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COMPARATIVE STUDIES ON THE ACTION OF PHYTOECDYSONE, '*SAMPOORNA*' IN SYNCHRONIZING RIPENING IN BIVOLTINE, MULTIVOLTINE AND CROSS-BREED COMMERCIAL SILKWORM, *BOMBYX MORI*, L. - A CHRONOBIOLOGICAL PERSPECTIVE

[1]Shanthan Babu, M.A., [2]Srinath, B., Lakshminarayana Reddy, P., [3]Prasad Rao, G.D.V., Sujatha, B., Ravi, G. and Sankar Naik, S.

Department of Sericulture, Sri Krishnadevaraya University, Anantapur -515 503, Andhra Pradesh, India.
[1]RSRS, CSB, PO Box 50, Anantapur – 515 501, Andhra Pradesh, India.
[2]REC, CSB, Agriculture Market Yard, Vikarabad – 501 101, Telangana, India.
[3]Department of Zoology, S.G.S. College, Jaggaiah Pet, Krishna district, Andhra Pradesh, India.

ABSTRACT

The intensity of non-uniformity in rhythmic expression of developmental marker events increase from initial stages of life cycle to final stages in the population of commercial silkworm, *Bombyx mori* L. Silkworm larvae in population do not ripen uniformly, taking 2 days and above, causing problems to farmers for more time, labour, expenditure and further risk. Keeping these difficulties of the farmers in mind, comparative studies were conducted to determine the patterns of ripening in multivoltine, bivoltine and cross-breed commercial silkworm through chronobiological approach, using CSR2 representing bivoltine breed, PM representing multivoltine breed and their hybrid, PM x CSR2. Simultaneously the impact of phytoecdysone, *Sampoorna*, (a brand name of phytoecdysone of CSRTI, Mysore, India), applying at the appearance of 5% ripening were also studied. Experimental larvae were reared under natural solar-day, LD 12:12 conditions at 25 °C and 80% RH, feeding V1 mulberry leaves. Ripening patterns in silkworm larvae initiated in the early hours of the day, LD 12:12, expressing diurnal predominance of the overt phenomenon, reoccurred in 24 h intervals disclosing circadian nature. Ripening prolonged for 3 consecutive days for CSR2, reflecting gating characteristics. However, the ripening patterns for PM and PM x CSR2 lasted for 2 consecutive days (2 gates) only, however, expressing circadian nature and gating. Ripening duration for CSR2 was 47 h and that for PM and PM x CSR2 was 27 h. Upon application of *sampoorna*, the silkworm larvae did not follow circadian characteristics in ripening process, expressing continuous ripening activity, lasting for 30 h for CSR4 and 23 h for PM and PM x CSR2. It is proposed that the treated larvae utilize *sampoorna* as external source of ecdysone, ignoring (?) internally secreted ecdysone and further, ignoring the instructional signals from the brain and persist ripening continuously. Ripening durations between experimental and control larvae of CSR2 were statistically highly significant (< 0.01) while that for PM and PM x CSR2 were not. Comparing the ripening durations of PM and PM x CSR2 under control and treated (*sampoorna*) conditions, it is surmised that for synchronizing ripening, *sampoorna* treatment may not be necessary for PM (multivoltine breed) and PM x CSR2 (cross-breed).

KEY-WORDS: *Bombyx mori*, ripening, phytoecdysone, *sampoorna*, synchronization.

INTRODUCTION

Insect growth is discontinuous and is characterized by a series of moults like larval-to-larval, larval-to-pupal and finally, pupal-to-adult eclosion. Ecdysis (larval-to-larval moults and/or larval-to-pupal) in insects is a complex process with many intermittent stages. Hinton (1973) and Truman and Taghert (1981) viewed that the ecdysis begins with apolysis, extends through the production of new cuticle, and ends with the casting of old skin and expansion and hardening of the new skin. Larval-to-pupal ecdysis is still complex, and is too lengthy process as it includes steps like stopping feeding, ripening, wandering for a fitting place of cocooning, cocoon making, pharate pupal formation, production of new cuticle, secretion of glue material between old and new cuticle, casting of old skin, expansion and hardening of the new skin and finally pupation (Sivarami Reddy *et al*., 1993, Shanthan Babu, 2014, Srinath, 2014). The prothoracicotrophic hormone (PTTH) from the brain exerts a tropic influence on the prothoracic gland. In turn, the PTTH drives the prothoracic gland to release a passive ecdysone hormone. The passive ecdysone is converted into an active ecdysone by the peripheral tissue. This active ecdysone causes the apolysis and the beginning of secretion of a new cuticle by the epidermis (Reynolds, 1980, Truman and Taghert, 1981, Happ, 1984).

The mulberry silkworm, *Bombyx mori* enters a period of rapid growth after its fourth and final (Shanthan Babu, 2014, Srinath, 2014) larval-to-larval moult. At the end of fifth instar, feeding is stopped followed by 'ripening'. The ripening stage is recognized by the change in larval colour, from light ash to yellow (Shanthan Babu, 2014, Srinath, 2014). Following the colour change phase, the larvae show no interest in available food. This situation is comparable with that reported for saturniid silkworms, *Hyalophora cecropia* and *Antheraea pernyi* (Lounibos, 1976). The

larvae of *Bombyx* are picked and mounted on mountage for cocoon spinning (Krishnaswami *et al*., 1973, Krishnaswami, 1986, Sivarami Reddy *et al*., 1993, Sivarami Reddy, 1993, Shanthan Babu, 2014, Srinath, 2014) at this stage (ripening stage). When the cocoon construction is initiated by the ripened larvae at its selected site of the cocooning frame, the wandering stage is stopped followed by cocoon construction in *Bombyx mori*.

