ecdysial process. Previous studies were mostly of laboratory studies. In this study we tried to initiate to shift these studies from laboratory to the farmers rearing houses.

In the silkworm growth, there are several aspects of economic importance such as hatching duration, larval-to-larval moulting durations and larval-to-pupal durations. These measurements in terms of time are dealt separately using index computation in various studies. On the other economic quantitative traits such as silkworm egg hatching percentage, silkworm brushing percentage, larval ripening percentage, cocoon weight, shell weight and shell ratios etc., are to be dealt in a different way, with evaluation index. All the heterozygotic hybrid improvement studies are the economic silkworm rearing characters in standard rearing packages at laboratory level only. Evaluation of such characters under different temperature and humidity conditions at farmer level is studied. In this direction, a common evaluation index, based on the evaluation index value, a method used in educational system in Japan to determine student's merit. It is to be remembered that the multiple trait evaluation index was adapted to judge the superiority of various silkworm hybrids in a different context of farmers' rearing house conditions, using CSR2 x CSR4. In a precise expression, the present study is aimed at quantification measurements of reducing durations (hatching duration, moulting durations, ripening durations etc.) on one hand through computing index values and evaluation of quantitative economic traits (such as egg hatching percentage, brushing percentage, larval ripening percentage, cocoon weight, shell weight, shell ratio etc.,) on other through computing evaluation index are at farmers' rearing house conditions proposed.

Keywords: phytoecdysteroid, Index computation, multiple trait evaluation index, quantitative economic traits.

Introduction

For the world class commercial value of silk from the mulberry silkworm, *Bombyx mori* L., it is appropriately termed as the queen of textile. Though the mulberry silkworm is completely domesticated, it is greatly influenced by external sources or environment like photoperiod, temperature and humidity too. Among these factors, photoperiod is recognized as the strongest, affecting the overt phenomena in growth and development and other multiple traits. The temperature and humidity are secondary in implicating changes in silkworm growth and development. Though genetic makeup determines the expression of rearing characteristics in *Bombyx* silkworm, the influence of environmental factors seems to affect all the rearing characters in a positive or negative manner. The implications of photoperiod were extensively studied (Sivarami Reddy, 1993; Lakshminarayana Reddy, 2001; Shanthan Babu, 2014; Srinath, 2014).

Similarly, the growth, development and synchrony of larval-to-larval as well as larval-to-pupal ecdysis are greatly affected by the other rearing environmental characters, such as larval spacing and larval feed quantities (Krishnaswami, 1990; Kawakami and Yanagawa, 2003). Ravi, 2014; Lakshminarayana Reddy *et al*., 2015 have studied the implications of larval population densities on the synchronization of larval-to-larval and larval-to pupal ecdysis as well. The utilization of phytoecdysteroid in synchronizing larval-to-pupal ecdysis is the recent technology (Nirmal Kumar *et al*., 2006). Considerably, commendable work has been conducted on utilization of phytoecdysteroid in synchronizing larval ripening, just before the beginning of pupal stage in *B. mori* (Shanthan Babu, 2014; Srinath, 2014) pointed out. In the present study results on avoiding mixed age characters two approaches were inducted.

The first approach is to analyze and emphasize the hypothesis that the silkworm, *Bombyx mori* L. is yielding to express of mixed age characters even from the beginning of the initial developmental marker event, the egg hatching to the ultimate larval developmental marker event, the larval ripening through the influence of external environmental factors.

Similarly, the second way is that how these expressed mixed-age characters, to the maximum possible level, are avoided and to synchronize the developmental events such as egg hatching, larval-to-larval moults and larval-to-pupal ecdysis.

For above two approaches, two specific and simple analytical measurements were adopted. For example, **'event index'** measurement system was followed to establish the intensity of mixed-age characters in developing silkworm larval population. On the other, certain recent technologies were adopted to avoid such expression of mixed-age characters in the larval population. And the impact of such technologies in avoiding mixed-age characters in silkworm larval population of CSR2 x CSR4 at both Laboratory and Farmers' level as well were determined through **Evaluation Index (*EI*) method**.

Materials and methods:

EVENT INDEX STUDIES:

I. Intensity of mixed-age characters appearance in egg hatching of CSR2 x CSR4 population.

a. **Intensity of expression of mixed-age characters in egg hatching of CSR2 x CSR4 at Laboratory level:** In the present context of CSR2 x CSR4 hatching at Laboratory level, four environmental (photoperiodic) factors, LD 12: 12, DD (continuous dark), LL (continuous light) and a technology of incubation and hatching, Black-Box system were studied. The other major influential factors; temperature and humidity, were completely controlled. Data on hatching duration alone were considered and analyzed for hatching duration index (incident index). For calculating hatching duration, the regular way hatching under LD 12 : 12 condition was considered as control and the remaining three photoperiodic conditions DD, LL and Black-Box system) as experimental. The analyzed data on hatching duration index are presented in Figure 1.

Data clearly demonstrated that the hatching duration of CSR2 x CSR4 was 0.00. The hatching duration index of the next two photoperiodic conditions, DD and LL expressed negative values (-8% and -24% for DD and LL respectively), indicating that the hatching durations under these two photoperiodic conditions (DD and LL) are more than that of control (LD 12 : 12) photoperiodic condition (8% more for DD and 24% more for LL over LD 12 : 12). The Black-Box system recorded a very high positive hatching duration index, indicating that the hatching duration under Black-Box system is very low compared to LD 12 : 12, DD and LL conditions. The hatching duration recorded an index over 80% which should be considered as a remarkable one.

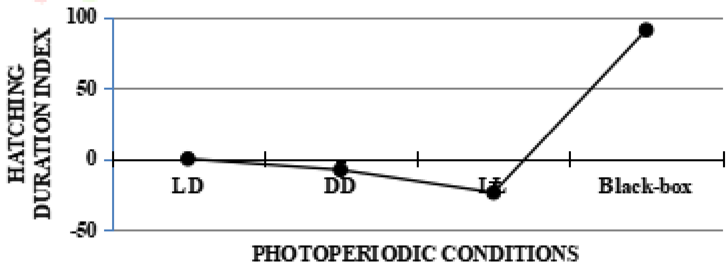

Figure 1: Graphic representation of hatching duration index for CSR2 x CDR4 under four photoperiodic conditions, LD 12: 12, DD (continuous dark), LL (continuous light) and Black-Box system at Laboratory level. (Mean of 5 replication ± SD).

b. Intensity of expression of mixed-age characters in egg hatching of CSR2 x CSR4 at Farmers' level: The studies on hatching in CSR2 x CSR4 hatching at Farmers' level were also experimented with four environmental (photoperiodic) factors, LD 12: 12, DD (continuous dark), LL (continuous light) and Black-Box system. However, the other major factors such as temperature and humidity were not controlled at farmers' rearing house conditions and for these, the farmers practices were only used. Analyzed data on hatching duration index in CSR2 x CSR4 at farmers' level are presented in Figure 2.

In the case of hatching duration index for CSR2 x CSR4 at farmers' level, the trend in expression of hatching duration index was identical to that at laboratory level. However, the degree of index value varied from farmers' rearing house conditions to laboratory level conditions. The hatching duration index for CSR2 x CSR4 at farmers' level too was on base (0.00%). The hatching duration index for CSR2 x CSR4 at farmers' rearing house conditions with DD and LL recorded on negative side. Thus, hatching duration index for CSR2 x CSR4 at farmers' rearing house conditions was recorded -14% (± 15.007) under DD and that for LL, it was -28% (± 9.468). With Black-Box, the hatching duration index was on the more positive side, recording 91 % ± 2.194). When the data are statistically analyzed, the differences were statistically highly significant at 1% level ($p < 0.01$)

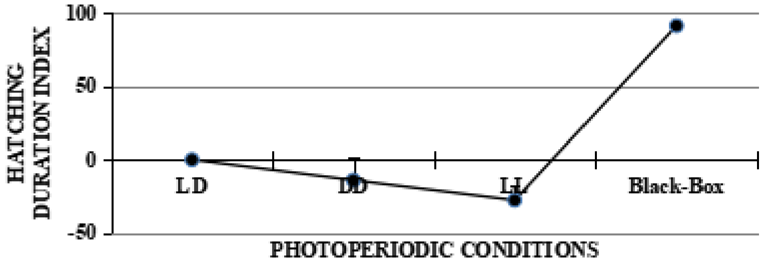

Figure 2: Graphic representation of hatching duration index for CSR2 x CDR4 under four photoperiodic conditions, LD 12: 12, DD (continuous dark), LL (continuous light) and Black-Box system at Farmers' Rearing House conditions. (Mean of 5 replication ± SD).

