INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

EXTENDED STUDIES ON THE EFFECTIVENESS OF PHYTOECDYSONE ON PUPATION COMPONENTS IN TWO HYBRIDS OF MULBERRY SILKWORM, *BOMBYX MORI* L.

Suvarna, G., Lavanya Latha, K., *Lakshminaraya Reddy, P., Sujatha, B., Satyanarayana, B. & Sankar Naik, S.
Department of Sericulture, Sri Krishnadevaraya University, Anantapur - 515 003, Andhra Pradesh, India.
*Corresponding Author E-mail:- lakshminarayanareddyp@yahoo.in

ABSTRACT
Response of phytoecdysteroid, '*Sampoorna*' on reduction of larval ripening period are already available. However, all the reported studies on application of sampoorna to the silkworm at the onset of ripening were restricted to the investigations on larval ripening patterns, time taken and economic characters only. In the present communication, implication of *Sampoorna* on all the three components of pupation *viz*., larval ripening, pharate pupal formation and pupation are accounted. Two popular mulberry silkworm (*Bombyx mori* L.) hybrids, PM x CSR_2 (multivoltine x bivoltine) and CSR_2 x CSR_4 (bivoltine x bivoltine) hybrids, reared under LD 12 : 12 condition, on V1 mulberry variety at 25 ±1°C temperature and 80 ±5% RH conditions were taken as experimental materials. *Sampoorna* was administered, *peroral*, through feeding mulberry leaf sprayed with 250µg in 10ml distilled water on 100g of mulberry leaves/100 larvae (2.5µg of *Sampoorna*/larva) at the onset of larval ripening. Control batch silkworm larvae received similar quantity of mulberry leaf sprayed with 10ml distilled water. The three pupation components, larval ripening, pharate pupal formation and pupation were studied both for control and experimental larvae (5 replications each). All the pupation components expressed 2 gates for PM x CSR_2 and 3 gates for CSR_2 x CSR_4 of activity with circadian frequency, taking lights-on as initiation signal, diurnal, gated activity and mixed population characters. Durations of pupation components were more, with 26, 33 and 30 hours for PM x CSR_2 for larval ripening, pharate pupal formation and pupation respectively and 59, 52 and 52 hours CSR_2 x CSR4 for the same components respectively. Upon *Sampoorna* treatment, the durations of these components reduced from 26 to 23 hours for larval ripening, 33 to 14 hours for pharate pupal formation and 30 to 15 hours for pupation with PM x CSR_2 and 59 to 26 hours for larval ripening, 52 to 21 hours for pharate pupal formation and 52 to 14 hours for pupation with CSR_2 x CSR_4. Apart from distribution diagrams, data on cumulative frequency clearly indicated that under control conditions, the curves were step-wise incremental for control larvae while that for *sampoorna* treated, these responses were curvilinear ones. The percentage of reduction in pupation component events (%) in CSR_2 x CSR_4 are more (56% for larval ripening 60% for pharate pupal formation and 73% for pupation) than those for PM x CSR_2 (15% for larval ripening, 58% for pharate pupal formation and 50% for pupation) indicating that *sampoorna* may not be much useful for PM x CSR_2. Further, sampoorna has more influence on pharate pupal formation compared to high impact on pupation. Thus, sampoorna reduced all the component durations of larval ripening, pharate pupal formation and pupation in both PM x CSR_2 and CSR_2 x CSR_4 as well, however with less applicability to PM x CSR_2. Results are discussed on the importance of effectiveness of *sampoorna* in bivoltine x bivoltine hybrid sericulture.

KEYWORDS: Mulberry silkworm, *Bombyx mori* L., phytoecdysteroid, *Sampoorna*, pupation components, reduction in durations.

INTRODUCTION

In mulberry silkworm, *Bombyx* mori L., there are well defined three types of ecdysial processes; the larval-to-larval ecdysis, the larval-to-pupal ecdysis and the pupal-to-adult eclosion. The larval-to-larval ecdysis, commonly called as larva moulting, occurs three to four times based on moultinism. Since the two mulberry silkworms (multivoltine x bivoltine hybrid, PM x CSR_2 and bivoltine x bivoltine hybrid, CSR_2 x CSR_4) are tetra-moulters, they moult for four times in their larval or eating period. Larval-to-larval ecdysing processes are simple, with only two components, settling for moult (SM) and completion of moult (CM). However, the larval-to-pupal ecdysis is a complex process and is too lengthy one; ecdysis includes sequences like cessations or stopping feeding, larval ripening, hastened locomotor activity towards periphery of rearing space in search of a convenient place for cocooning, construction of cocoon, pharate pupal formation after completion of cocooning, fabrication of new cuticle inside the old pharate pupal cuticle, secretion of glue material between old and new cuticle, casting of old skin, expansion and hardening of the new pupal cuticle and finally pupation. There are seen clear defined three components in larval-to-pupal ecdysis, larval ripening, pharate pupal formation and pupation (Sivarami Reddy *et al*., 1993). Well characterized evidences are available that the prothoracicotrophic hormone (PTTH) from the brain exercises a tropic influence on the prothoracic gland. Thus released PTTH directs the prothoracic gland for the release an inactive ecdysone hormone. The passive ecdysone is then converted into an active ecdysone by the peripheral tissue. This active ecdysone leads the lengthy larval-to-

pupal ecdysis, causes the apolysis and the initiating secretion of a new cuticle by the epidermis (Reynolds, 1980; Truman and Taghert, 1981; Happ, 1984).

In any moult (larval-to-larval or larval-to-pupal), the ecdysis or moult is not considered as gated but viewed occurring after certain fixed hours of gated PTTH release (Beck, 1980; Truman, 1972; Truman and Taghert, 1981). This type of action resembles the mimic of gated appearance of PTTH. Therefore, rhythm in components of pupation such as ripening, pharate pupal formation and pupation in the present study should be considered as mimicking of gated phenomenon. This type of mimicking the actual PTTH gated release is termed as a 'fortuitous synchrony' in the mixed age population of *Bombyx mori*. In such case of 'fortuitous synchrony', the picking-up of the ripened silkworm became time taking and laborious factor. The situation also adds to the cocoon production cost (Kanika Trivedi *et al*., 2003; Sashindran Nayar *et al*., 2005; Nirmal Kumar *et al*., 2006, 2007). They (Kanika Trivedi *et al*., 2003; Sashindran Nayar *et al*., 2005; Nirmal Kumar *et al*., 2006, 2007) viewed that larval ripening in *Bombyx mori* larvae should be quick and continuous, limited to a single day. In accurate words, the expression of larval ripening from 2 days (in PM x CSR_2) and from 3 days (in CSR_2 x CSR_4) in *B. mori* should be eliminated and curb the phenomenon to a single day.

Phytoecdysteroids are used in *B. mori* cocoon crops in China, Japan and South Korea to decrease larval ripening period and to increase productivity in sericulture (Zhuang *et al*., 1992). In India also, the phytoecdysteroids have been recently employed. The premier research institute in sericulture, the Central Sericultural Research and Training Institute, Mysore released a phytoecdysteroid with a brand name, '*Sampoorna*' (Kanika Trivedy *et al*., 2003, Bindroo and Satish Verma, 2014). At present use of *sampoorna* is a recommended technology for commercial application on silkworm for early and uniform maturation (larval ripening) of silkworm without affecting the cocoon economic characteristics (Bindroo and Satish Verma, 2014). Sampoorna is not only effective for uniform maturation, but also in certain situations like leaf shortage, occurrence of non-cocooning silkworm and possibility of diseases outbreak. However, researches on phyto-ecdysteroids were restricted to only the first component of larval-to-pupal ecdysis, larval ripening alone (Kanika Trivedi *et al*., 2003; Sashindran Nayar *et al*., 2005; Nirmal Kumar *et al*., 2006, 2007, Shanthan Babu, 2014; Srinath, 2014; Srinath *et al*., 2018). The other two pupation process components *viz*., pharate pupal formation and pupation were left untouched. Keeping this in view, an attempt is made in the present investigation to assess the implications of *Sampoorna* in reducing the ripening period in two mulberry silkworm hybrids, multivoltine x bivoltine (PM x CSR_2) and bivoltine x bivoltine (CSR_2 x CSR_4) under natural day (LD 12:12) conditions through chrono-biological approach. The studies thus were extended to complete three components of pupation (larval ripening, pharate pupal formation and pupation).

MATERIALS AND METHODS

The experimentation was conducted with two popular mulberry silkworm hybrids, one each from multivoltine x bivoltine hybrid, PM x CSR_2 and the other from bivoltine x bivoltine hybrid, CSR_2 x CSR_4. Silkworm eggs (DFLs, disease free layings) were procured on the third day of oviposition, from the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Madanapalli, Chittoor District, Andhra Pradesh. The DFLs were transported to the experimentation site, the Department of Sericulture, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, during evening cool hours. The DFLs were immediately spread into the pre disinfected rearing trays.