The major features of moulting cycles are regulated by a sequence of three hormones, PTTH, ecdysone and JH (Riddiford, 1980, Reynolds, 1980, Truman and Taghert, 1981, Happ, 1984).While there will be only one installment of PTTX release and consequently only one installment release of moulting hormone for larval-to-larval moulting, two installments of PTTH and consequently two installments of ecdysone are released for larval-to-pulal ecdysial process (Riddiford, 1980, Truman and Taghert, 1981, Shimada, 1989, Sivarami Reddy, 1993, Shanthan Babu, 2014, Srinath, 2014). With the larval-to-pupal ecdysis, the first installment of ecdysone is reported to be 5 to 8 times less in quantity than the second installment of ecdysone and this initiates ripening process (Riddiford, 1980, Truman and Taghert, 1981, Shimada, 1989, Sivarami Reddy, 1993, Shanthan Babu, 2014, Srinath, 2014). The release of second installment of PTTH (Riddiford, 1980, Truman and Taghert, 1981, Shimada, 1989, Sivarami Reddy, 1993, Shanthan Babu, 2014, Srinath, 2014) causes the releases of second installment of ecdysone which is 5 to 8 times more than the first installment (Riddiford, 1980) executes the larval-to-pupal ecdysis, initiating apolysis (Hinton, 1973, Sivarami Reddy, 1993, Shanthan Babu, 2014, Srinath, 2014).

The ecdysis itself is not gated but occurs after certain fixed hours of gated release of PTTH (Beck, 1980, Truman, 1972, Truman and Taghert, 1981) which resembles the gated appearance. Therefore, rhythm in ripening should be considered as 'fortuitous synchrony' in the mixed age population of *Bombyx mori* (Beck, 1980, Truman, 1972, Truman and Taghert, 1981). In such case of 'fortuitous synchrony', the picking-up of the ripened silkworm became time taking, laborious and ads to the cocoon production cost (Kanika Trivedi *et al*., 2003, Sashindran Nayar *et al*., 2005, Nirmal Kumar *et al*., 2006, 2007, Srinath *et al*., 2018). It is desired that the ripening in *Bombyx mori* larvae should be continuous and restricted to a single day. Phytoecdysteroids are used in *B. mori* cocoon crops in China, Japan and South Korea to increase productivity in sericulture (Zhuang *et al*., 1992). In India also, the phytoecdysteroids have been recently employed. The Central Sericultural Research and Training Institute (CSRTI), Mysore released a phytoecdysteroid with a brand name, '*Sampoorna*' (Kanika Trivedy *et al*., 2003). At present it is a recommended technology for commercial use for early, quick and uniform maturation of silkworm larvae, without affecting the cocoon economic characteristics. *Sampoorna* is effective not only for uniform maturation, but also in certain unpredicted situations like leaf shortage, occurrence of non-cocooning silkworm and possibility of diseases outbreak. Srinath *et al*. (2018) reported that sampoorna definitely reduce the ripening duration in bivoltine breeds and hybrids with more synchronization in ripening. Keeping this in view, an attempt has been made, in the present investigation, to study the rhythmicity of ripening process and to assess the implications of *sampoorna* in reducing the ripening period in a bivoltine silkworm breed, CSR2, a multivoltine breed PM and their hybrid, PM x CSR2 of *Bombyx mori* under natural day (LD 12 : 12) conditions. Further, emphasis is given in deciding the requirement of *sampoorna* for multivoltine breed and cross-breed *Bombyx* rearing.

MATERIALS & METHODS

Two pure breeds of popular bivoltine silkworm (*Bombyx mori* L.) in the contemporary sericulture industry of India, CSR2 (a bivoltine breed) and PM (a multivoltine breed and their hybrid, PM x CSR2 (cross-breed) were used for the rhythmic patterns of ripening and comparative studies on *sampoorna* on ripening process. Disease free layings (DFLs, commonly called) of two pure breeds, CSR2 and PM, and their hybrid, PM x CSR2 were procured, on the third day of oviposition, from the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Bangalore, Karnataka, India and the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Madanapalle, Chittoor District, Andhra Pradesh, India. The DFLs were transported to the Department of Sericulture, Sri Krishnadevaraya University, Anantapur, where the experimentations were carried out. The DFLs were transported from the source to the laboratory during evening cool hours, immediately spread into the pre disinfected rearing trays and maintained under natural solar-day photoperiodic condition, LD 12:12, with a rearing room temperature of 25 ^{o}C and relative humidity (RH) of 80%. Natural solar day (24 h) was divided into two equal parts, 12 h dark phase (scotophase) and 12 h light phase (photophase). The photophase was initiated from 06.00 h and lasted for 12 h at 18.00 h local time. Similarly, the scotophase was imposed from 18.00 h and continued up to 06.00 h local time. A 60 W florescent bulb, as light source for illuminating the experimental animals during photophase of rearing period was arranged above the rearing tray, its height from the surface of experimental silkworm larvae was so monitored that the light intensity at the surface measured 50 lux. Hatched-out larvae were fed on V1 mulberry variety leaves 4 times a day (Srinath, 2014). Use of phytoecdysone, available in the brand name *Sampoorna* (a product of the Central Sericultural Research and Training Institute, Mysore, India) has become a routine practice among sericultural farmers of India (Nirmal Kumar *et al*., 2006). *Sampoorna*, procured from CSRTI, Mysore was administered at the onset of ripening (at 5% of larval ripening developmental marker event). The larvae were fed with mulberry leaves sprayed with *sampoorna* @ 250 µg in 10 ml distilled water on 100 g of mulberry leaves (V1 variety) for 100 larvae so that each larva would get 2.5 µg of *sampoorna* (Nirmal Kumar *et al*., 2006). Five replications of 100 larvae each (for easy counting and calculation purpose) were maintained for each silkworm breed/hybrid. As control, five replications with 100 larvae for each breed/hybrid were also maintained. The control batches received 100 g of mulberry leaf (V1 variety) sprayed with 10 ml distilled water alone. Data on number of larvae ripened, on hourly