II. Intensity of mixed-age characters appearance in CSR2 x CSR4 population with varied fifth instar larval population spacing and feeding quantities at farmers' rearing house conditions.

a. **Intensity of mixed-age characters appearance in CSR2 x CSR4 population with different fifth instar larval spacing regimes at farmers' rearing house conditions**: The results obtained in three fifth instar larval spacing regimes, a. high larval spacing (low larval population density of 40 number of larvae/ft^2, b. optimum larval spacing (optimum larval population density of 70 number of larvae/ft^2 and c. low larval spacing (high larval population density of 130 number of larvae/ft^2) were considered for deciding the intensity of mixed-age characters in larval ripening in the present perspective. For better understanding, these spacing regimes are taken as high, optimal and sub-optimal fifth instar larval population spacing. No doubt, there exists certain quantum of mixed age characters in the ripening larvae of CSR2 x CSR4 because of three fifth instar larval spacing conditions. But, the quantification of these mixed-age characters are calculated as ripening duration index and presented in Figure 3.

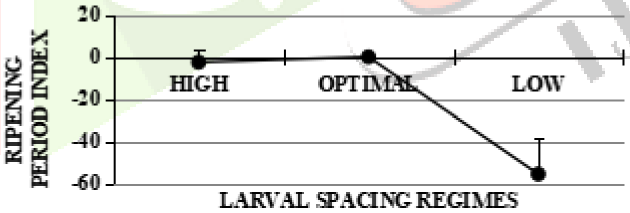

Figure 3: Larval ripening duration index for CSR2 x CDR4 with three fifth instar larval spacing regimes; a. high larval spacing (low larval population density of 40 number of larvae/ft^2, b. optimum larval spacing (optimal larval population density of 70 number of larvae/ft^2) and c. low larval spacing (high larval population density of 130 number of larvae/ft^2) at Farmers' Rearing House conditions. (Mean of 5 replication ± SD).

The optimal fifth instar larval spacing (optimum larval spacing (optimal larval population density of 70 number of larvae/ft^2 was considered as the control while computing the larval ripening duration index while the other two fifth instar larval population spacing regimes (high larval spacing (low/sub-optimal larval population density of 40 number of larvae/ft^2 and low larval spacing (high larval population density of 130 number of larvae/ft^2) were considered as experimental population. From the figure (graph) it can be seen that there exists clear-cut differences in larval ripening duration index values. The larval ripening index of control (optimal) larval population set at base of the graph (0.00%) and the other two fifth larval ripening duration index of CSR2 x CSR4 population scattered away from the optimal fifth instar larval spacing population in terms of larval ripening duration index. However, the scattering was towards negative side of the graph. Importantly, the high larval spacing (low/sub-optimal larval population density of 40 number of larvae/ft^2, the index value is just negative (-2.5% ± 6.007), indicating that the larval ripening duration index values of both optimal and high fifth instar larval population spacing regimes on CSR2 x CSR4 are not statistically different. On the other side, the larval ripening duration index of CSR2 x CSR4 at low fifth larval instar spacing was thrown far below negative value (-55% ± 16.971), indicating a high level of mixed-age characters.

b. Intensity of mixed-age characters appearance in CSR2 x CSR4 population with different fifth instar larval feeding quantity regimes at farmers' rearing house conditions: In the case of larval ripening duration index for CSR2 x CSR4 with three regimes of fifth instar larval feed quantity (high, optimal and sub-optimal V1 mulberry feed quantity) (Figure 4), the larval ripening duration index of control (optimal) was on the base line. The index value of more feed was just below the base line (-9% ± 7.483) and the differences are not significant, statistically. On the other hand, the larval ripening duration index was very low (-55% ± 16.971) indicating the severity of high mixed-age characters, and the differences are highly statistically significant ($p < 0.01$).

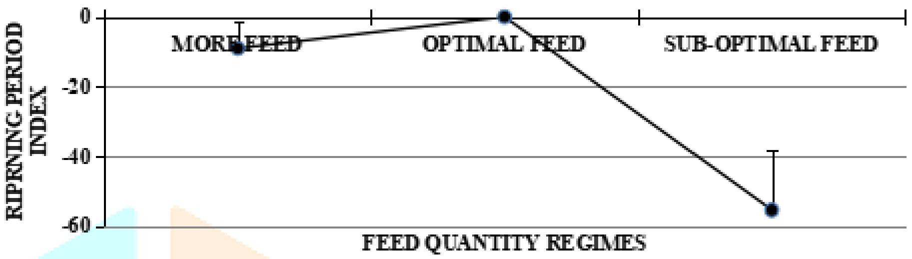

Figure 4: Larval ripening duration index for CSR2 x CDR4 with three fifth instar larval feeding quantity regimes; a. high fifth instar larval feeding quantity (69.12 kg/1000 larvae), b. optimum fifth instar larval feeding quantity (57.6 kg/1000 larvae) and c. low fifth instar larval feeding (46.08 kg/1000 larvae) quantity at Farmers' Rearing House conditions. (Mean of 5 replication ± SD).

III. Intensity of mixed-age characters appearance in CSR2 x CSR4 population with varied fifth instar larval population spacing and feeding quantities coupled with sampoorna treatment at farmers' rearing house conditions.

For the computation of index values under various fifth instar larval density and fifth instar larval feeding quantity regimes coupled with treatment of **Sampoorna**, only two fifth instar larval spacing regimes, optimal fifth instar larval spacing and less fifth instar larval population spacing from larval spacing regimes and optimal fifth instar larval feeding quantity and less fifth instar larval feeding quantity were alone taken. The more fifth instar larval spacing and more fifth instar larval feeding quantity regimes are eliminated as these two conditions resulted in on-par counts with their counterpart optimal conditions.

a. Intensity of mixed-age characters appearance in CSR2 x CSR4 population with fifth instar larval population spacing coupled with Sampoorna treatment at farmers' rearing house conditions: In the context of CSR2 x CSR4 larval ripening duration index under less fifth instar larval spacing and optimal fifth instar larval spacing, coupled with Sampoorna treatment at Farmers' level, data were (from Chapter IV) analyzed for larval ripening duration index). For calculating ripening duration index, the optimal fifth instar larval spacing was considered as control and the remaining two fifth instar larval spacing and Sampoorna treatment were taken as experimental. The analyzed data on hatching duration index are presented in Figure 5.

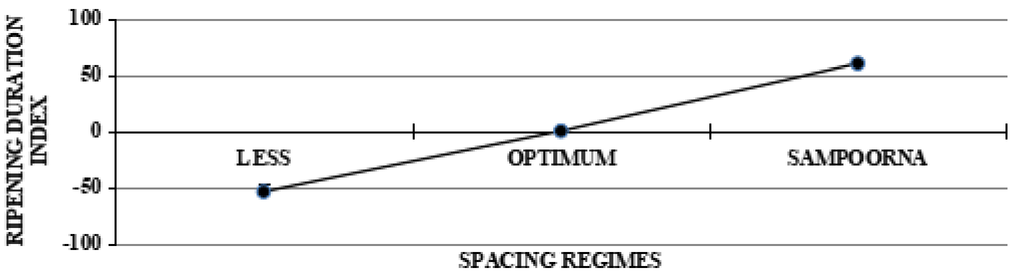

Figure 5: Larval ripening duration index for CSR2 x CDR4 with two fifth instar larval spacing regimes, optimum fifth instar larval spacing (optimal larval population density of 70 number of larvae/ft^2), low fifth instar larval spacing (high larval population density of 130 number of larvae/ft^2) coupled with Sampoorna treatment at Farmers' Rearing House conditions. (Mean of 5 replication ± SD).