Standard silkworm rearing method followed was that of Krishnaswami (1986). For keeping uniform conditions such as temperature (25 ±1°C), humidity (80 ±5%), the chawki (young age; I & II instar silkworm larvae) rearing was not conducted. Hatched out larvae from the egg sheet were collected into pre-disinfected plastic (Neelkamal) rearing trays (2' x 3'), daily fed three times (06.00, 14.00 and 22.00 h) of the day on fresh mulberry (*Morus* sp., V1 variety) leaves except during moulting. While cleaning the unspent leaves 2 times during I and II instar periods and once in every day after II moult, the larvae were transferred into separate pre-disinfected rearing trays. Larvae under moult were not disturbed. The same day when the DFLs were brought to the laboratory, they were introduced into light-dark schedule, natural solar day photoperiodic condition, LD 12:12 (photophase from 06.00 to 18.00h and scotophase from 18.00 to 06.00 h), Two batches for each hybrid were maintained. Experimental larvae were provided with a dim red light source (> 0.1 lux) for handling animals whenever needed.

Towards the end of the final or 5^{th} instar eating period, the larvae turn into light yellow colour from light ash and this stage is recognized as 'ripening stage' (Krishnaswami, 1986). At this stage, the caterpillars stop feeding and start wandering around for a proper cocooning site. Also the larvae start oozing silk from their spinnerate as a silk thread indicating its readiness for initiating cocooning. This developmental marker event is designated as the 'ripening' in the present study, one of the larval-to-pupal ecdysial components. The second component in pupation pattern is that caterpillar inside cocoon goes on spinning cocoon and when spinning is completed larva loses its grip and start shrinking. This stage is called pharate pupal formation. The third and final component of pupation is described that pharate pupa casts its old skin and this particular stage is termed as pupation. Observations, at one hour intervals, on the number of larvae ripened were recorded. Simultaneously, the ripened larvae were separated from the experimental batches and mounted onto cocooning device (mountage) kept at the distance identical to that between the light source and experimental animals under feeding. It is reported that the cocooning, in *B. mori* completes roughly after 48 hours of mounting (Krishnaswami, 1986). The cocoons from the mountages were carefully taken out before expected hours of cocoon spinning completion. The spinning larvae inside the cocoon are undisturbed. The cocoons thus separated were carefully cut opened on top side of the cocoon, vertically. If the worms inside the cocoon are still continuing with spinning, the operation of cut-opening cocoons was delayed further. Half (vertical) portion of cocoon shell was

removed to expose the animals, enabling easy observations. The animals in the cut-opened cocoons were kept in a tray in a single layer for rest of observations on pharate pupal formation, the second developmental marker event in the larval-to-pupal ecdysial process. At this stage of pharate pupal formation, the silkworm larvae lose their grip with inner layers of the cocoons and start shrinking. The observations on the number of 'pharate pupae' formed were noted at one hour intervals till the completion of the experimental batch. Nearly after 24 hours after pharate pupal formation, the third and the last developmental marker event in the larval-to-pupal ecdysial process, the animals shed their larval cuticle (larval-to-pupal ecdysis) to become pupa. Observations on the number of pupae formed (*i.e*., the number of pharate pupae shed their pharate pupal/larval cuticle) were made at one hour intervals. Data on ripening, pharate pupal formation and pupation were represented as distribution diagrams (hourly histograms, resolved for 24 h; $\omega = 360^{o}$).

Use of phytoecdysteroid available in the brand name *Sampoorna* (Bindroo and Satish Verma, 2014) becomes a routine practice among sericultural farmers of India (Nirmal Kumar *et al*., 2006). *Sampoorna* is administered to silkworm towards the commencement of maturation process at the end of the fifth larval instar period so that all the larvae mature almost simultaneously and the spinning process is synchronized enabling the farmers to market the final product, the cocoon in a single lot. *Sampoorna* was procured from CSRTI, Mysore for the experimentation. *Sampoorna* was administered at the onset of cocooning (at 5% of larval ripening developmental marker event). The larvae were fed with mulberry leaves treated (sprayed) with *Sampoorna* at the rate of 250 µg in 10 ml distilled water on 100 g of mulberry leaves for 100 larvae so that each larva would get 2.5µg of *Sampoorna*. Five replications of 100 larvae each were maintained for each silkworm hybrid. As control batches, five replications with 100 larvae for each replication were also maintained for each hybrid. The control batches received 100g of mulberry leaf sprayed with 10ml distilled water alone. Data on number of larvae ripened, on hourly basis were recorded and represented as distribution diagrams (hourly histograms, resolved for 24h; $\omega = 360^{o}$). Data were subjected to statistical analysis, employing ANOVA to test the significance or otherwise of the results.

RESULTS

The main aim of the experimentation is not only to examine the effectiveness of phytoecdysteroid, *sampoorna* on the reduction of the first pupation component, the larval ripening period in two mulberry silkworm (*Bombyx mori* L.) hybrids; multivoltine x bivoltine hybrid (PM x CSR_2) and bivoltine x bivoltine hybrid (CSR_2 x CSR_4) in the chronobiological perspectives but also extension of these studies to second and third components of pupation, pharate pupal formation and pupal formation. Natural day photoperiodic condition (LD 12:12) was employed for this study. The rhythmicity of larval ripening, pharate pupal formation and pupation of PM x CSR_2 and CSR_2 x CSR_4 and the response of pupation components (larval ripening, pharate pupal formation and pupation to per-oral application of sampoorna was recorded. The data were expressed in distribution diagrams as well as the cumulative frequency graphs.

I. Studies on the action of sampoorna on larval ripening in multivoltine x bivoltine, PM x CSR_2 and bivoltine x bivoltine silkworm hybrid, CSR_2x CSR_4:

1. Studies on the action of sampoorna on larval ripening in multivoltine x bivoltine silkworm hybrid, PM x CSR_2:

a. Studies on the rhythmic patterns on larval ripening in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: Data on larval ripened in percentage against time in the multivoltine x bivoltine silkworm hybrid, PM x CSR_2 under LD 12: 12 conditions are in Fig. 1. Larval ripening occurred for two consecutive days, with less ripening on day 1 and more on day 2. Ripening was day active one, occurring in the light part of the day. Peak ripening activity was at 06.00 h of the day while initiation was at 06.00 h local time on day 1 and at 02.00 h local time on day 2. The rhythmicity in ripening was seen very sharp and precise.

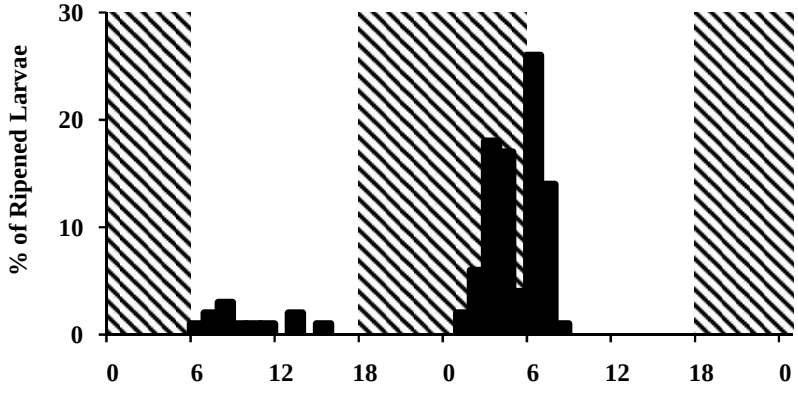

FIGURE 1: Larval ripening behaviour in multivoltine x bivoltine silkworm hybrid, PM x CSR2 of *Bombyx mori* L. under natural day (LD 12 : 12) condition.

b. Studies on the implications of sampoorna on larval ripening in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: Per-oral application of sampoorna on the just initiated ripening larvae of PM xCSR_4 inflicted

significantly on the larval ripening process (Fig. 2). The larvae after sampoorna application did not follow any of the rhythmic characteristics of circadian rhythmicity. Ripening larva continued as if they are in a hurry to complete ripening process immediately. Thus, ripening initiated at 06.00 h local time. There was no break in rhythmicity (gating) as observed for those under LD 12: 12 condition (two consecutive days). Only one day ripening (below 24 hours, Fig. 2.), from 06.00 h of the initiation day to 04.00 h (early hours) on immediate day was seen. For academic purpose, the peak of larval ripening is seen at or around 10.00 h of the day. No larval ripening was seen on day 2 as against that observed for PM x CSR_2 under LD 12:12. Thus, ripening was continuous, without any break when sampoorna is applied.