basis were recorded and represented as distribution diagrams (hourly histograms, resolved for 24 h, ω = 360º). Further, the data were plotted for cumulative frequency curves for precise and decisive comparison. From the recorded data, the durations of ripening, in h, for control and *sampoorna* treated batches were calculated. Also, the differences in ripening durations between control and *sampoorna* treated silkworm larval batches were calculated. Macroscopic data were treated statistically (ANOVA). All the values, below 5% (< 0.05) are designated as significant, those below 1% (< 0.01) level as highly significant and the rest as non-significant.

RESULTS

A. Ripening patterns in CSR2:

a. Ripening patterns in CSR2 under LD 12: 12 conditions: The first developmental marker event in the larval-to-pupal ecdysis, the ripening in CSR2 under LD 12 : 12 condition is depicted in Fig. 1. The ripening occurred just after the lights-on phase of the imposed photoperiod. Notably, the activity occurred for three consecutive days. The peak of the ripening on the third day advanced into the dark phase, expressing 'peak-bias' condition. The occurrence of peak in the imposed light phase implies that the activity is a diurnal one. Further, the interval between two consecutive peaks is around 24 h, hinting towards circadian expression.

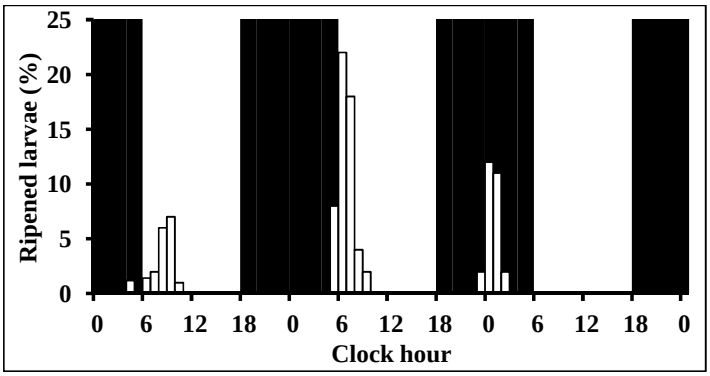

FIGURE 1. Distribution of ripening in CSR2 (*Bombyx mori*), a bivoltine breed under LD 12: 12 condition. Note occurrence of ripening for 3 consecutive days. The rhythm was circadian. Black area indicates dark phase imposed.

b. Impact of sampoorna on ripening patterns in CSR2 under natural day conditions (LD 12: 12): The response of ripening to *sampoorna* application in CSR2 is depicted in Fig. 2. Unlike that observed for CSR2 without application of 'sampoorna', the ripening was completely continuous, restricting the entire activity to duration of 30 h. There appeared neither circadian periodicity nor diurnal pattern. Even, the gating pattern is not observed.

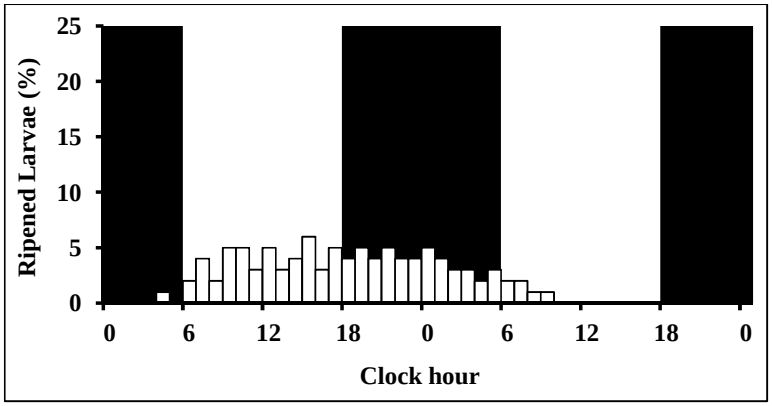

FIGURE 2. Implications of sampoorna application on the distribution of ripening in CSR2 (*Bombyx mori*), a bivoltine breed under LD 12: 12 condition. Note ripening initiated at 04.00 h and it continued till 10.00 h on the next day, expressing neither diurnal nor gating patters. The ripening completed in just 30 h. Black area indicates dark phase imposed.

c. Impact of sampoorna on cumulative ripening patterns in CSR2 under natural day conditions (LD 12: 12): Data on the cumulative ripening patterns both in control and sampoorna treated batches of CSR2 are presented in Fig. 3. Interestingly, the cumulative ripening in control batch has given a curve that resembled a step-wise increment because of the gating phenomenon in the ripening process, lasting for 47 h. In the sampoorna treated batch, the curve was an additive one, as the ripening was continuous as against that of larvae without *Sampoorna* treatment. Ripening continued for just 30 hours only.