From the graph, it is clear that CSR2 x CSR4 larvae with less fifth instar larval spacing (high larval population density of 130 number of larvae/ft^2) recorded very awkwardly negative larval ripening duration index (-54% ± 7.060) and it is fixed at negative bottom level in the graph (Figure 5). The larval ripening duration index for optimal fifth instar spacing (optimal larval population density of 70 number of larvae/ft^2) with CSR2 x CSR4 was 0.0%. The index for Sampoorna treated CSR2 x CSR4 was surprisingly more towards positive side (60% ± 2.824). The observation indicates that the more mixed-age population was converted into a synchronized and straight population.

b. Intensity of mixed-age characters appearance in CSR2 x CSR4 population with fifth instar larval feeding quantity coupled with Sampoorna treatment at farmers' rearing house conditions: For calculating the larval ripening duration index with optimal fifth instar larval feeding quantity and less fifth instar larval feeding quantity coupled with Sampoorna treatment at farmers' level and computed. The computed data on larval ripening duration under optimal fifth instar larval feeding quantity and less fifth instar larval feeding quantity coupled with Sampoorna treatment are graphed in Figure 6

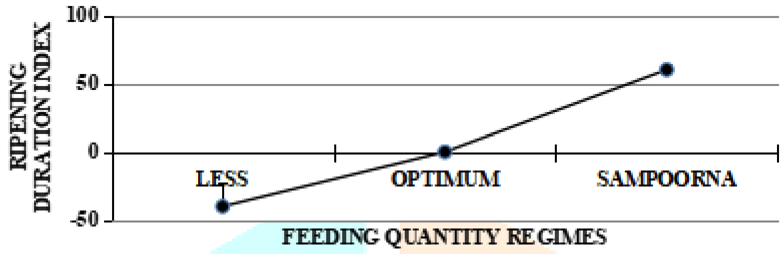

Figure 6: Larval ripening duration index for CSR2 x CDR4 with two fifth instar larval feeding quantity regimes, optimum fifth instar larval feeding quantity and low fifth instar larval feeding quantity coupled with Sampoorna treatment at Farmers' Rearing House conditions. (Mean of 5 replication ± SD).

In the case of larval ripening duration index with two fifth instar larval feeding quantity regimes, optimum fifth instar larval feeding quantity and low fifth instar larval feeding quantity coupled with Sampoorna treatment at Farmers' Rearing House conditions, larval ripening duration of CSR2 x CSR4 with low fifth instar larval feed quantity recorded a low and negative value (-40% ± 16.960) indicating that this particular treatment alone showing late of mixed-age characters. The larval ripening duration index of CSR2 x CSR4 with optimum fifth instar larval feed was neutral, fixed on base line of graph (Figure 6.) whereas, the index value of CSR2 x CSR4 treated with Sampoorna was positively high (60% ± 4.902). The condition definitely indicates much synchronized state of ripening.

EVALUATION INDEX STUDIES:

I. Evaluation of mixed-age characters appearance in egg hatching of CSR2 x CSR4 through Evaluation Index.

1. Intensity of mixed-age characters in egg hatching of CSR2 x CSR4 at Laboratory level: In the present context of measuring hatching and brushing percentage disparities of mixed-age characters using Evaluation Index (*EI*) for CSR2 x CSR4 hatching at Laboratory level has been concentrated using four environmental (photoperiodic) factors, LD 12: 12, DD (continuous dark), LL (continuous light) and a technology of incubation and hatching, Black-Box system were studied. The other influential factors such as temperature and humidity were completely controlled. Data on hatching percentage and brushing was considered and analyzed for EI for hatching and brushing percentages.

a. Studies on computing *EI* for total brushing percentage of CSR2 x CSR4 at Laboratory conditions: In sericulture, the final products are total hatching percentage and total brushing percentage. The hatching percentage is referred to the sum total hatching percentage of all the days over total number of eggs kept for incubation while brushing percentage is from the highest hatching of the day that is considered for continuation of silkworm rearing. Total hatching data in CSR2 x CSR4 incubated in four photoperiodic conditions (LD 12 : 12, DD, LL and Black Box) are presented in Table 1.

Table 1: Total hatching percentage in CSR2 x CSR4 incubated under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box conditions at Laboratory level.

Replication	Total hatching Percentage under photoperiodic conditions.			
	LD 12 : 12	DD	LL	Black-Box
1.	95	97	98	99
2.	94	96	93	100
3.	96	99	96	100
4.	92	95	92	98
5.	97	97	91	95
Average	95	97	94	98
± SD	1.924	1.483	2.915	2.074

From the Table 1, total hatching percentage in CSR2 x CSR4 under all four experimental photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) seem to be identical as the hatching percentage crossed 95% with a range of 94 to 98%. Statistically, all four experimental photoperiodic conditions did not show any significant differences. In such circumstances, one can be misled to assume that all photoperiodic conditions studied are similar in terms of total hatching in CSR2 x CSR4.

Similarly, the next important economic aspect of hatching in CSR2 x CSR4, the brushing percentage is furnished in Table 2.

Table 2: Brushing percentage in CSR2 x CSR4 incubated under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box conditions at Laboratory level.

Replications	Total Brushing Percentage under photoperiodic conditions.			
	LD 12 : 12	DD	LL	Black-Box
1.	92	61	87	100
2.	96	71	91	98
3.	97	73	87	100
4.	94	60	89	91
5.	98	79	88	100
Average	95.4	68.8	88.4	97.8
± SD	2.408	8.136	1.673	3.899

From the above two tables (data on total hatching percentage and brushing percentage), the evaluation index (*EI*) values were computed and given in Table 3.

Table 3: Evaluation Index (EI) values computed for CSR2 x CSR4 incubated under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Laboratory level.

Conditions	EI for Total Hatch	EI for Brushing	Tot EI	Average EI	± SD
LD 12 : 12	43.960	55.930	99.890	49.945	8.465
DD	54.027	35.706	89.733	44.867	12.955
LL	49.542	50.608	100.150	50.075	0.754
Black-Box	62.081	57.755	119.836	59.918	3.059

The computed data on EI for total hatching in CSR2 x CSR4 under four photoperiodic conditions at Laboratory conditions are presented in graph (Figure 7).

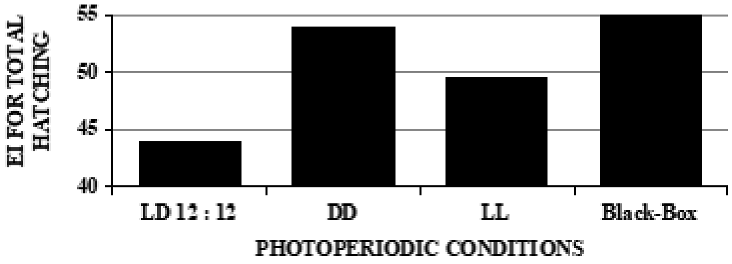

Figure 7: Computed data on EI for total hatching percentage in CSR2 x CSR4 under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Laboratory conditions.

From the data furnished in Figure 7, it is clear that EI values for CSR2 x CSR4 are different with different photoperiodic condition. Thus, the lowest EI recorded surprisingly for LD 12 : 12 condition. Highest EI was recorded in CSR2 x CSR4 under Black-Box condition.