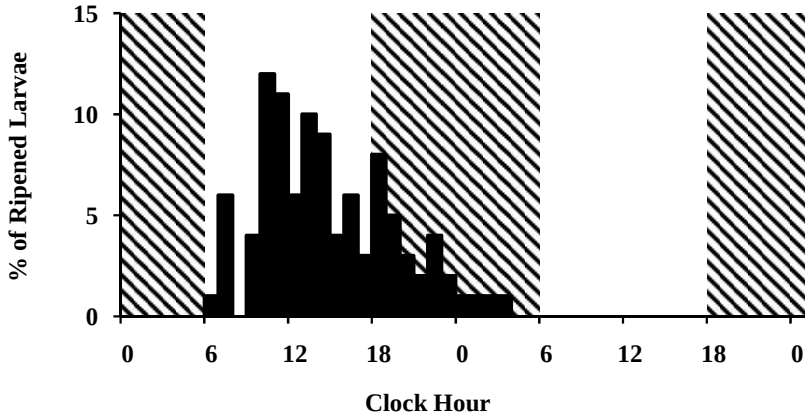

FIGURE 2: Larval ripening pattern in multivoltine x bivoltine silkworm hybrid, PM x CSR_2 of *Bombyx mori* L. as a result of application of sampoorna (a phytoecdysteroid extract) under natural day (LD 12: 12) condition.

2. Studies on the action of sampoorna on larval ripening in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4:

a. Studies on the rhythmic patterns on larval ripening in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: Data on larval ripening in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4 under LD 12: 12 conditions are in Fig. 2. Ripening in CSR_2 x CSR_4 occurred for three consecutive days. Ripening on day 1 was very low, indicating stray larval ripening. The same on day 2 and day 3 was high. Ripening in CSR_2 x CSR_4 also initiated at or around dawn (06.00 h) part of the solar day, indicating predominantly a diurnal response and further lights-on is taken as synchronizing signal. However, appearance of peak was different for different days of ripening. Thus, peak larval ripening was noted at 10.00 h on day 1 and also on day 2. Peak of ripening occurred at 03.00 h of late dark peak. This may be a case of peak bias. Thus, the rhythmicity in larval ripening in bivoltine x bivoltine mulberry silkworm hybrid, CSR_2 x CSR_4 was diurnal, taking lights on as signal for larval ripening synchronization, prolonged for three continuous consecutive days, thus expressing gating phenomenon and it expressed mixed-age characteristics.

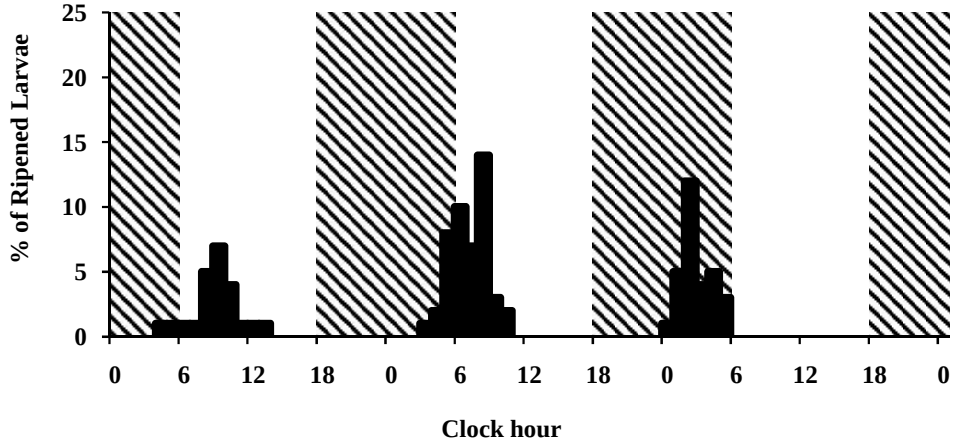

FIGURE 3: Distribution diagram of larval ripening behaviour in pupation patterns of the bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4 of *Bombyx mori* L. under natural day (LD 12: 12) condition.

b. Studies on the effect of sampoorna on larval ripening in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: Application of sampoorna on the just initiated ripening larvae of CSR_4 x CSR_4 induced significant changes in regular (LD 12: 12) larval ripening pattern (Fig. 4). The larvae after sampoorna application not at all followed the

pattern expressed under LD 12:12 photoperiodic conditions. Further, ripening initiated at 04.00 h on the day 1 and completed in the same day at 05.00h. Thus, ripening which was continued for three consecutive days concluded within 24 hours of time, thus saving 2 days.

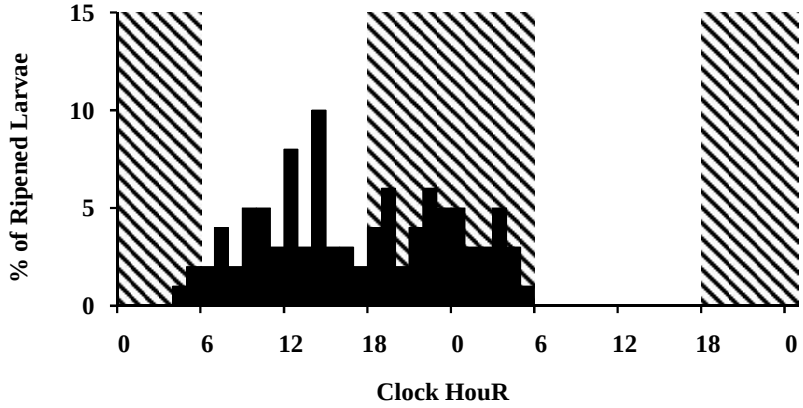

FIGURE 4: Larval ripening pattern in bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4 of *Bombyx mori* L. as a result of application of sampoorna (a phytoecdysteroid extract) under natural day (LD 12: 12) condition.

3. Cumulative expression of larval ripening in mulberry silkworm hybrids, PM x CSR_2 and CSR_2 x CSR_4:

a. Cumulative expression of larval ripening in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: The larval ripening (%) data presented in distribution diagrams (Fig. 1 and 2) were treated for cumulative scoring in larval ripening and presented in Fig. 5. A clear-cut and exclusive curve pattern describing the exact impact of the sampoorna on larval ripening in multivoltine x bivoltine silkworm hybrid, PM x CSR_2 are noticed. Thus, the cumulative response in larval ripening under control, (without sampoorna application, ●) revealed a stepwise positively graded curve was seen with two steps of increase in larval ripening activity. The horizontal line indicates the silence time of action between two peaks pertaining to two consecutive days of ripening rhythmicity. The other curve, indicating the cumulative larval ripening in PM x CSR_2 (ɵ) with the application of sampoorna is an additive curvilinear in appearance. The time taken for completion under control condition extended over 30 hours, while that for batch of sampoorna treated was just below 24 hours.

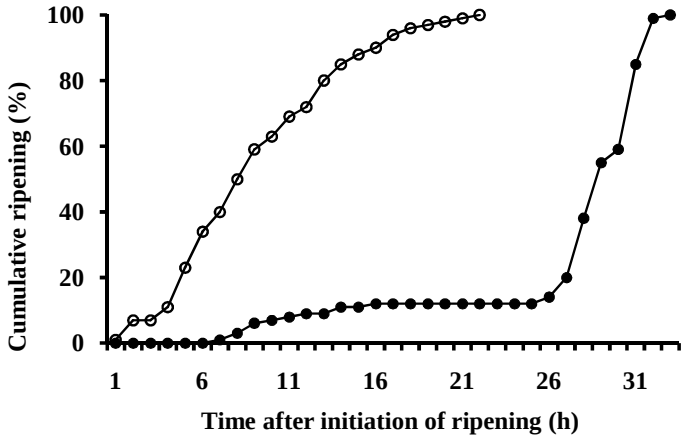

FIGURE 5. Cumulative ripening in PM x CSR_2 (*Bombyx mori*) larvae under LD 12:12 conditions. Note the differences in curve characteristics ripening under normal conditions (without sampoorna; ●) and that treated with *Sampoorna* (ɵ).

b. Cumulative expression of larval ripening in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: In the case of bivoltine x bivoltine mulberry silkworm hybrid, the cumulative readings in larval ripening without sampoorna application (sprayed with exact quantity of distilled water) and that of applied with sampoorna just resembled that of PM x CSR_2 (Fig. 6). The only difference is seen in cumulative larval ripening without sampoorna. The control batches of larval ripening expressed two stepwise increments (because the larval ripening in CSR_2 x CSR_4 was a continuous for three consecutive days), while that of sampoorna treated curve was additive curvilinear one.