B. Ripening patterns in PM:

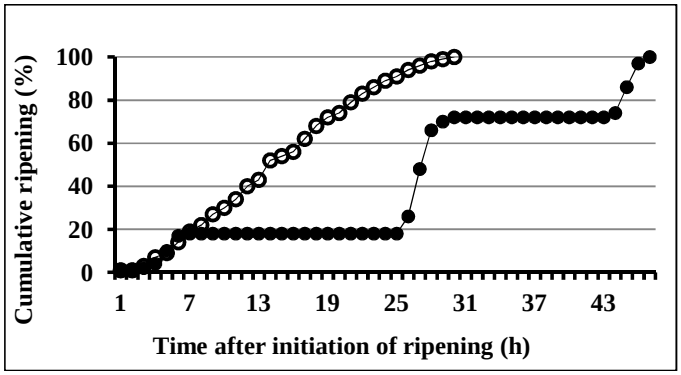

FIGURE 3. Cumulative ripening in CSR2 (*Bombyx mori*) larvae under natural day (LD 12: 12) conditions. Note the ripening under normal conditions (without sampoorna, ●) is rhythmic and the batches treated with sampoorna (o) have followed an additive curve. The control batches completed ripening in 47 h where as the sampoorna treated batched in just 30 h.

a. Ripening patterns in PM under natural day conditions (LD 12: 12): Data on ripening patterns in PM under LD 12 : 12 condition are depicted in Fig. 4. The ripening occurred just after lights-on phase of the imposed photoperiod, as that observed for CSR2. Further, the activity occurred for two consecutive days only as against to that of CSR2. The occurrence of peak in the light phase imposed indicates that the activity is a diurnal one and phase locked to lights-on. The interval between two peaks is around 24 h, implicating that the rhythm is circadian. The ripening period, from the initiation of ripening and its conclusion was 27 h. obviously, less duration is because the larvae took only two gates of ripening.

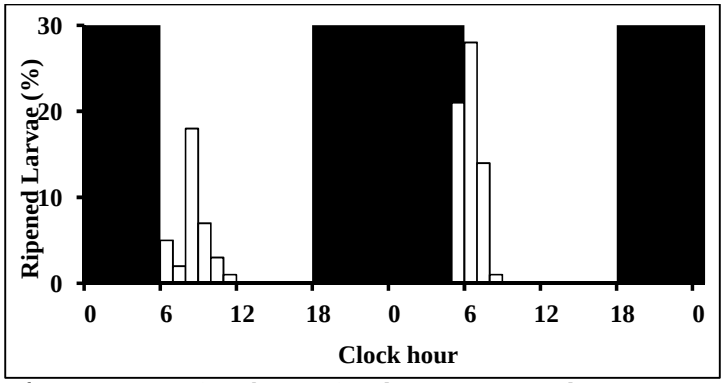

FIGURE 4. Distribution of ripening in PM (*Bombyx mori*) under LD 12: 12 condition. Note occurrence of ripening for 2 consecutive days. The rhythm was circadian. Black area indicates dark phase imposed. Total ripening period was 27 h.

b. Impact of sampoorna on ripening patterns in PM under natural day conditions (LD 12: 12): The response of ripening to sampoorna application in PM is depicted in Fig. 5. The ripening was continuous, restricting the entire activity to duration of 23 h. Due to the application of *Sampoorna, both the* circadian periodicity and diurnal pattern are not expressed. The gating pattern was also not observed.

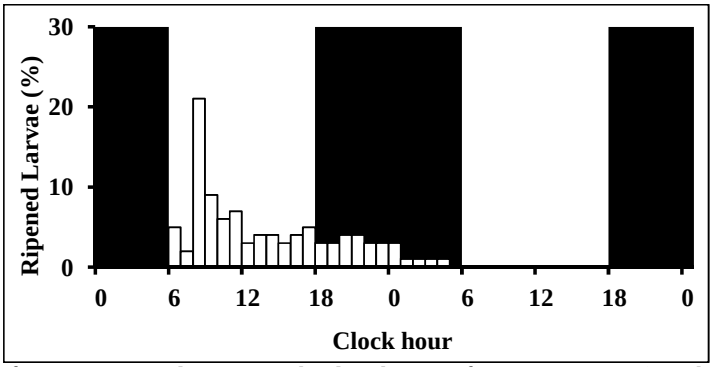

FIGURE 5. Implications of sampoorna application on the distribution of ripening in PM (*Bombyx mori*) under LD 12 : 12 condition. Note ripening initiated at 06.00 h and it continued till 05.00 h on the next day, expressing diurnal rhythmicity. However, gating phenomenon is not seen. Ripening completed in just 23 h. Black area indicates dark phase imposed.

c. Impact of sampoorna on cumulative ripening patterns in PM, a multivoltine breed under natural day conditions (LD 12 : 12): Data on the cumulative ripening patterns both in control and sampoorna treated batches of PM is presented Fig. 6. Interestingly, the cumulative ripening in control batch has given a curve that resembled a step-wise increment because of the gating phenomenon, lasting for 27 h. In the sampoorna treated batch, the curve was an additive one and ripening concluded in just 23 h. only.

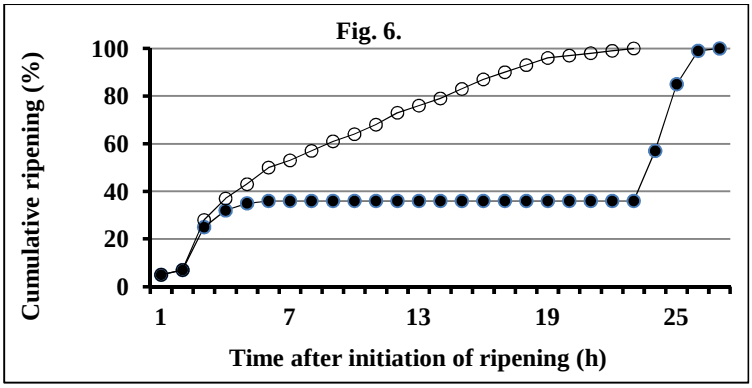

FIGURE 6. Cumulative ripening in PM (*Bombyx mori*) larvae under natural day (LD 12 : 12) conditions. Note the ripening under normal conditions (without sampoorna, ●) is rhythmic and the batches treated with sampoorna (o) have followed an additive curve. The control batches completed ripening in 28 h where as the sampoorna treated batched in just 32 h.