The computed EI values for brushing percentage with CSR2 x CSR4 under all the four experimental photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) for CSR2 x CSR4 at Laboratory conditions are presented in Figure 8.

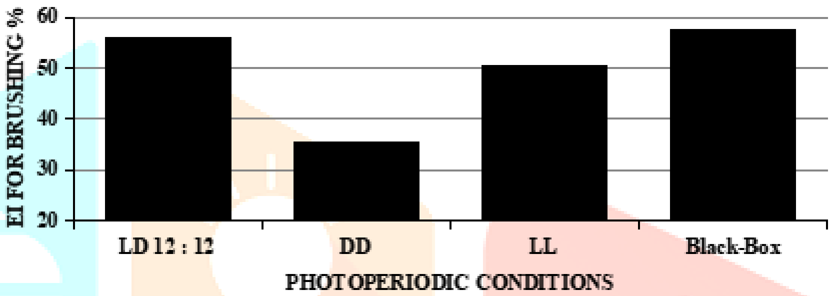

Figure 8: Computed data on EI for brushing percentage in CSR2 x CSR4 under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Laboratory conditions.

In the case of EI for brushing percentage of CSR2 x CSR4 silkworm hybrid under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Laboratory conditions also varied among different photoperiodic conditions. Thus, EI for brushing percentage with LD 12 : 12 condition and Black-Box incubation conditions have recorded highest followed by LL and DD. This type of EI leads to confusion. To avoid such ambiguity, the problem is resolved through computing average EI value, which are given in Figure 9.

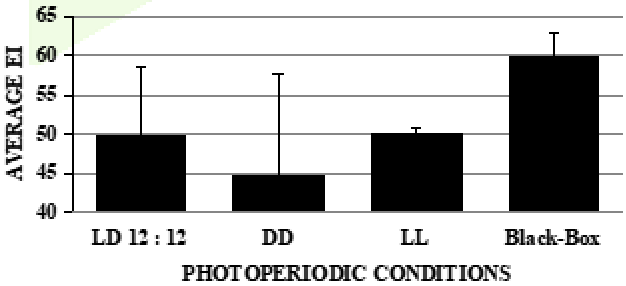

Figure 9: Computed data on average EI for brushing % in CSR2 x CSR4 under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Laboratory conditions.

In the case of average EI computation (Figure 8), it is clear that the average EI in CSR2 x CSR4 is high with Black-Box condition alone with EI value of 60. The other three photoperiodic conditions (LD 12 : 12, DD and LL) did not even scored EI crossing 50, which is designated allowable range.

II. Evaluation of mixed-age characters appearance in egg hatching of CSR2 x CSR4 through Evaluation Index.

1. Intensity of mixed-age characters in egg hatching of CSR2 x CSR4 at Farmers' Rearing House conditions: The mixed age characters of egg hatching in CSR2 x CSR4 at farmers' rearing house conditions also computed through Evaluation Index. Crude data on total hatching and brushing percentage of CSR2 x CSR4 incubated under four photoperiodic conditions, LD 12 : 12, DD, LL and Black-Box system are tabulated in Table 4 and Table 5 respectively.

Table 4: Total hatching percentage in CSR2 x CSR4 incubated under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box conditions at Farmers' level.

Replications	Total hatching Percentage under photoperiodic conditions.			
	LD 12 : 12	DD	LL	Black-Box
1.	90	97	89	99
2.	94	95	88	99
3.	92	94	91	97
4.	89	97	86	96
5.	96	98	84	93
Average	92.200	96.200	87.600	96.800
± SD	2.864	1.643	2.702	2.490

From the data presented in Table 4, it is clear that total hatching percentage is very low (88% ± 2.702). However, the other three photoperiodic conditions, LD 12 : 12, DD and Black-Box, show no statistical differences. Examining the brushing percentage in CSR2 x CSR4 under four photoperiodic conditions, LD 12 : 12, DD, LL and Black-Box system (Table 5), data on brushing was not consistent in CSR2 x CSR4 with all four photoperiodic conditions. Thus, brushing percentage was far below the economic level of brushing (92%) under DD and LL conditions in CSR2 x CSR4 at farmers' level. With photoperiodic conditions of LD 12 : 12 and Black-Box system, brushing percentage in CSR2 x CSR4 were 93.4% ± 1.673 and 96.4% ± 3.362 respectively crossing economic levels of brushing at farmers level.

Table 5: Brushing percentage in CSR2 x CSR4 incubated under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box conditions at Farmers' level.

Replications	Total Brushing Percentage under photoperiodic conditions.			
	LD 12 : 12	DD	LL	Black-Box
1	92	61	87	97
2	96	71	91	98
3	93	73	87	96
4	94	60	89	91
5	92	79	88	100
Average	93.400	68.800	88.400	96.400
± SD	1.673	8.136	1.673	3.362

The crude data on total hatching percentage and brushing percentage are treated for Evaluation Index values. Thus, the EI values of total hatching and brushing percentage computed for CSR2 x CSR4 under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at farmers level are furnished in Table 6.

Table 6: Evaluation Index (EI) values computed for CSR2 x CSR4 incubated under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Farmers' Rearing House conditions.

Conditions	EI for Total hatching %	EI for brushing %	Total EI	Average EI	± SD
LD 12 : 12	47.650	55.360	103.007	51.5035	5.450
DD	57.050	35.540	92.600	46.295	15.210
LL	36.840	51.330	88.170	44.084	10.250
Black-Box	58.460	57.774	116.234	58.117	0.485

Data presented in Table 6 gives very confusing indications on EI of CSR2 x CSR4. For example, EI of CSR2 x CSR for total hatching percentage indicated that total hatching scored more than the 50 EI as well as EI under DD condition. On the other way, EI was more for CSR2 x CSR4 with three photoperiodic conditions (LD 12 : 12, LL and Black-Box). When the EI data is averaged, only two photoperiodic conditions emerged as promising (LD 12 : 12 and Black-Box). Black-Box has resulted in highest EI values for CSR2 x CSR4. This clarity is clear when EI values are graphed (Figure 10, 11 and 13).

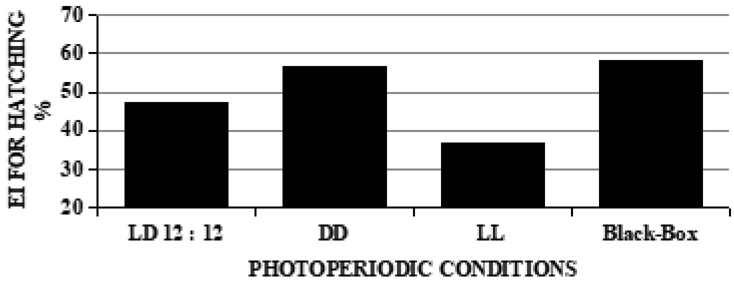

Figure 10: Computed data on EI for total hatching percentage in CSR2 x CSR4 under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Farmers' conditions.

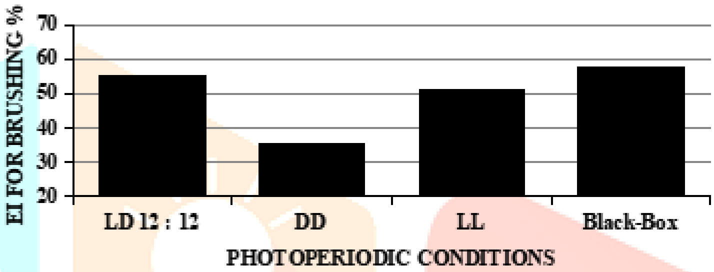

Figure 11: Computed data on EI for brushing percentage in CSR2 x CSR4 under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Farmers Rearing House conditions.