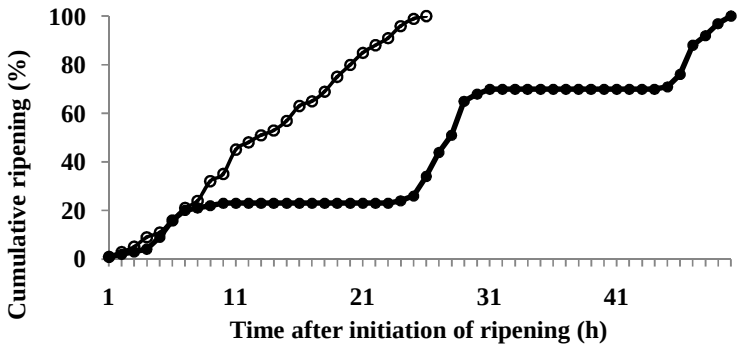

FIGURE 6. Cumulative ripening in CSR_2 x CSR_4 (*Bombyx mori*) larvae under LD 12:12 conditions. Note the differences in curve characteristics ripening under normal conditions (without sampoorna; ●) and that treated with *Sampoorna* (ɵ).

II. Studies on the action of sampoorna on pharate pupal formation in multivoltine x bivoltine, PM x CSR_2 and bivoltine x bivoltine silkworm hybrid, CSR_2x CSR_4:

1. Studies on the action of sampoorna on pharate pupal formation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2:

a. Studies on the rhythmic patterns on pharate pupal formation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: Pharate pupal formation, in percentage, against time in the multivoltine x bivoltine silkworm hybrid, PM x CSR_2 under LD 12: 12 conditions are presented in Fig. 7. As observed from the distribution graph, it is clear that the distribution of pharate pupal formation in PM x CSR_2 continued for two consecutive days, with gating and circadian in nature. Peak of pharate pupal formation exhibited a distinct peak at or around 06.00 h of local day.

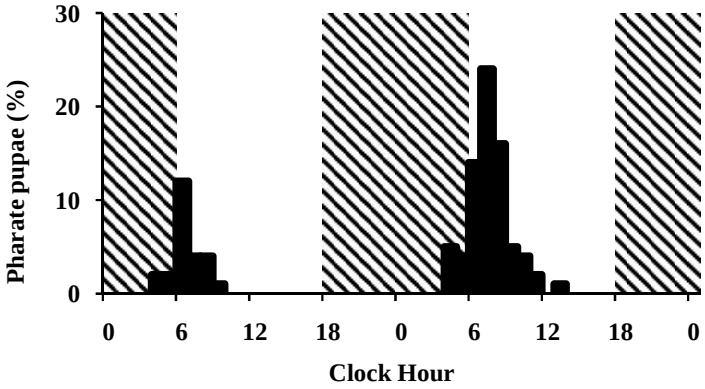

FIGURE 7: Behaviour of pharate pupal formation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2 of *Bombyx mori* L. under natural day (LD 12: 12) condition.

b. Studies on the implications of sampoorna on pharate pupal formation in multivoltine x bivoltine silkworm hybrid, PM xCSR_2: Implications of sampoorna application on pharate pupal formation in PM x CSR_4 resulted in a single day expression of pharate pupal formation (Fig. 8). Larvae initiate pharate pupal formation in the beginning of light-on phase of the day and completed by the beginning of dusk. The (pharate pupal forming larvae) did not utilize the day 2 for the action.

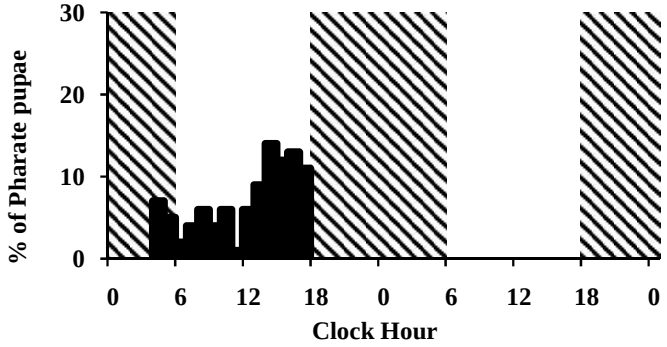

FIGURE 8: Pharate pupal formation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2 of *Bombyx mori* L. as a result of application of sampoorna under natural day (LD 12: 12) condition.

2. Studies on the action of sampoorna on pharate pupal formation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4:

a. Studies on the rhythmic patterns on pharate pupal formation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_3: distribution data on pharate pupal formation in bivoltine x bivoltine mulberry silkworm hybrid, CSR_2 x CSR_4 are graphed in the distribution diagram, Fig. 9. It can be noticed that the distribution of pharate pupal formation in CSR_2 x CSR_4 was continuous for three consecutive days. The quantum of pharate pupal formation on day 1 in CSR_2 x CSR_4 was very low and it increased in the day 2 and continued further. Notably, the rhythm in pharate pupal formation in CSR_2 x CSR_4 under LD 12:12 photoperiodic condition followed all the circadian rules; it was diurnal as the pharate pupal formation appeared in day time, it was initiated at the onset of light phase and hence termed as taking lights-on as signal for pharate pupal formation initiation, it was circadian as it re occurred in around 24 hours interval, it was gating as the gate appeared for three consecutive day and it was an expression of mixed age population characteristics as it (pharate pupal formation) took three days because the entire population is a mixture of three age groups.

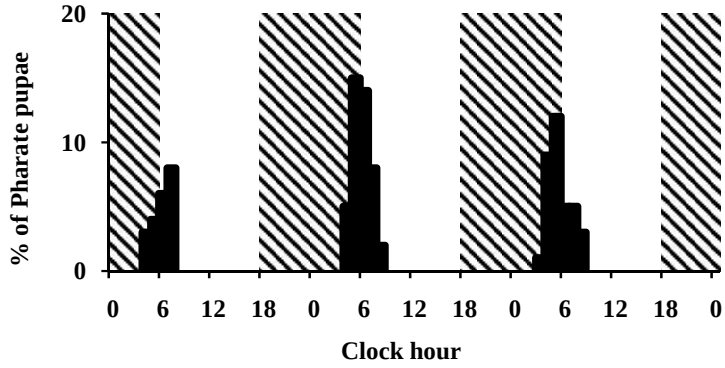

FIGURE 9: Behaviour of pharate pupal formation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4 of *Bombyx mori* L. under natural day (LD 12: 12) condition.

b. Studies on the implications of sampoorna on pharate pupal formation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: As observed for PM x CSR_2, the pharate pupal formation in CSR_2 x CSR_4 also, pharate pupal formation just initiated at dawn and completed at middle of the scotophase on the same day (Fig. 10). Thus, three consecutive days pharate pupal formation in CSR_2 x CSR_4 comfortable completed in a single day.

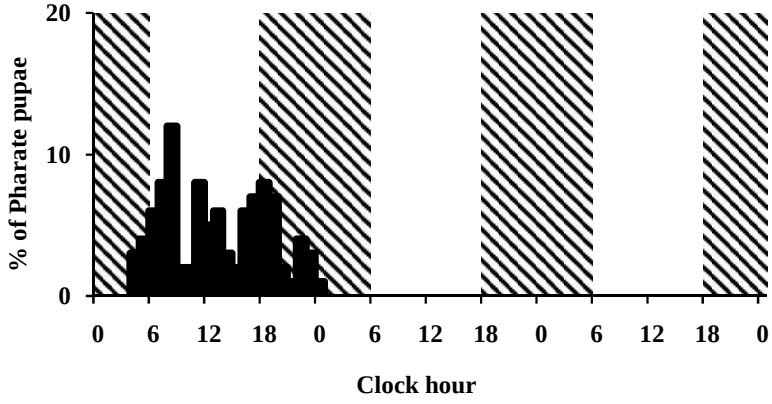

FIGURE 10: Effect of sampoorna on pharate pupal formation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4 of *Bombyx mori* L. under natural day (LD 12: 12) condition.

3. Cumulative expression of pharate pupal formation in mulberry silkworm hybrids, PM x CSR_2 and CSR_2 x CSR_4:

a. Cumulative expression of pharate pupal formation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: Pharate pupal formation (%) data presented in distribution diagrams (Fig. 7 and 8) are treated for cumulative scoring in pharate pupal formation and presented in Fig. 11. The pharate pupal formation curve in control batch is an expression of two consecutive day's action while that of sampoorna treated batch is a clear additive curvilinear one that completed well before 16 hours of time in multivoltine x bivoltine silkworm hybrid, PM x CSR_2.