C. Ripening patterns in PM x CSR2:

a. Ripening patterns in PM x CSR2 under natural day conditions (LD 12: 12): The ripening patterns in PM under LD 12: 12 conditions are presented in Fig. 7. The ripening occurred immediately after lights-on phase of the imposed photoperiod. Further, the activity occurred for two consecutive days, as in PM. The activity is a diurnal one because the peak activity occurred in the light part of the imposed photoperiod. Similarly, the interval between two peaks is around 24 h, indicating circadian nature of the expression. In PM x CSR2 also, the ripening was observed for 27 h.

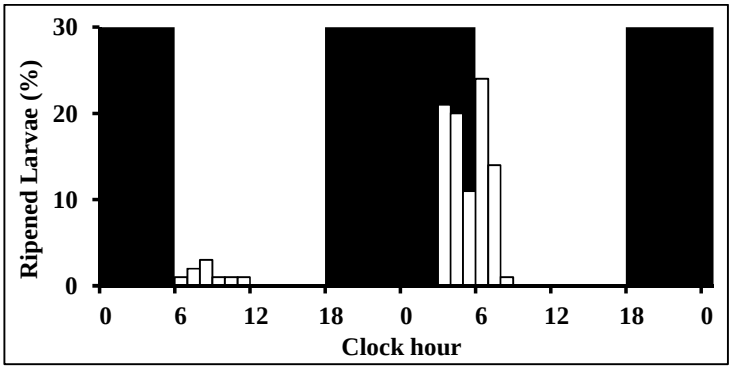

FIGURE 7. Distribution of ripening in PM x CSR2 (*Bombyx mori*) under LD 12 : 12 condition. Note occurrence of ripening for 2 consecutive days. The rhythm was circadian. Total ripening was 27 h. Black area indicates dark phase imposed.

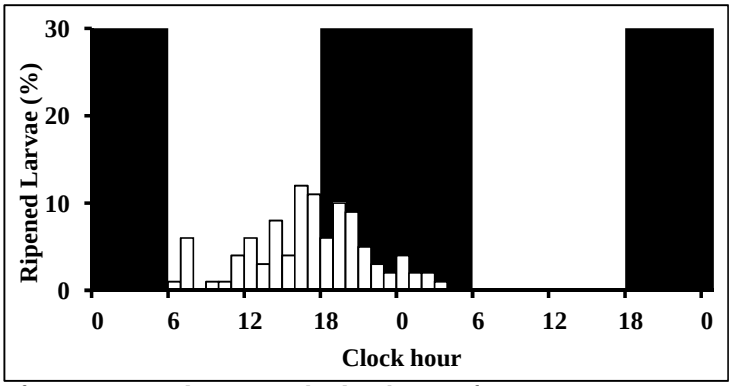

FIGURE 8. Implications of sampoorna application on the distribution of ripening in PM x CSR2 (*Bombyx mori*) under LD 12 : 12 condition. Note ripening initiated at 06.00 h and it continued till 05.00 h on the next day, expressing diurnal rhythmicity. Total ripening, thus, was 23 h. Gating phenomenon is not seen. Black area indicates dark phase imposed.

b. Impact of sampoorna on ripening patterns in PM x CSR2 under natural day conditions (LD 12: 12): The response of ripening to sampoorna application in PM x CSR2 is depicted in Fig. 8. The ripening was continuous for 23 h like that observed for PM. There appeared neither circadian periodicity nor diurnal pattern. The gating pattern is also not observed.

c. Impact of sampoorna application on cumulative ripening patterns in PM x CSR2, under LD 12: 12: Data on the cumulative ripening patterns both in control and sampoorna treated batches of CSR2 is presented Fig. 6. Interestingly, the cumulative ripening in control batch has given a curve that resembled a step-wise increment because of the gating phenomenon, lasting for 27 h. In the sampoorna treated batch, the curve was an additive one. Ripening continued for only 23 h.

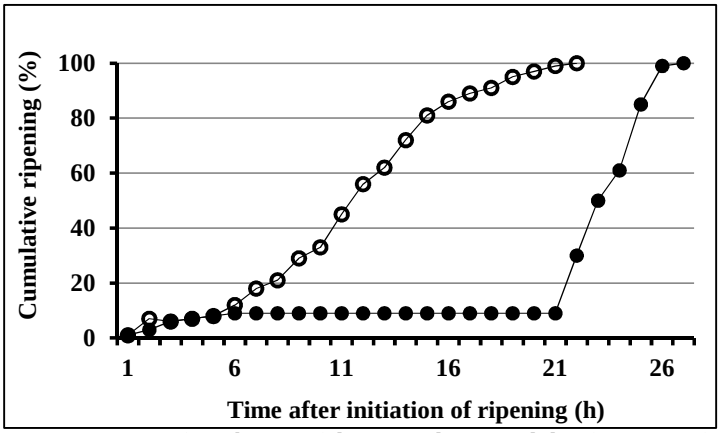

FIGURE 9. Cumulative ripening in PM x CSR2 (*Bombyx mori*) larvae under natural day (LD 12 : 12) conditions. Note the ripening under normal conditions (without sampoorna, ●) is rhythmic and the batches treated with sampoorna (o) followed an additive curve. The control batches completed ripening in 27 h where as the sampoorna treated batched in just 23 h.