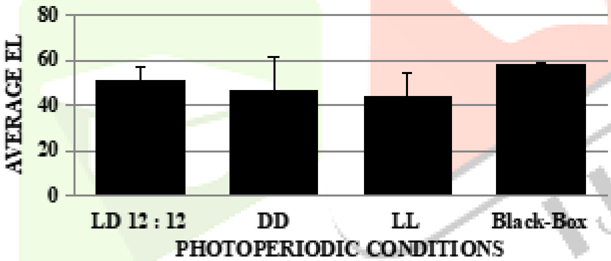

Figure 12 Computed data on average EI in CSR2 x CSR4 under four photoperiodic conditions (LD 12 : 12, DD, LL and Black-Box) at Farmers' conditions.

III. Evaluation of mixed-age characters appearance during larval period of CSR2 x CSR4 through Evaluation Index.

Towards understanding the mode of appearance of mixed-age characters in CSR2 x CSR4, three fifth instar larval spacing; a. high larval spacing (low larval population density of 40 number of larvae/ft^2, b. optimum larval spacing (optimum larval population density of 70 number of larvae/ft^2 and c. low larval spacing (high larval population density of 130 number of larvae/ft^2) and three fifth instar V1 mulberry feeding quantity regimes; a. low V1 mulberry shoot feeding (46.08 kg/1000 larvae), b. optimum V1 mulberry shoot feeding (57.6 kg/1000 larvae) and c. high V1 mulberry shoot feeding (69.12 kg/1000 larvae) were enlisted for examination at farmers' level. Only one character, larval ripening was considered towards this examination. The data on day-to-day larval ripening were taken from chapter III and treated for Evaluation Index values. The data on replication-wise, mean and standard deviation EI for larval ripening in CSR2 x CSR4 with three fifth instar larval spacing are given in Table 7.

Table 7: Calculated *EI* for ripening % (replication wise) and average EI in CSR2 x CSR4 with three fifth instar larval spacing; high V1 mulberry shoot feeding (69.12 kg/1000 larvae).

Larval spacing	EI – replications-wise					Average EI	± SD
	1.	2.	3.	4.	5.		
More	54.730	56.657	52.773	54.588	49.487	53.647	2.701
Optimal	56.757	54.841	58.320	56.882	60.246	57.409	2.010
Sub-Optimal	38.512	38.500	38.906	38.529	40.266	38.942	0.7589

From the graph, it can easily be realized that the average EI values for larval ripening percentage in CSR2 x CSR4 are almost identical under two fifth instar larval spacing, optimal fifth instar larval spacing and high fifth instar larval spacing. With the less fifth instar larval spacing, however, average EI values for larval ripening percentage in CSR2 x CSR4 at farmers rearing house conditions are very low, below admissible value (below 50), indicating that the less fifth instar larval spacing provided an adversely mixed-age characters.

Regarding fifth instar larval feed quantity, three fifth instar larval feeding quantity regimes were selected. The replication wise EI for larval ripening percentage, along with average EI for larval ripening percentage of CSR2 x CSR4 reared with three fifth instar larval feeding quantity regimes, a. low V1 mulberry shoot feeding (46.08 kg/1000 larvae), b. optimum V1 mulberry shoot feeding (57.6 kg/1000 larvae) and c. high V1 mulberry shoot feeding (69.12 kg/1000 larvae) are furnished in Table 8.

The data on average EI for larval ripening in CSR2 x CSR4 with three fifth instar larval spacing are also given in Figure 13.

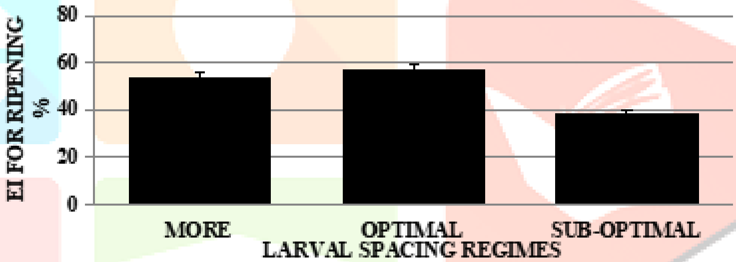

Figure 13: Average EI values for larval ripening % with CSR2 x CSR4 reared under three fifth instar larval spacing; a. high larval spacing (low larval population density of 40 number of larvae/ft^2, b. optimum larval spacing (optimum larval population density of 70 number of larvae/ft^2 and c. low larval spacing (high larval population density of 130 number of larvae/ft^2).

Table 8: Calculated EI (replication wise) and average EI in CSR2 x CSR4 with three fifth instar larval feeding quantity regimes, a. low V1 mulberry shoot feeding (46.08 kg/1000 larvae), b. optimum V1 mulberry shoot feeding (57.6 kg/1000 larvae) and c. high V1 mulberry shoot feeding (69.12 kg/1000 larvae).

Larval Feeding	EI – replications-wise					Average EI	± SD
	1.	2.	3.	4.	5.		
More	52.182	53.919	56.290	54.730	48.350	53.094	3.037
Optimal	58.729	57.447	55.241	56.757	60.722	57.779	2.071
Sub-Optimal	39.089	38.634	38.469	38.512	40.927	39.126	1.036

The average data on EI for CSR2 x CSR4 under three fifth instar larval feeding quantity regimes, a. low V1 mulberry shoot feeding (46.08 kg/1000 larvae), b. optimum V1 mulberry shoot feeding (57.6 kg/1000 larvae) and c. high V1 mulberry shoot feeding (69.12 kg/1000 larvae) are also presented in Figure 14. In the case of different fifth instar larval feeding quantity regimes also, the average EI values in CSR2 x CSR4 under two fifth instar larval feeding quantity regimes; a. high V1 mulberry shoot feeding (69.12 kg/1000 larvae) and b. optimum V1 mulberry shoot feeding (57.6 kg/1000 larvae), recorded over 50 indicating that these two larval feeding conditions are showing less mixed-age characters. The differences in average EI for these two fifth instar larval feeding regimes are not statistically significant. On the other hand, the average EI recorded for sub-optimal fifth instar larval feeding quantity ((46.08 kg/1000 larvae) was far below the acceptable level. The average EI was 39.126 ± 1.036.

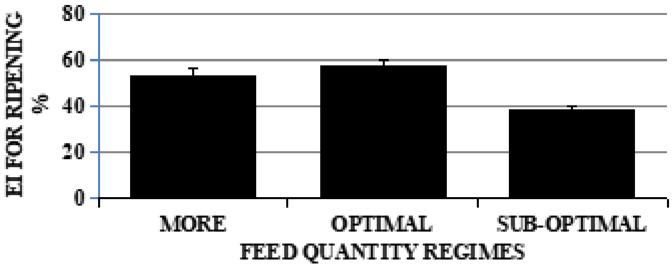

Figure 4: Average EI in CSR2 x CSR4 with three fifth instar larval feeding quantity regimes, a. low V1 mulberry shoot feeding (46.08 kg/1000 larvae), b. optimum V1 mulberry shoot feeding (57.6 kg/1000 larvae) and c. high V1 mulberry shoot feeding (69.12 kg/1000 larvae).

IV. Evaluation of mixed-age characters appearance in the larval ripening treated with Sampoorna in CSR2 x CSR4 through Evaluation Index.

For this study, only two fifth instar larval spacing regimes, a. optimum larval spacing (optimum larval population density of 70 number of larvae/ft^2 and b. low larval spacing (high larval population density of 130 number of larvae/ft^2) and two fifth instar larval feed quantity regimes, a. optimum V1 mulberry shoot feeding (57.6 kg/1000 larvae) and b. high V1 mulberry shoot feeding (69.12 kg/1000 larvae) were considered as the high fifth instar larval spacing and optimal larval spacing on one hand and low fifth instar larval feed quantity and optimal fifth instar larval feed quantity regimes have almost equally registered similar EI values. Instead, treatment of Sampoorna was added to two selected fifth instar larval spacing regimes and the other two fifth instar larval feeding quantity regimes for the study of Evaluation Index values for CSR2 CSR4 at farmers' rearing house conditions. Data on ripening percentage against each experimental condition was taken from Chapter IV and computed for EI of individual entries as well as calculating the average EI. Such data on EI are presented in Table 9.