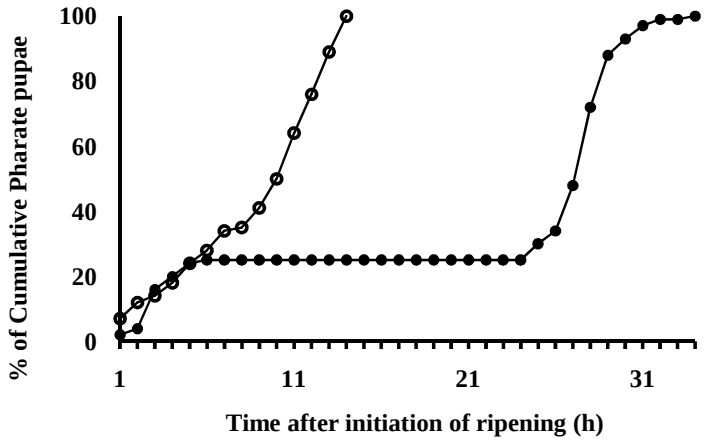

FIGURE 11. Cumulative pharate pupal formation in PM x CSR_2 (*Bombyx mori*) under LD 12:12 conditions. Note the differences in curve characteristics ripening under normal conditions (without sampoorna; ●) and that treated with Sampoorna (ɵ).

b. Cumulative expression of pharate pupal formation in bivoltine x bivoltine silkworm hybrid, x CSR_2 x CSR_4: Data on pharate pupal formation (%), presented in distribution diagrams (Fig. 9 and 10) are treated for cumulative scoring in pharate pupal formation and presented in Fig. 12. The pharate pupal formation curve in control batch is an expression of three consecutive days action while that of sampoorna treated batch is a clear additive curvilinear one that completed well before 21 hours of time in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4.

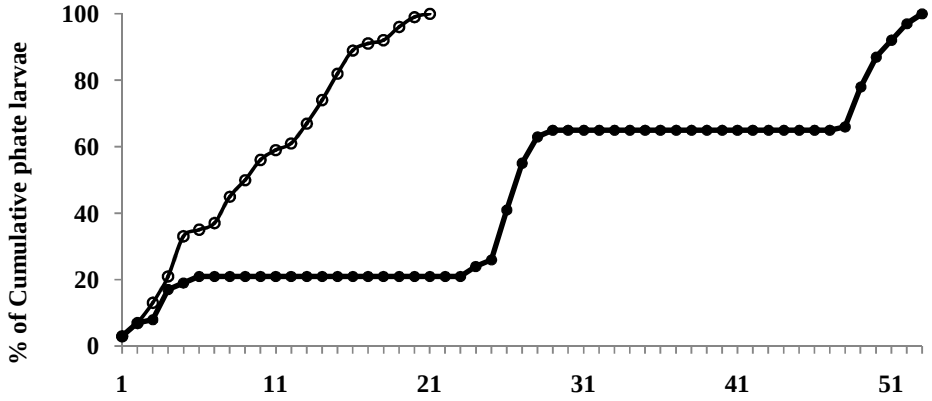

FIGURE 12. Cumulative pharate pupal formation in CSR_2 x CSR_4 (*Bombyx mori*) under LD 12:12 conditions. Note the differences in curve characteristics ripening under normal conditions (without sampoorna; ●) and that treated with Sampoorna (ɵ).

III. Studies on the action of sampoorna on pupation in multivoltine x bivoltine, PM x CSR_2 and bivoltine x bivoltine silkworm hybrid, CSR_2x CSR_4:

1. Studies on the implication of sampoorna on pupation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2:

a. Studies on the rhythmic patterns on pupation patterns in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: Pupation (%), against time of the day in the multivoltine x bivoltine silkworm (*Bombyx* mori) hybrid, PM x CSR_2 under LD 12: 12 conditions are graphed in distribution diagram, Fig. 13. As observed for larval ripening and pharate pupal formation, pupation phenomenon in PM x CSR_2 also continued occurring for two consecutive days. Pupation was more on day 2 than on day 1. Pupation initiated very close to dawn. However, the peak pupation expressed at 06.00 h local time, the exact lights-on phase. No strange, but, the rhythm in pupation patterns in PM x CSR_2 under LD 12 : 12 photoperiodic condition observed following circadian specifications; thus, pupation was diurnal (as the pharate pupal formation appeared in day time), it was instigated at the onset of light phase (hence termed as taking lights-on as signal for pupation too), circadian (as it re-occurring in 24 hours interval), gated (as the gate appeared for two consecutive day) and it revealed mixed age population characteristics (as pupation took two days).

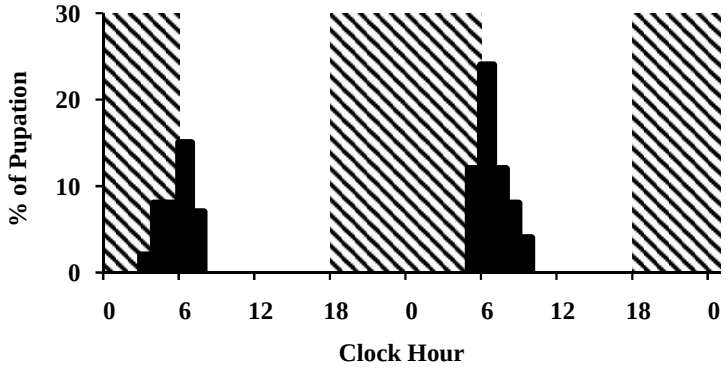

FIGURE 13: Behaviour of pupation in multivoltine x bivoltine silkworm hybrid, PM x CSR2 of *Bombyx mori* L. under natural day (LD 12: 12) condition.

b. Studies on the implications of sampoorna on pupation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: Implications of sampoorna application on pupation in PM x CSR4 drastically reduced the pupation duration to a single day expression of (Fig. 14). Larvae initiate pupation in the beginning of light-on phase of the day and completed by the beginning of dusk. The (pharate pupal forming larvae) did not utilize the day 2 for the action.

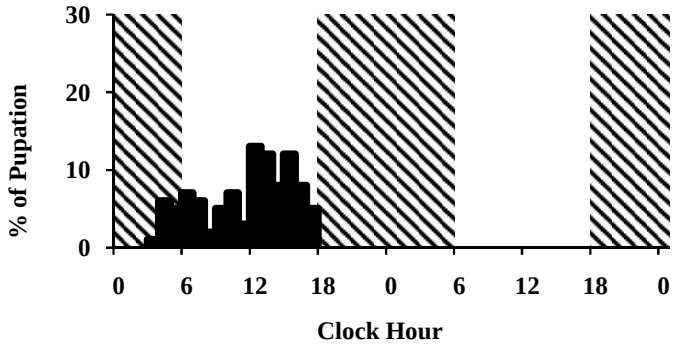

FIGURE 14: Pupation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2 of *Bombyx mori* L. as a result of application of sampoorna under natural day (LD 12: 12) condition.

2. Studies on the action of sampoorna on pupation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4:
a. Rhythmic patterns on pupation patterns in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: Percentage of pupation in the bivoltine x bivoltine silkworm (*Bombyx* mori) hybrid, CSR_2 x CSR_4 (Fig. 15) followed the description given for that of PM x CSR_2, except the exemption that the pupation continued expressing for three consecutive days. Compared to the other two pupation components, larval ripening and pharate pupal formation, pupation in CSR_2 x CSR_4 did not get itself widen as the expression is very sharp and precise. The peak of pupation on all the days was also distinct.

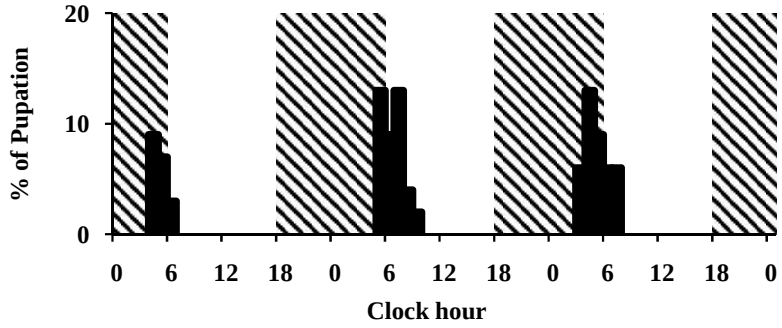

FIGURE 15: Behaviour of pupation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4 of *Bombyx mori* L. under natural day (LD 12: 12) condition.

b. Studies on the implications of sampoorna on pupation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: Implications of sampoorna application on pupation in CSR_4 x CSR_4 drastically reduced the pupation duration to a single day expression of (Fig. 16).

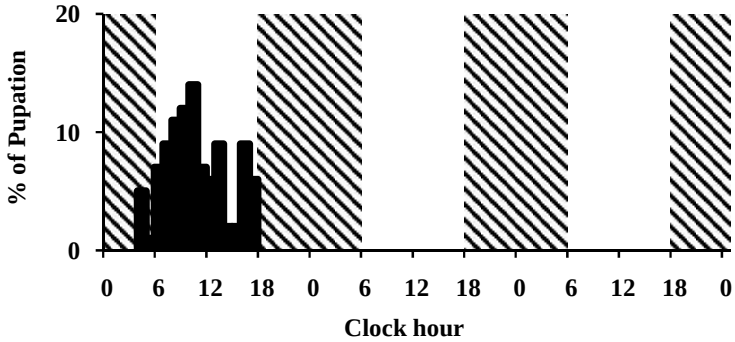

FIGURE 16: Pupation in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4 of *Bombyx mori* L. as a result of application of sampoorna under natural day (LD 12: 12) condition.