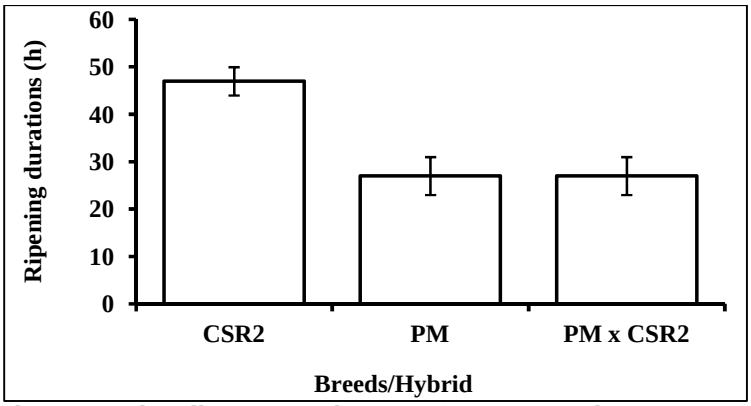

FIGURE 10. Ripening duration in the silkworm, *Bombyx mori* (CSR_2, PM and PM x CSR2) under control (without *Sampoorna* application). Note ripening durations of 47 h for CSR2 and only 27 h for PM and PM x CSR2. Data are the mean of 5 replications (± SD). The ripening durations are statistically highly significant among the breeds/hybrid. The values between PM and PM x CSR2, however are not significant.

D. Ripening durations:

Ripening durations in control silkworm batches: The important aspect of silkworm rearing in terms of economy, time saving etc., is the duration of ripening period. The results on the ripening in the control batches are presented in Fig. 10. It can be noticed that the bivoltine breed, CSR2 took long period of ripening, lasting for 47 h. This is the result of the gated phenomenon in ripening and occurring for consecutive three days. The ripening periods in PM and the hybrid, PM x CSR2 are less, recording only 27 h. Both PM and PM x CSR2 took two consecutive days for completion of ripening process, with two gates.

E. Ripening durations in *sampoorna* treated silkworm batches:

The ripening duration of CSR2, PM and PM x CSR2, after *sampoorna* treatment is presented in Fig. 11. Compared to the ripening durations with the control silkworm, the ripening period drastically reduced in treated batches. Thus, for CSR2, the ripening period has been reduced to 30 h. under treatment conditions from 47 h. of control silkworm larvae. Similarly, the ripening period was 23 h in treated batches of PM and PM x CSR2 when the same was 27 h. in control batches. The observed data are statistically highly significant among themselves as well as between control and treated batches.

F. Differences in ripening durations between control and sampoorna treated silkworm batches:

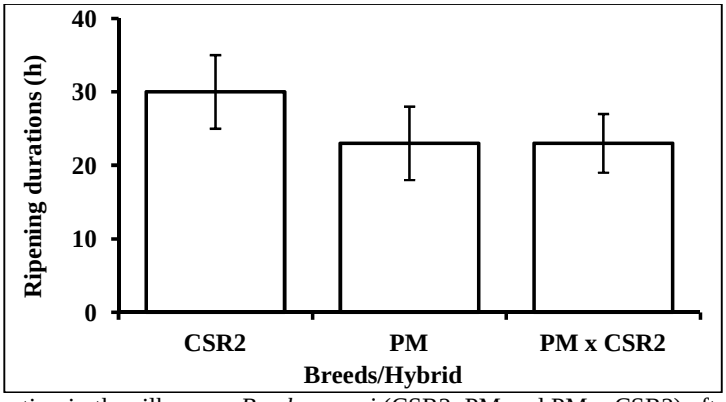

FIGURE11. Ripening duration in the silkworm, *Bombyx mori* (CSR2, PM and PM x CSR2) after *Sampoorna* application. Note ripening durations of 30 h for CSR2 and only 23 h for PM and PM x CSR2. Data are the mean of 5 replications (± SD). The ripening durations are statistically highly significant among the breeds/hybrid. The values between PM and PM x CSR2, however are not significant.

The differences in ripening duration between control and *sampoorna* treated batches of CSR2, PM and PM x CSR2 are presented in Fig. 12. The differences in ripening duration between control batches and *sampoorna* treated batches in CSR2 were 17 h. The same for both PM and PM x CSR2 was 4 h. The differences in ripening durations are statistically highly significant only between CSR2 and remaining two experimental larvae. Those values between PM and PM x CSR2, however are not significant.