Table 8: Calculated *EI* ripening percentage (replication wise) and average EI in CSR2 x CSR4 with three fifth instar larval regimes, optimal, sub-optimal fifth instar larval spacing regimes and Sampoorna treated (5 replication ± SD).

Larval spacing	**EI – replications-wise**					**Average EI**	**± SD**
	1.	2.	3.	4.	5.		
Optimal	45.598	46.797	47.818	47.226	44.226	46.333	1.431
Sub-optimal	42.956	41.994	41.271	41.679	44.226	42.425	1.183
Sampoorna	61.446	61.209	60.911	61.094	61.547	61.241	0.258

For better understanding, the same data has been given in Figure 14. It can be seen that the average EI value for both optimal and sub-optimal fifth instar larval spacing did not even crossed while that for CSR2 x CSR4 treated with Sampoorna recorded average EI over 60, indicating best suited for synchronization and reducing mixes-age characters in ripening larval population of CSR2 x CSR4.

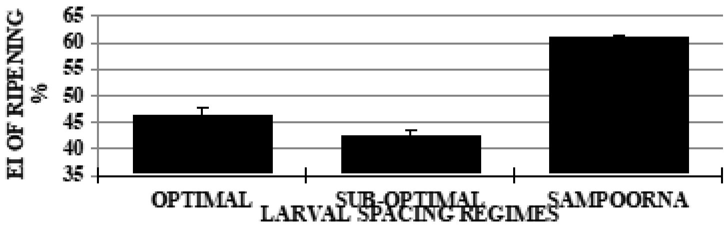

Figure 15: Average EI values for larval ripening % with CSR2 x CSR4 reared under two fifth instar larval spacing; a. optimum larval spacing (optimum larval population density of 70 number of larvae/ft^2 and b. low larval spacing (high larval population density of 130 number of larvae/ft^2) and sampoorna treated larvae.

The data on larval ripening percentage for CSR2 under a. optimal fifth instar feed quantity, b. sub-optimal fifth instar feed quantity and Sampoorna treated larvae are taken from Chapter IV and computed for replication wise EI values and average EI values as well. Such data on replication wide EI and average EI values for CSR2 x CSR4 under optimal and sub-optimal fifth instar larval feed regimes at farmers' rearing house conditions are presented in Table 9.

Table 9: Calculated *EI* ripening percentage (replication wise) and average EI in CSR2 x CSR4 with two fifth instar larval regimes, optimal, sub-optimal fifth instar larval feeding quantity regimes and Sampoorna treated (5 replication ± SD).

Larval feeding	**EI – replications-wise**					**Average EI**	**± SD**
	1.	2.	3.	4.	5.		
Optimal	59.272	47.226	47.818	49.051	47.226	50.119	5.171
Sub-optimal	39.404	41.679	41.271	40.508	41.679	40.908	0.968
Sampoorna	51.325	61.094	60.911	60.441	61.094	58.973	4.284

Average EI data of CSR2 x CSR4 with two fifth instar larval feeding regimes, optimal, sub-optimal fifth instar larval feeding quantity regimes and sampoorna treated batches at farmers' rearing house conditions are given in Figure 15.

The average EI values (Figure 16) clearly indicated that it was very low for sub-optimal feed quantity batches, just crossing 40. Similarly, the silkworm larval batch which was provided with optimal fifth instar larval feed quantity have shown EI value of just over 50. On the other hand, CSR2 x CSR4 larvae treated with sampoorna have recorded highest average EI nearing 60 at farmers rearing house conditions.

Evaluation of cocoon economic traits through evaluation index at farmers' rearing house conditions:

For the purpose of evaluating cocoon economic traits, such as cocoon weight, shell weight and shell ratio with five experimental conditions, optimal fifth instar larval spacing, sub-optimal fifth instar larval spacing, optimal fifth instar larval feeding quantity regime, sub-optimal fifth instar larval feeding quantity regime and Sampoorna treatment at farmers' rearing house conditions were considered with CSR2 x CSR4 silkworm hybrid. The cocoon economic traits of CSR2 x CSR4 at farmers rearing house conditions under the above five experimental conditions are given in Table 10. Since the data are to be processed for further evaluation index, statistical treatment was not applied to avoid ambiguity. From these primary records on cocoon economic traits, Evaluation Index (EI) values were computed and presented in Table 11). From the Table 11, it can be seen that the EI values for all cocoon economic traits (cocoon weight, shell weight and shell ratio) registered below 50 level, indicating that the sub-optimal fifth instar larval spacing and sub-optimal fifth instar larval feeding quantity regime as well induce much mixed age characters in the population and therefore unacceptable EI values. On the other hand, optimal conditions of both larval spacing and feeding conditions have recorded EI values just touching 50. Interestingly, EI of Sampoorna treated CSR2 x CSR4 silkworm hybrid larvae registered more than 60. The situation is till clear when the data on EI for cocoon economic traits like cocoon weight, shell weight and shell ratio are converted into graph (Figure 15 for EI of cocoon weight, Figure 16 for EI of shell weight, Figure 17 for EI of shell ratio and Figure 18 for average EI values).

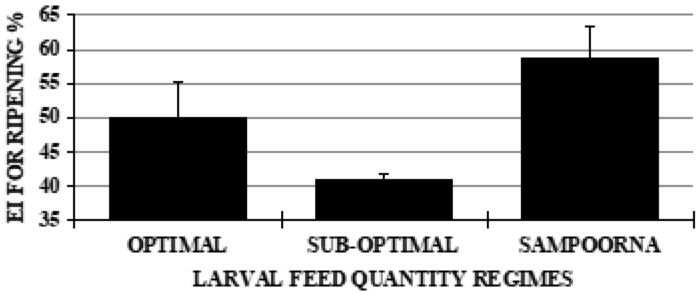

Figure 16: Average EI values for larval ripening % with CSR2 x CSR4 reared under two fifth instar larval feeding regimes; a. optimum fifth instar larval feeding quantity, b. sub-optimal fifth instar larval feeding quantity regime and c. sampoorna treated larvae.

Table 10: Cocoon economic traits of CSR2 x CSR4 reared under five experimental conditions (optimal fifth instar larval spacing, sub-optimal fifth instar larval spacing, optimal fifth instar larval feeding quantity regime, sub-optimal fifth instar larval feeding quantity regime and sampoorna treatment) at farmers' rearing house conditions.

Conditions	cocoon wt (g)	Shell weight (g)	Shell Ratio (%)
Optimal spacing	1.890	0.380	20.112
sub-optimal spacing	1.790	0.355	19.841
optimal feed	1.900	0.384	20.220
sub-optimal feed	1.820	0.332	18.221
sampoorna	1.990	0.422	21.225
Average	1.878	0.375	19.924
± SD	0.078	0.034	1.087

Table 11: EI values for cocoon economic traits of CSR2 x CSR4 reared under five experimental conditions (optimal fifth instar larval spacing, sub-optimal fifth instar larval spacing, optimal fifth instar larval feeding quantity regime, sub-optimal fifth instar larval feeding quantity regime and sampoorna treatment) at farmers' rearing house conditions.