3. Cumulative expression of pupation in mulberry silkworm hybrids, PM x CSR_2 and CSR_2 x CSR_4:

a. Cumulative expression of pupation in multivoltine x bivoltine silkworm hybrid, PM x CSR_2: Pupation (%) data on pupation patterns of both control and sampoorna treated multivoltine x bivoltine mulberry silkworm, that were presented in distribution diagrams (Fig. 13 and 14) are treated for cumulative scoring in pupation and presented in Fig. 17. Pupation curve in control batch is an expression of two consecutive days action while that of sampoorna treated batch is a clear additive curvilinear one that completed well before 16 hours of time in multivoltine x bivoltine silkworm hybrid, PM x CSR_2.

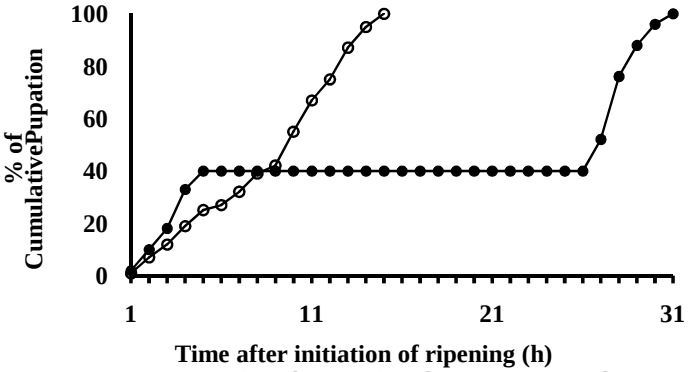

FIGURE 17. Cumulative pupation in PM x CSR_2 (*Bombyx mori*) under LD 12:12 conditions. Note the differences in curve characteristics ripening under normal conditions (without sampoorna; ●) and that treated with Sampoorna (ɵ).

b. Cumulative expression of pupation in bivoltine x bivoltine silkworm hybrid, x CSR_2 x CSR_4: Data on pharate pupal formation (%), presented in distribution diagrams (Fig. 15 and 16) are treated for cumulative scoring in pharate pupal formation and presented in Fig. 18. The pharate pupal formation curve in control batch is an expression of three consecutive day's action while that of sampoorna treated batch is a clear additive curvilinear one that completed well before 21 hours of time in bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4.

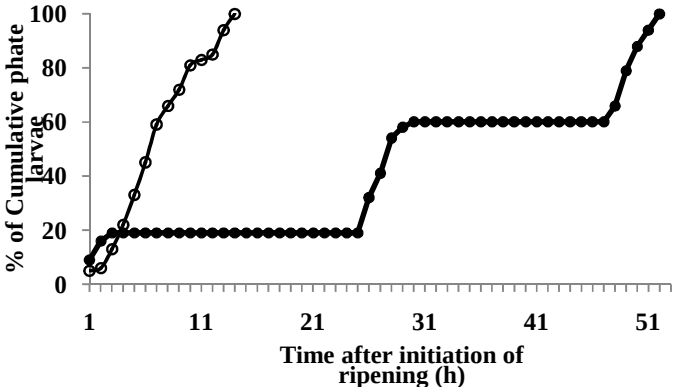

FIGURE 18. Cumulative pupation in CSR_2 x CSR_4 (*Bombyx mori*) under LD 12:12 conditions. Note the differences in curve characteristics ripening under normal conditions (without sampoorna; ●) and that treated with Sampoorna (ɵ).

VI. Comparison of durations of pupation and pupation components:

a. Comparison of ripening durations of control and sampoorna treated silkworm hybrids, PM x CSR_2 and CSR_2 x CSR_4: The durations of larval ripening is an important one to compare in the present study that indicate the considerable reduction in larval ripening in sampoorna treated larvae to that of control larval batch. Data on larval ripening durations, from the initiation to the completion of ripening in both *Bombyx* hybrids, PM x CSR_2 and CSR_2 x CSR_4 under control and experimental (sampoorna treated) are presented in Fig. 19. When the larval ripening durations for the multivoltine x bivoltine hybrid, PM x CSR_2 are compared, larval ripening period for control (non-sampoorna treated) was 26 hours (± 2.845) and the same for larvae treated with sampoorna was 23 (± 2.841) hours. The differences are quite comparable and thus not statistically significant, except that the larval ripening duration reduced by 3 hours. On the other hand, larval ripening durations of bivoltine x bivoltine hybrid, CSR_2 x CSR_4 are surprisingly different. Thus, larval ripening duration for non-sampoorna treated larvae was 59 (± 3.834) hours and that for sampoorna treated larvae was 25 ± 2.892 hours. The differences in larval ripening period of control and sampoorna treated larvae of CSR_2 x CSR_4 are statistically highly significant ($p < 0.01$).

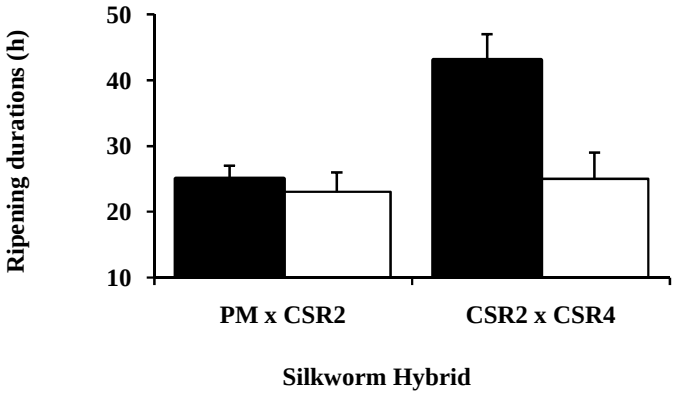

FIGURE 19. Larval ripening durations in two mulberry silkworm (*Bombyx mori*) hybrids, PM x CSR_2 and bivoltine x bivoltine, CSR_2 x CSR_4 under control (open bars) and sampoorna treated (closed bars). Note that the larval ripening durations in PM x CSR_2 are simply comparable (between experimental and control) and are not statistically significant. Those for CSR_2 x CSR_4 showed maximum differences and are statistically highly significant ($p < 0.01$).

b. Comparison of durations of components of pupation (larval ripening, pharate pupal formation and pupation) of control and sampoorna treated multivoltine x bivoltine silkworm hybrid, PM x CSR_2: As explained in previous paragraph, the larval ripening durations between non-treated larvae and sampoorna treated larvae are comparable (Fig. 20). However, these differences for pharate pupal formation with non-treated silkworm hybrids of PM x CSR_2 and treated ones are highly significant (33 hours against 14 hours, $p < 0.05$). Similar observations are made with pupation patterns, Pupation duration in PM x CSR_2 under no-sampoorna treated (control) conditions was 30 hours. The same for PM x CSR_2 when treated with sampoorna was 15 hours. Thus, the differences in pupation durations under experimental PM x CSR_2 and that under sampoorna treated PM x CSR_2 were statistically highly ($p < 0.01$) significant.

c. Comparison of durations of components of pupation (larval ripening, pharate pupal formation and pupation) of control and sampoorna treated bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: Data on the components (larval ripening, pharate pupal formation and pupation) durations of pupation pattern for bivoltine x bivoltine mulberry silkworm (*Bombyx mori*) hybrid, CSR_2 x CSR_4 are presented in Fig. 21. Note that all the durations (larval ripening, pharate pupal formation and pupation) CSR_2 x CSR_4 under experimental and control are statistically highly significantly ($p < 0.01$) different.

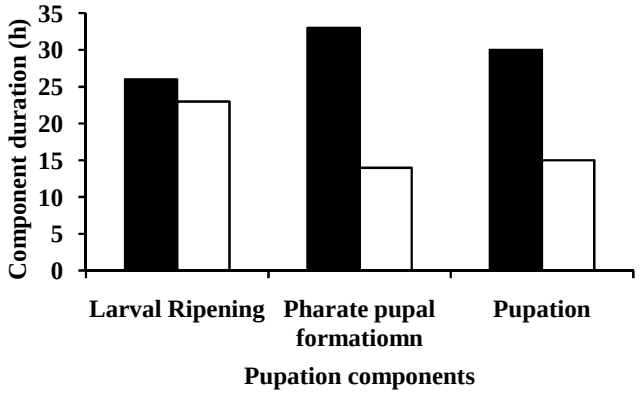

FIGURE 20. Comparison of durations of pupation components (larval ripening, pharate pupal formation and pupation) in multivoltine x bivoltine mulberry silkworm (*Bombyx mori*) hybrid, PM x CSR_2 under control (closed bars) and sampoorna

treated (open bars). Note that the larval ripening durations in PM x CSR_2 are comparable (between experimental and control, not statistically significant) while those for pharate pupal formation and pupation are statistically highly significantly ($p < 0.01$) different.