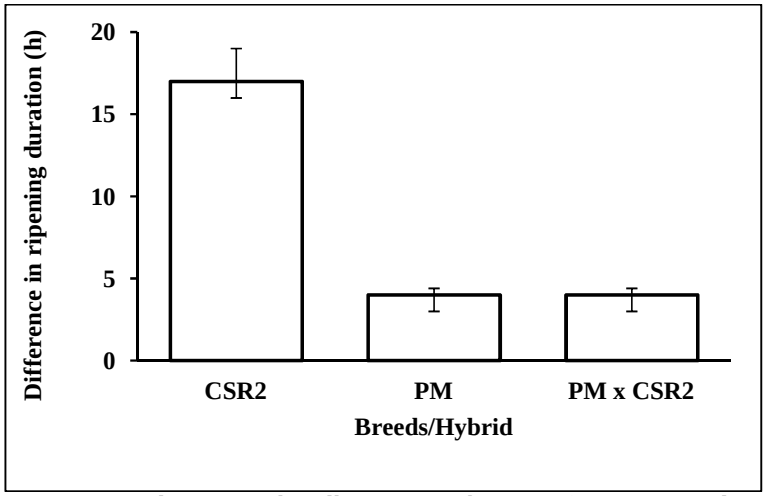

FIGURE 12. Differences in ripening duration in the silkworm, *Bombyx mori* (CSR2, PM and PM x CSR2) between un-treated (control) batches and treated (*sampoorna*) batches. Note differences in ripening duration of 17 h for CSR2 and only 4 h for PM and PM x CSR2. Data are the mean of 5 replications (± SD). The differences in ripening durations are statistically highly significant only between CSR2 and remaining. Those values between PM and PM x CSR2, however are not significant.

DISCUSSION

Behavioral phases between eating period (larval) and that of pre-pupal are very distinct in *B. mori*, the transitional phase is identified by cessation of feeding, change in larval colour and wandering of larvae in search of an appropriate site for cocooning. This stage was recognized as 'wandering stage' by many researchers (Piepho *et al*., 1960, Lounibos, 1976, Riddiford, 1980, Truman and Taghert, 1981, de Wilde *et al*., 1980). However, it is coined as 'ripening stage' in *B. mori*, since this term is broadly used in contemporary Indian sericulture (Krishnaswami *et al*., 1973, Krishnaswami, 1986, Sivarami Reddy, 1993, Sivarami Reddy *et al*., 1993, Shanthan Babu, 2014, Srinath, 2014, Srinath *et al*., 2018).

Although insects are extremely diverse group of organisms, the endocrine control of growth, eclosion and metamorphosis, within the group are remarkably parallel (Riddiford, 1980). It is well established that the major features of moulting cycles are regulated by a sequence of three hormones, PTTH, ecdysone and JH (Riddiford, 1980, Reynolds, 1980, Truman and Taghert, 1981, Happ, 1984). In the presence of high haemolymph titer of JH, the moulting cycle proceed to produce an additional larval/nymphal stage (Williams, 1961, Riddiford, 1980, Happ, 1984). On the other hand, if the JH titer is very low or virtually absent, morphogenesis is initiated, leading to the production of pupal or adult stage. The JH, responsible for the maintenance of larval characters (Riddiford, 1980) remains high, up to ultimate larval-to-larval moult, and thereafter drastically decreases to plateau level when the final instar larva attains its absolute size. At this instance, the brain releases PTTH during the next allowable gate

and causes the secretion of first installment of ecdysone, of course, 5 to 8 times less in quantity (as in *M. sexta*, Riddiford, 1980) than that necessary to elicit ecdysis which itself has, in the absence of JH, a profound effect on the animal, leading to the beginning of metamorphosis process.

The second release of PTTH, approximately 1.5 to 2 days before the pupal ecdysis, as in *M. sexta* (Riddiford, 1980, Truman and Taghert, 1981) and in *B. mori* (Shimada, 1989) in turn releases the second installment of ecdysone that is 5 to 8 times more than the first installment (Riddiford, 1980), and this hormone executes the larval-to-pupal ecdysis, initiating apolysis (Hinton, 1973). Truman (1972) and Truman and Taghert (1981) demonstrated that ecdysis itself is not gated but occurs after certain fixed hours of gated PTTH release (Beck, 1980, Truman, 1972, Truman and Taghert, 1981). The issue of second release of 2nd installment of PTTH and the consequential issues, in the present study, are not considered.

The synchrony of ecdysis appears to be solely dependent on the gated release of PTTH (Beck, 1980). The release of PTTH in the insect system immediately causes the release of ecdysone in turn, initiating ecdysial process. As discussed earlier, during the final instar larval period, the ecdysone at low concentration, in the absence of JH, initiates metamorphosis behavior (ripening, in the present study). The time between the first release of PTTH and appearance of wandering stage (as in *M. sexta*) or ripening (as in *B. mori*, in the present study) should be apparently more over that between the second installment release of PTTH and the pharate-pupal formation which time obviously is utilized for cocooning. Comparing the timings in ecdysis and eclosion, Truman and Taghert (1981) viewed that ecdysis itself is not a gated phenomenon, but occurs after certain time of gated release of PTTH. Therefore, the release of PTTH is the initiation point of ecdysial process. Once PTTH is released, or thus once the moult is initiated, the brain exercises or exerts the least control over subsequent ecdysis (Truman and Taghert, 1981). This statement is having full support with the fact that the removal of the brain from developing adults of *A. pernyi* seriously disrupted the eclosion time (Truman and Riddiford, 1970). In the case of ecdysis of fifth instar *Manduca*, removal of the entire head by neck ligation shortly after PTTH secretion (about 36 h before ecdysis) had no effect on the timing of the ecdysial attempts (Truman, 1972).