Experimental Conditions	EI for cocoon weight	EI for shell weight	EI for shell ratio	Total EI	Average EI	± SD
Optimal spacing	51.540	51.595	51.732	154.867	51.622	0.099
sub-optimal spacing	38.705	44.258	49.238	132.201	44.067	5.269
optimal feed	52.824	52.789	52.726	158.339	52.780	0.050
sub-optimal feed	42.556	37.341	34.329	114.226	38.075	4.162
Sampoorna	64.376	64.016	61.975	190.367	63.456	1.295

In all the cases, it is noted that EI for CSR2 x CSR4 at farmers' rearing house conditions under sub-optimal conditions of fifth instar larval spacing and fifth instar larval feed quantity regimes was far below 50. The same for CSR2 x CSR4 at farmers' rearing house conditions under optimal conditions of fifth instar larval spacing and fifth instar larval feed quantity regimes has just 50. EI for CSR2 x CSR4 at farmers' rearing house conditions under sampoorna treatment crossed 60 which is much clear when average EI values are considered.

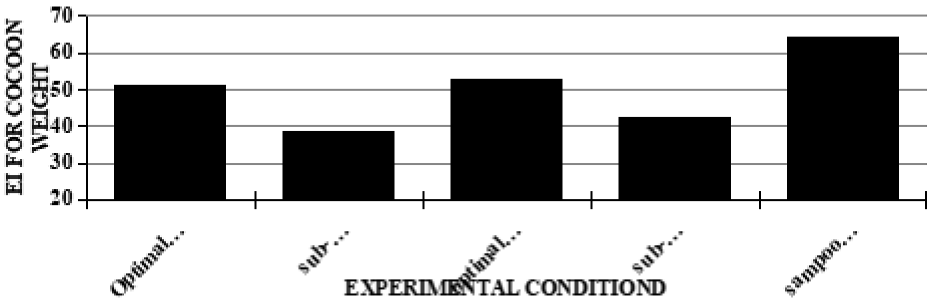

Figure 17: EI for cocoon weight of CSR2 x CSR4 reared at farmers' rearing house conditions under optimal fifth instar larval spacing, sub-optimal fifth instar larval spacing, optimal fifth instar larval feeding quantity regime, sub-optimal fifth instar larval feeding quantity regime and Sampoorna treatment.

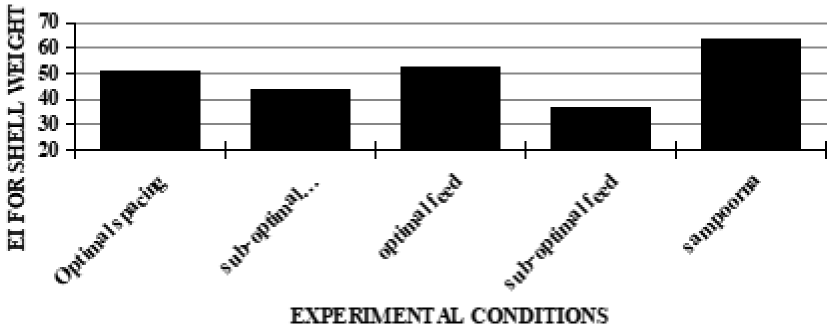

Figure 18:EI for shell weight of CSR2 x CSR4 reared at farmers' rearing house conditions under optimal fifth instar larval spacing, sub-optimal fifth instar larval spacing, optimal fifth instar larval feeding quantity regime, sub-optimal fifth instar larval feeding quantity regime and Sampoorna treatment.

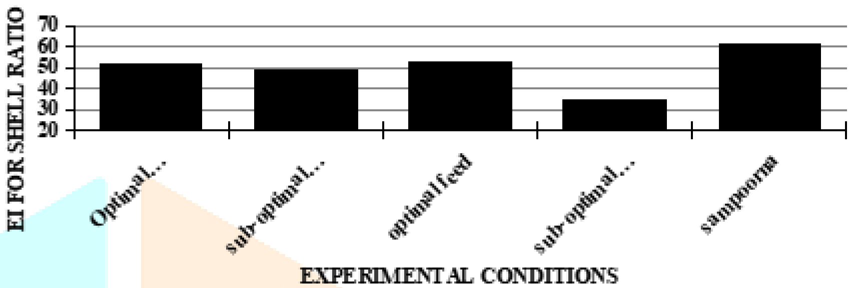

Figure 19:EI for shell ratio of CSR2 x CSR4 reared at farmers' rearing house conditions under optimal fifth instar larval spacing, sub-optimal fifth instar larval spacing, optimal fifth instar larval feeding quantity regime, sub-optimal fifth instar larval feeding quantity regime and s=Sampoorna treatment.

DISCUSSIONS:

The entire discussion deals with two major objective, a. event durations and their index and quantitative traits and their evaluation index. These studies include hatching patterns at laboratory conditions and farmers' rearing house condition, fifth instar larval spacing and larval feed quantity regimes at farmers' condition and finally the application of phytoecdysteroid, Sampoorna on larval ripening with the bivoltine x bivoltine hybrid, CSR2 x CSR4 at farmers' silkworm rearing conditions.

At laboratory conditions, rhythmicity in silkworm egg showed that egg hatching is linked to lights-on signal and the rhythm is a circadian and continued for two consecutive days. With LL, however, the egg hatching rhythm further expressed a near damp-out condition. This nature of silkworm egg hatching condition leads to hatching durations more than 24 hours in LD 12 : 12 DD and LL photoperiodic regimes and near Damper-out condition in LL photoperiodicity which is unacceptable bargain in the commercial rearing. Anantha Narayana, (1980) and Anantha Narayana *et al*. (1978) viewed that silkworm hatching is a day-active and its activity phase-locked to lights-on signal. Egg hatching is under circadian control in PM x NB4D2 (Sivarami Reddy, 1993; Sivarami Reddy and Sasira Babu, 1990; Sivarami Reddy *et al.*, 1998). Shanthan Babu, (2014), Srinath, (2014), emphasized a new hypothesis of mixed-age expression. Further worked the economic aspects of silkworm egg hatching which included total egg hatching percentage and brushing percentages. Kanika Trivedi, (2015) strongly felt that egg hatching in silkworm should be restricted to a single day. Economic hatching should be around 95% (Krishnaswami, 1990); brushing percentage should be over 90% (Kawakami and Yanagawa, 2003). Even at farmers' conditions too, reports that silkworm egg hatching are now available. They reported two consecutive days hatching under LD 12 : 12, DD an LL. Thus, a two-day hatching was not acceptably and a new silkworm incubation system, Black-Boxing is introduced. With Black-Box system, not only the silkworm egg hatching was restricted to a single day, but also hatching duration was further reduced to 3 to 4 hours only with more than 95% of egg hatching and more than 93% brushing.

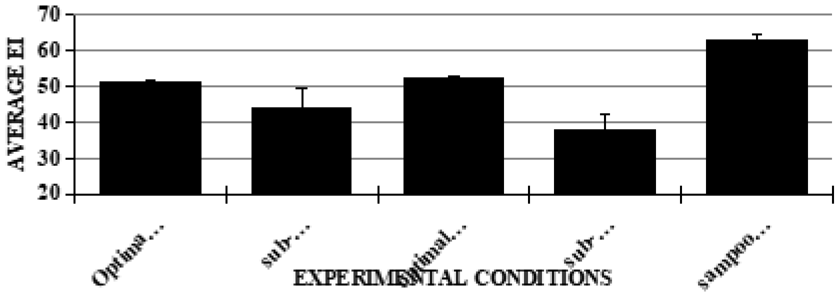

Figure 19: Average EI for cocoon economic traits (cocoon weight, shell weight and shell ratio) of CSR2 x CSR4 reared at farmers' rearing house conditions under optimal fifth instar larval spacing, sub-optimal fifth instar larval spacing, optimal fifth instar larval feeding quantity regime, sub-optimal fifth instar larval feeding quantity regime and sampoorna treatment.