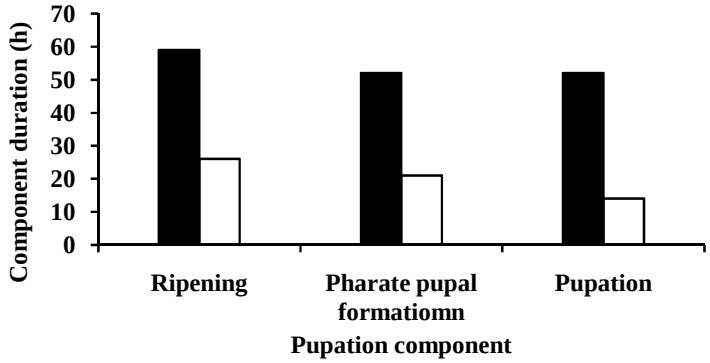

FIGURE 21. Comparison of durations of pupation components (larval ripening, pharate pupal formation and pupation) in bivoltine x bivoltine mulberry silkworm (*Bombyx mori*) hybrid, CSR_2 x CSR_4 under control (closed bars) and sampoorna treated (open bars). Note that all the durations (larval ripening, pharate pupal formation and pupation) for CSR_2 x CSR_4 under experimental and control are statistically highly significantly ($p < 0.01$) different.

d. Comparison of differences in durations of components of pupation (larval ripening, pharate pupal formation and pupation) of control and sampoorna treated multivoltine x bivoltine hybrid, PM x CSR_2 and bivoltine x bivoltine silkworm hybrid, CSR_2 x CSR_4: The data on durations in pupation components (larval ripening, pharate pupal formation and pupation) in sampoorna treated larvae in multivoltine x bivoltine (PM x CSR_2) and bivoltine x bivoltine (CSR_2 x CSR_4) were always low. However, the differences in percentage between control and treated over control gives a precise picture. In this case, such differences (%) between control and experimental over control (differences in percentage = (control–experimental) / control x 100) are presented in Fig. 22. The percentage difference in larval ripening between control and experimental was very low (15.221%) in PM x CSR2. Hence, the differences are not statistically significant. However, those for larval ripening in CSR2 x CSR4 (56.320%) are highly significant ($p< 0.01$). The differences in other pupation components (pharate pupal formation and pupation are also high (over 50%), and thus are statistically highly significant ($p < 0.01$).

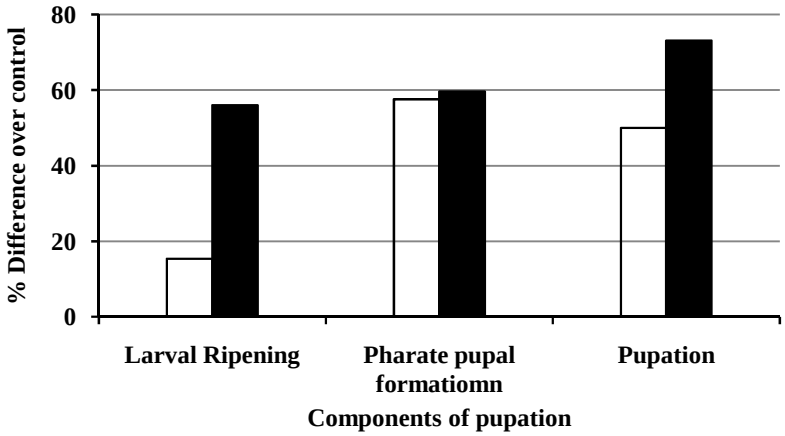

FIGURE 22: Differences in percentage between control and sampoorna treated over control in pupation components (larval ripening, pharate pupal formation and pupation) in multivoltine x bivoltine (PM x CSR_2) and bivoltine x bivoltine (CSR_2 x CSR_4).

DISCUSSION

Distinct behavioral characteristics are seen in *Bombyx mori* while the larvae enter into pre-pupal stage from eating larval stage at the end of final larval instar stadium. The phase between eating period (larval) and that of pre-pupal are differently observed in *B. mori*. Imperative changes that are designated as stopping feeding, change in larval colour and wandering in search of a suitable site for cocoon formation that protects the pupa inside. This particular transitional stage was termed as 'wandering stage' by many (Piepho *et al*., 1960; Lounibos, 1976; Riddiford, 1980; Truman and Taghert, 1981; de Wilde *et al*., 1980). In the present study, however 'ripening stage' (as larvae ripen) or 'mounting' (as ripened larvae are picked up and mounted on cocooning devices) are mostly used as these terms are broadly used in present Indian sericulture industry (Krishnaswami *et al*., 1973;

Krishnaswami, 1986; Sivarami Reddy, 1993; Sivarami Reddy *et al*., 1993, Shanthan Babu, 2014; Srinath, 2014; Srinath *et al*., 2018).

A theory of involvement of three hormones, Prothoracicotrophic Hormone (PTTH), Juvenile hormone (JH) and ecdysone is accepted as primarily regulating the moulting processes (Riddiford, 1980; Reynolds, 1980; Truman and Taghert, 1981; Happ, 1984). Secretion of ecdysone by PTTH for two installments is reported in larval-to-pupal ecdysis, against only one installment of ecdysone for larval-to-larval moulting. When eating final instar larvae attain absolute weight, PTTH is released in the next allowable gate and causes the secretion of the first installment of ecdysone (Riddiford, 1980). In the absence of JH, this first installment of ecdysone exerts a distinct effect on the animal, leading to the initiation of metamorphosis process, such as cessation of feeding, change in larval body colour and wandering for a cocooning site etc. The second release of PTTH, in *B. mori* (Shimada, 1989) releases the second installment of ecdysone in 8 times more in quantity than the first installment, and this hormone implements the pupal ecdysial process initiating apolysis (Hinton, 1973).

Therefore, at the release of second installment of PTTH/ ecdysone, the animal or insect is directed towards apolysis (Hinton, 1973) causing pharate pupal formation and finally pupal ecdysis. In other words, the release of the first installment of PTTH is the initiation point of behavioral changes while the second installment of PTTH/ ecdysone is the directive point for ecdysial process. Once PTTH/ecdysone is released the brain exercises and exerts no control over subsequent ecdysis (Truman and Taghert, 1981). Precisely, after the release of PTTH/ ecdysone, the clock virtually has no control over ecdysis and the process of clock-independent proceeds to complete ecdysis (Fujishita and Ishizaki, 1981).

Shanthan Babu (2014), Srinath (2014) and Srinath *et al*. (2018) described that at 5% larval ripening stage, all the *Bombyx* silkworm larvae in the mixed age population are committed for larval ripening at that stage. All the larvae might have got released the first dose of PTTH and thus the first dose of ecdysone. The first batches (gate) of larvae might be in advanced stage. The second gate larvae in moderately advanced stage and the next (third) batch (gate) caterpillars got first dose of ecdysone few hour earlier to 5% level ripening stage. Thus, the entire population in the 'mixed age' *Bombyx* larvae, however, is waiting for their second dose of PTTH/ ecdysone release and their virtual (fortuitous) gates of pharate pupal formation and finally pupation. They (Shanthan Babu, 2014; Srinath, 2014; Srinath *et al*., 2018) advanced that phytoecdysone (*Sampoorna*) is administered at 5% ripening level of ripening, the batch (silkworm larvae) did not wait for internal secretion of ecdysone and they utilized the external applied ecdysone (*Sampoorna*) for completion of ripening period in additive manner rather than in a gated mode. Thus, larvae, treated with *Sampoorna* might have not followed the 'fortuitous synchrony' after PTTH release as revealed in the present study also. The resultant curves revealed an additive curvy-linear ripening process. Thus, a lengthy ripening period of 43 h in CSR2 x CSR4and 26 hours of ripening duration in PM x CSR_2 under controlled conditions (without *Sampoorna*) has been reduced to 26 h for CSR_2 x CSR_4 and 21 h for PM x CSR_2.