It is apparent that the release of PTTH in the population of CSR2 in the final instar larval period is in three gates as supported by three gate of ripening for three consecutive days. These findings are strongly supported by Srinath *et al*. (2018). Thus, the non-uniformity in rhythmic expression of developmental marker events, from the initial developmental marker event, the hatching (Shanthan Babu, 2014, Srinath, 2014) is increased towards the later developmental marker event, ripening (Srinath *et al*., 2018), as this event arrives at the end of the ultimate larval stages in the population of commercial silkworm, *Bombyx mori*. Hatching, being the initial developmental marker event in CSR2, was mostly restricted to a single day (Shanthan Babu, 2014, Srinath, 2014) or single peak. When the ripening is considered, the non-uniformity increased (Fig. 1). In the case of multivoltine breed (PM) and the cross-breed (PM x CSR2), the uniformity is not such complicated (Fig. 4 and 7 respectively). Truman (1972) reported that uniformity will be dependent on instar and larval size. The larval size of PM and PM x CSR2 is less compared to bivoltine breed, CSR2. Further, the fifth instar larval instar duration of multivoltine silkworm is less compared to bivoltine silkworm (Shanthan Babu, 2014). Hence, it is predicted that the synchronization in ripening of PM and PM x CSR2 larvae would be considerably good compared to CSR2. Thus, all the three silkworm varieties, CSR2, PM and PM x CSR2 have irrevocably under circadian clock control with a gating periodicity of around 24 h and thus following the gating phenomenon.

The results on the impact of *sampoorna* on the synchronization of ripening are interesting. The control silkworm larvae followed expression of mixed age characters with circadian nature and revealing gating phenomenon (Fig. 1, 4 and7). *Sampoorna* treatment on silkworm larvae at 5% larval ripening stage (at which point, the *sampoorna* treatment is administered), all the larvae of *Bombyx* silkworm in the mixed age population are committed for ripening. The imaginary first batch of larvae might be in an advanced stage. The second batch larvae are in moderately advanced stage and the third batch animals at least few hours earlier to 5% level ripening stage. Thus, the entire population in the 'mixed age' *Bombyx* larvae is free from the influence of the internal clock. When the phytoecdysone (*Sampoorna*) is administered at 5% ripening level, the larvae did not wait for internal secretion/supply of either PTTH or ecdysone as they have sufficient quantities of external ecdysone and are at liberty to utilize the external ecdysone (*sampoorna*), without depending on internal brain signals or release of PTTH or even consequent release of ecdysone, for completion of ripening in additive manner rather than gated phenomenon. It implies that *sampoorna* application give the opportunity to silkworm larval population not to depend on the release of either PTTH or the ecdysone. Therefore, the *sampoorna* applied larvae did not follow the 'fortuitous synchrony' after PTTH release and consequential ripening was additive type, against a gated one, as revealed in Figs. 2, 3, 5, 6, 8 and 9. And therefore, they expressed a single peak, of course a broad one (Figs. 2, 5 and 8) and have revealed an additive curvy-linear pattern in ripening process (Figs. 3, 6 and 9) indicating that external source of *sampoorna* delinked the larvae from direct control (?) of brain, indirect control of either PTTH and internal secretion of ecdysone. Thus, a lengthy ripening period of 47 h in CSR2 and 27 h in PM and PM x CSR2 under controlled conditions (without *sampoorna*) has been squeezed to 30 h for CSR2 and 27 h for PM and PM x CSR2.

Comparing the ripening durations (Figs. 10 and 11), it is inferred that more ripening durations in control batches (47 h for CSR2 and 27 h for PM and PM x CSR2) has been reduced to 30 h for CSR2 and 23 h for PM and PM x CSR2. The differences in ripening durations between control and treated batches of CSR2 alone are, thus, highly (< 0.01) significant, indicating the most effectiveness of the *sampoorna* in reducing ripening durations. On the other hand, the differences in ripening durations between control and *sampoorna* treated batches of PM and PM x

CSR2 are not significant. *Sampoorna* has acted as the best synchronizer in reducing the mixed-age population ripening durations from 3 days to mere a day. 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 e*t al*., 2003, Sashindran Nair *et al*., 2005, Nirmal Kumar *et al*., 2006, 2007, Srinath *et al*., 2009). Comparing the results of bivoltine and multivoltine cross-breed, one can get the impression that *sampoorna* treatment is highly essential for bivoltine silkworms, as seen from significant reduction in ripening duration from control to sampoorna treated CSR2 batches and significant in differences in ripening durations between control and treated batches of CSR2. However, less ripening durations (for control and treated batches) and non-significant differences in ripening durations between control and sampoorna treated batches of PM and PM x CSR2 hints that sampoorna treatment is not necessary for PM and PM x CSR2 unless in exigencies like disease out-break (Mithilesh Kar *et al*., 2009).

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REFERENCES

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

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.

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.

Mithilesh Kar, Sudhakara Rao, P., Kishore, S., Selva Kumar, T Nisha Gopal, Narasimha Nayaka, A.R., Chandrasekaran, K. (2009) Effect of Phytoecdysteroid on Disease Incidence, Melting and Economic, *Int. J. Indust. Entomol*., 18 (1), 8-12.

Nirmal Kumar, S., Shashindran Nair, K. and Rabha, J. (2006) Role of phytoecdysteroid treatment in the maturation process of multi x bivoltine (BL67 x CSR101) 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 groth 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. Thesis, Sri Krishnadevaraya University, Anantapur, India.

Shimada, J. (1989) Release of prothoracicotropic hormone in 5th 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 NB4D2), Ph. D., Thesis, Sri Venkateswara University, Tirupati, India.

Sivarami Reddy, N., Pavan Kumar, T., Murali Mohan, P. and Sasira Babu, K. (1993) 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. and Riddiford, L.M. (1970) Neuroendocrine control of ecdysis in silkmoths, *Science*, **167**: 1624-1626.

Truman, J. W. and 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.

Williams, C.M. (1961) The juvenile hormone. II. Its role in the endocrine control of moulting, pupation and adult development in the cecropia silkworm. *Biol. Bull*. Mar. Biol. Lab. Woods Hole, **121**: 521-585.

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.