In the present research work, computing the hatching duration data for index values revealed that while the hatching durations index was on base line (0.00%), the same for DD and LL conditions were towards negative level at Laboratory (Figure 1) and Farmers (Figure 2) conditions. The hatching duration index for DD was on very positive side (Figures 1 and 2) indication that the hatching duration is so bettered that a hatching duration of more than 24 hours under LD 12 : 12, DD and LL has been restricted a mere 2 to 3 hours in Black-Box system of photoperiodic condition. On the other side, the computation of silkworm egg hatching economic traits such as total egg hatching and total brushing percentage in CSR2 x CSR4 were computed for Evaluation Index (*EI*) values. It is amply revealed that *EI* for total hatching percentage for CSR2 x CSR4 at laboratory conditions with LD 12 : 12 and LL photoperiodic conditions are far below acceptable range (*EI* is below 50). The same for DD and Black-Box conditions crossed the acceptable range of *EI* (*EI* for total hatching in DD is 54 and that for Black-Box condition is 62, Table. 3 and Figure 7). For *EI* of brushing percentage in CSR2 x CSR4 at laboratory level, *EI* of both under LD 12 : 12 and Black-Box alone secured more than 50 and acceptable in terms of brushing only (Table 3 and Figure 8). The other two photoperiodic conditions, DD and LL registered *EI* of below 50 and with DD it was too below. The prime reasons for such far below *EI* with DD was that the hatching divided itself almost equally for two consecutive days of hatching and the highest hatching was considered for brushing percentage. The average *EI* values clearly showed that *EI* for CSR2 x CSR4 under Black-Box condition alone at laboratory condition is highly acceptable since average *EI* for Black-Box system alone crossed 50 marks (average *EI* is 60; Table 3 and Figure 9). At farmers rearing conditions, *EI* for total hatching percentage were over 50 marks under DD and Black-Box system (Table 6 and Figure 10). On the other hand *EI* for CSR2 x CSR4 under LD 12 : 12 and Black-Box system have crossed 50 marks at farmers level (Table 6 and Figure 11). The tray hatching on day-one under LD 12 : 12 condition did not accounted for brushing percentage and therefore brushing percentage and further *EI* for brushing were more. The average *EI* examination revealed that average *EI* for LD 12 : 12 and Black-Box system alone scored over 50 and Black-Box scored 58 (Table 6 and Figure 12).

Studies and their reports on the implications of fifth instar larval spacing requirements for the silkworm *B. mori* at laboratory conditions are available (Ravi, 2014; Lakshminarayana Reddy, *et al*., 2015). However, these studies are not available at farmers' condition. Also, reports on the fifth instar larval feed quantity and its necessity on fifth instar larval growth and development are not at all available both at laboratory and farmers conditions too. In the present study, such studies are taken with five selected farmers taking bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4.

Earlier reports categorized fifth instar silkworm larval densities into 3 broad divisions; uneconomical larval population density zone (ULPDZ), b. optimum larval population density zone (OLPDZ), and c. loss larval population density zone (LLPDZ). In the present study also three fifth instar larval spacing schedules; low fifth instar larval population spacing, optimum fifth instar larval population spacing and high fifth instar population spacing were used. Computation of index values using larval ripening revealed three types of relations to the fifth instar larval spacing recorded two types of index values, index value 0.00% or near 0.00% and negative index (Figure 3). Similar results on index values were reported for both PM x CSR2 and CSR2 x CSR4. The results on fifth instar larval population spacing indicates that with less larval spacing (or high population density), the larval growth is reduced as the competition for the available feed is increased (Krishnaswami, 1990). Similar results for computed larval ripening duration index with three types of fifth instar larval feeding quantity regimes. With more feeding quantity and optimum feed quantity, the larval ripening index values are on/near 0.00% level and that for the larvae reared with sub-optimal feed recorded negative index values (Figure 4). Less available feed tend to malnutrition in growing larval population leading increased ripening as also mortality incidences (Krishnaswami, 1990).

In the case of evaluation index on the larval ripening quantity for CSR2 x CSR4 with three fifth instar larval population spacing regimes at farmers' level, the *EI* for both more spacing regime and optimal-spacing regimes scored more than 50 marks. The *EI* for larvae of CSR2 x CSR4 reared on sub-optimal fifth instar larval spacing regime was very low (below 50 marks, Figure 12). In the same way, *EI* for ripening quantity for CSR2 x CSR4 at farmers' level under three fifth instar larval feeding quantity regimes behaved similarly. Thus, *EI* of larval ripening percentage for more feed quantity and optimal feed quantity recorded high values (more than 50 marks) indicating best available feed and best growth, resulting in more larval ripening percentage (Krishnaswami, 1990).

For evaluating the impact of Sampoorna on larval ripening period, larval ripening quantum and cocoon economic characters, only two experimental conditions from fifth instar larval spacing and fifth instar feeding quantity regimes (optimal and sub-optimal conditions) were considered along with Sampoorna treatment in CSR2 x CSR4 at farmers' rearing environment. The index for computing revealed that the index value was on negative side in CSR2 x CSR4 for sub-optimal larval spacing and sub-optimal feeding schedules at farmers' conditions while it (index value) on base line for optimal spacing and feeding regimes (Figure 5 and Figure 6). The index values for Sampoorna treated larvae surprisingly was a huge positive value (Index value above 60%) The observation indicates that the duration of larval ripening in Sampoorna treated larvae is far less compared to that of optimal and sub-optimal larval spacing and feeding regimes. Similarly, the computation of Evaluation Index (*EI*) revealed the highest *EI* for Sampoorna treated CSR2 x CSR4 larvae under both fifth instar larval spacing and fifth instar larval feeding quantity experiments (Figure 14 and 15), indicating that the larval ripening quantum if for higher in Sampoorna treated experimental conditions treated with Sampoorna.

The ultimate step in these studies is evaluation of cocoon economic characters. Two experimental conditions from fifth instar larval spacing regimes (optimal fifth instar larval spacing and sub-optimal fifth instar larval spacing) and two experimental conditions from fifth instar larval feeding quantity regimes (optimal fifth instar larval feeding quantity regime and sub-optimal fifth instar larval feeding quantity regime) were taken in this study and application of Sampoorna was added. Upon physical comparison of CSR2 x CSR4 cocoon quantitative traits such as cocoon weight, shell weight and shell ratio (Table 10) it is confused that values are very close under five experimental treatments (optimal spacing, sub-optimal spacing, optimal feed, sub-optimal feed and Sampoorna treatment). For example, the range of cocoon weight (from 1.8 to 2.0 g,),the range of shell weight (from 0.33 to 0.42 g) and shell weight (from 18.2 to 21.2%) forces a common man to assume that the population is almost a homogenous one. However, when data are computed for Evaluation Index (*EI*), the differences are drastic. Thus, *EI* values for cocoon weight crossed 50 marks for three experimental conditions; optimal spacing, optimal feeding and Sampoorna treatment (Figure 16). Similar trends in *EI* values for Shell weight (Figure 17) and shell ratio (Figure 18) was observed. When the individual *EI* values are averaged (average *EI*) a clear-cut trend is predominantly visible. Thus, the average *EI* values are, no doubt, more than 50 both for optimal spacing and optimal feeding regimes, it (average *EI*) of Sampoorna treated CSR2 x CSR4 larvae out scored (average *EI* = 64).

To conclude, the uniformity is increased in egg hatching with Black-Box method of incubation though there appeared much mixed-age character condition with traditional photoperiodic conditions such as LD 12 : 12, continuous dark (DD) and continuous light (LL) in CSR2 x CSR2, both at laboratory and farmers' rearing environments. For uniformity in fifth instar larval growth and larval ripening, optimal fifth instar larval spacing and optimal fifth instar larval feed quantity is a promising one. Finally, treatment of CSR2 x CSR4 with sampoorna at the onset of ripening resulted in strict uniformity of larval population ripening phenomenon through avoiding the traditional internal clock and internal PTTH control over larval ripening process and thereby restriction of ripening duration from more than 48 hours to mere 18 to 20 hours. The cocoon economic characters are also bettered in CSR2 x CSR4 when treated with Sampoorna.

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