Shanthan Babu (2014) and Srinath (2014; Srinath *et al*., 2018) did not extend their studies up to the completion of the entire pupation process. They left two pupation components, pharate pupal formation and pupation, which are covered in the present study. As expected, the rhythmic characters of pharate pupal formation and pupation too followed those of larval ripening under LD 12: 12 (control) conditions. Thus, the two components, pharate pupal formation and pupation in the present study exhibited a circadian rhythmicity, phase locked to dawn, thus diurnal and mixed age characters as these components followed gating phenomenon.When the case of sampoorna treated *Bombyx* larvae are considered, the rhythmic characters are not seen. They followed a curvy-linear pattern. Thus, the duration of pharate larval formation and pupation under treated (sampoorna) condition significantly reduced. Interestingly, the duration of pupation under sampoorna treatment drastically reduced. This may, perhaps be due to the fact that the pupation process is a simple mechanical one compared to the other two components of pupation viz., larval ripening and pharate pupal formation. The economic characteristics of the cocoons were reported to be non significant between control and *Sampoorna* treated batches of the silkworm, *Bombyx mori* (Kanika Trivedi *et al*., 2003; Sashindran Nair *et al*., 2005; Nirmal Kumar *et al*., 2006, 07; Srinath *et al*., 2009; Srinath *et al*., 2018).

Use of sampoorna for the multivoltine x bivoltine silkworm hybrid, PM x CSR_2 towards reduction of larval ripening period, pharate pupal formation period and pupation period is another issue to be discussed in the present study. The control silkworm larvae of PM x CSR_2 took two consecutive days for their larval ripening, pharate pupal formation and pupation as well. However, when the duration of three pupation components under control conditions are compared with those, the differences are not statistically different. This observation implies that sampoorna may not be required for multivoltine x bivoltine hybrids.

Notably, the durations of all the three pupation components (larval ripening, pharate pupal formation and pupation) occurred for 2 consecutive days for PM x CSR_2 and 3 consecutive days for CSR_2 x CSR_4. Upon sampoorna treatment, these lengthy durations were reduced to mere around 24 hours. For pharate pupal formation and pupation, these durations further came down to below 24 hours with sampoorna treatment. This indicated that sampoorna treated batches of silkworm larvae did not wait for internal secretion of PTTH/ ecdysone, nor follow the mixed age characters and straightway continued completion of individual pupation components (larval ripening, pharate pupal formation and pupation). In other words, sampoorna not only freed the larval ripening phenomenon from clock-dependency, continued to act as an independent process, but also it made the remaining two pupation components, pharate pupal formation and pupation to act so. The durations of the later two pupation components, especially pupation was the smallest (statistically highly significant) indicating

the mechanical nature of pupation process, as it is involved in only shedding old cuticle. Compare the reduction (%) in durations of components for three components of pupation (larval ripening, pharate pupal formation and pupation) revealed that the percent reduction of sampoorna treated batches over control batches was very high in CSR_2 x CSR_2 rather than in PM x CSR_2. This implies that sampoorna may not be immediately be of practical value to multivoltine x bivoltine silkworm hybrids.

REFERENCES

Beck, S.D. (1980) *Insect photoperiodism*, Academic Press, New York.

Bindroo, B.B. and Satish Verma. (2014) Sericultural technologies developed by CSRTI Mysore, Director, Central Sericultural Research & Training Institute, Central Silk Board–Ministry of Textiles–Govt. of India. Manandavadi Road, Srirampura, Mysore 570 08 India

de Wilde, J.; Hsiao, T.H. and Hsiao, C. (1980) The regulation of the metamorphic moults in the colorado potato beetle, *Leptinotarsa decemlineaty say*. In *Progress in ecdysone research*; J.A. Hoffmanna (ed.) (Elsevier/ North-Holland Biomedical Press, Amsterdam, pp. 83-98.

Fujishita, M. and Ishzaki, M. (1981) Circadian clock and prothoracicotropic hormone secretion in relation to the larval ecdysis rthyhm of saturniid, *Samia Cynthia ricini*, *J. Insect. Physiol*., **27**: 121-128.

Happ, G.M. (1984) Development and reproduction, Part II, Chapter 4, in *Insect Biology* H. E. Evans (ed.) (London: Addisen-Wesley Publishing Company Inc.,) pp. 93-113.

Hinton, H.E. (1973) Neglected phase in metamorphosis: a reply to V B Wigglesworth; *J. Ent. (A)*. **48**: 57-68.

Kanika Trivedi, Dhar, A.; Nirmal Kumar, S.; Sashindran Nair, K.; Ramesh, S. and Nisha Gopal (2003) Effect of phytoecdysteroid on pure breed performance of silkworm *Bombyx mori* L. *Int. J. Indust. Entomol*., **7(1)**: 29-36.

Krishnaswami, S. (1986) *New technology of silkworm rearing*, Bulletin No. 2, Central Silk Board, Bangalore, India.

Krishnaswami, S.; Narasimhanna, M.N.; Suryanarayana, S.K. and Kumararaja, S. (1973) *Manual on sericulture, Vol.2–Silkworm rearing*, (FAO of United Nations: Rome), pp. 1-131.

Lounibos, L.P. (1976) Initiation and maintenance of cocoon spinning behavior by saturniid silkworms, *Physiol. Entomol*., **1**: 195-206.

Nirmal Kumar, S.; Shashindran Nair, K. and Rabha, J. (2006) Role of phytoecdysteroid treatment in the maturation process of multi x bivoltine (BL_{67} x CSR_{101}) hybrid silkworm, *Bombyx mori* L. when maintained at low, medium and high temperature. *Int. J. Indust. Entomol*., **12 (2)**: 51-56.

Nirmal Kumar, S.; Shashindran Nair, K. and Mondal, S. (2007) Influence of different treatment regims of phytoecdysteroir on silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol*., **14 (1)**: 63-68.

Piepho, H.; Boden, E. and Holz, I. (1960) Uber die Hormonabhangigkeit des Verhaltens von Schwarmraupen von den Hautungen., *Z. Tierpsychol*., **17**: 261-269.

Reynolds, S.E. (1980) Integration of behavior and physiology in ecdysis; *Adv. Insect. Physiol*., **15**: 475-595.

Riddiford, L.M. (1980) Interaction of ecdysteroids and juvenile hormone in the regulation of larval growth and metamorphosis of the tobacco hornworm. In *Progess in ecdysone research*. (Ed. Hoffmann, J. A.), Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 409-430.

Sashindran Nair, K.; Yun-Gen, M. and Nirmal Kumar, S. (2005) Differential response of silkworm, *Bombyx mori* L. to phytoecdysteroid depending on the time of administration. *J. Appl. Sci. Environ. Mgt*. **9(3)**: 81-86.

Shanthan Babu, M.A. (2014) Expression of mixed-age characteristics in the developmental marker events of the silkworm, *Bombyx mori* L., Ph.D. thesis, Sri Krishnadevaraya University, Anantapur, India.

Shimada, J. (1989) Release of prothoracicotropic hormone in 5^{th} instar larvae of the silkworm, *Bombyx mori* L., *J. Seri. Sci. Jpn*., **58**: 315-321. (In Japanese with English summary).

Sivarami Reddy, N. (1993) Implications of photoperiod on the silkworm, *bombyx mori* L. (PM x $NB4D_2$), Ph.D., Thesis, Sri Venkateswara University, Tirupati, India.

Sivarami Reddy, N.; Pavan Kumar, T.; Murali Mohan, P. and Sasira Babu, K. (1993a) Photoperiodic implications on the pupal ecdysis in the silkworm, *Bombyx mori* L. (PM x NB4D2), *Insect Sci. Applic*., **14**: 21-29.

Srinath, B. (2014) Studies on the mixed-age population characteristics in the developmental marker events of certain bivoltine silkworm (*Bombyx mori* L.), Thesis, Sri Krishnadevaraya University, Anantapur, India.

Srinath, B., Naidu, B.V., Kishore, S. and Reddy, P.S. (2009) Influence of phytoecdysteroids for maturity and spinning in silkworm larvae. *Bull. Biol. Sci*. **7** (2): 103-106.

Srinath, B.; Shanthan Babu, M.A.; Lakshminarayana Reddy, P.; Sujatha, B. and Sankar Naik, S. (2018) Impact of phytoecdysone, Sampoorna' on the synchronization of ripening in the commercial silkworm, *Bombyx mori* L. – A chronobiological perspective, *I. J. S. N*., **9(1)**: 11-16

Truman, J. W. (1972) Physiology of insect rhythms. I. Circadian organization of the endocrine events underlying the moulting cycle of larval tobacco hornworms, *J. Exp. Biol*., **54**: 805-820.

Truman, J. W. an Taghert, H. (1981) Hormonal control of pupal ecdysis in the tobacco hornworm, *Manduca sexta*., In *Current topics in insect endocrinology and nutrition*, (Eds. Bhaskaran, G.; Friedman, S. and Rodriguez, J. G.), Plenum Press, New York and London, pp. 171-181.

Zhuang, D.; Xiang, M.; Gui, Z. Z. (1992) The practical studies on the growth and development by insect hormone in the silk worm, *Bombyx mori* L. A publication of the Sericulture Res. Institute, Chinese Academy of Agric. Sciences, Zhenjiang, presented at XIX Intern. Congress of Entomology, Beijing China.