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IMPACT OF PHOYTOECDYSONE, '*SAMPOORNA*' ON THE SYNCHRONIZATION OF RIPENING IN THE COMMERCIAL SILKWORM, *BOMBYX MORI* L. -A CHRONOBIOLOGICAL PERSPECTIVE

[1]Srinath, B., [2]Shanthan Babu, M.A., Lakshminarayana Reddy, P., Sujatha, B. & Sankar Naik, S.
Department of Sericulture, Sri Krishnadevaraya University, Anantapur -515 503, India.
[1]REC, CSB, Agriculture Market Yard, Vikarabad –501 101, India
[2]RSRS, CSB, PO Box 50, Anantapur – 515 501, India.

ABSTRACT
The intensity of non-uniformity in rhythmic expression of developmental marker events increase from initial stages of life cycle to the final stages in the population of commercial silkworm, *Bombyx mori* L. Silkworm larvae in population do not ripen uniformly, taking 3 days and above, posing difficulties to farmers for more time, labour, expenditure and further risk. Keeping these difficulties of the farmers in mind, studies were conducted to determine the patterns of ripening in the commercial silkworm through chronobiological approach. Simultaneously the impact of phytoecdysteroid, *Sampoorna*, (a brand name of phytoecdysone released by CSRTI, Mysore, India), applying at the appearance of 5% ripening were also studied on two popular bivoltine breeds, CSR_2 and CSR_4 and their hybrid, CSR_2 x CSR_4. Experimental animals 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 overt phenomenon, reoccurred in 24 hour intervals, thus expressing circadian nature and prolonged for 3 consecutive days, hence revealing gating rhythmic characteristics in all the three experimental silkworm breed/hybrid, lasting for 47 hours (CSR2 and CSR4) to 43 hours (CSR_2 x CSR_4). Upon application of *sampoorna*, the silkworm larvae did not follow circadian characteristics straightway in ripening process, expressing continuous ripening activity, lasting for 28 to 30 hours (CSR4 and CSR2 respectively) and 26 hours (CSR_2 x CSR_4) hours. It is proposed that the larvae treated with *Sampoorna* utilize only external source of ecdysone (*Sampoorna*), ignoring (?) internal secreted ecdysone and ignore the instruction signals from the brain and persist ripening continuously. Ripening durations between experimental batches and control larvae were statistically highly significant (< 0.01).

KEYWORDS: developmental marker, phytoecdysteroid, bivoltine breeds, ecdysone.

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. Processes that occur only once in life cycle of an insect (egg hatching, larval ecdysis, pupation, eclosion *etc*.) are under circadian control and are further, gated (Page, 1985; Truman, 1985; Zdarek, 1985). 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. The larval-to-pupal ecdysis is still complex, and is too lengthy process as it includes steps like stopping intake of food, ripening, wandering for a suitable 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*., 1993a, 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 (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 (Srinath, 2014). Following the colour change phase, the larvae show no interest in available food (leaf). This situation is compared 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*., 1993a; Sivarami Reddy, 1993; Srinath, 2014) at this stage (ripening stage). When the cocoon construction is initiated 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;Srinath, 2014). The first installment of ecdysone is reported to be 5 to 8 times less in quantity than the second installment of ecdysone initiates ripening (Sivarami Reddy, 1993, Srinath, 2014). The release of second installment of PTTH (Riddiford, 1980; Truman and Taghert, 1981; Shimada, 1989; Sivarami Reddy, 1993; 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; 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, pharate pupal formation and pupation in the present study should be considered '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, laborious and ads to the cocoon production cost (Kanika Trivedi *et al*., 2003; Sashindran Nayar *et al*., 2005; Nirmal Kumar *et al*., 2006, 2007). 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, 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 situations like leaf shortage, occurrence of non-cocooning silkworm and possibility of diseases outbreak. 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 two bivoltine silkworm breeds (CSR_2 and CSR_4) and their hybrid (CSR_2 x CSR_4) of *Bombyx mori* under natural day (LD 12 : 12) conditions.

MATERIALS & METHODS

Two pure breeds of popular bivoltine silkworm (*Bombyx mori* L.) breeds in the contemporary sericulture industry of India, CSR_2 and CSR_4 and their hybrid, CSR_2 x CSR_4 were used for the rhythmicity of ripening and comparative studies on *Sampoorna* on ripening process. Disease free layings (DFLs, commonly called) of two pure breeds, CSR_2 and CSR_4, and their hybrid, CSR_2 x CSR_4 were procured, on the third day of oviposition, from the Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Bangalore, Karnataka, India. The DFLs were transported to the Department of Sericulture, Sri Krishnadevaraya University, Anantapur; where the investigations 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°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 12h 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 phytoecdysteroid 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 treated (sprayed) with *Sampoorna* at the rate of 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 were maintained for each silkworm breed/hybrid. As control batches, five replications with 100 larvae for each breed/hybrid were also maintained for each breed/hybrid. 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; $\omega = 360^o$). Further, the data were plotted for cumulative frequency curves for precise and decisive comparison. 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.

RESULTS

I. Ripening patterns in CSR_2

A. Ripening patterns in CSR_2 under LD 12:12: Ripening in CSR_2 under LD 12:12 conditions (Fig. 1) occurred just after 'lights-on' phase of the photoperiod imposed. The activity occurred for three continuous days. The peak of the ripening rhythm advanced into the dark phase, expressing 'peak-bias'. Occurrence of peak in the light part of the day indicates the activity is a diurnal. The interval between two successive peaks is ≈ 24 h and thus circadian.

B. Impact of Sampoorna on ripening patterns in CSR_2: The response of ripening to *Sampoorna* in CSR_2 is given in Fig. 2. With the application of '*Sampoorna*', continuous ripening is recorded. The ripening activity duration was 30 h. In addition, circadian periodicity, diurnal and gating phenomenon patterns were not observed.

C. Impact of Sampoorna on cumulative ripening patterns in CSR_2: Cumulative ripening patterns both in control and *sampoorna* treated batches of CSR_2 (Fig. 3) indicated that cumulative ripening in control batch followed a curve that resembled a step-wise increase because of the gating phenomenon.

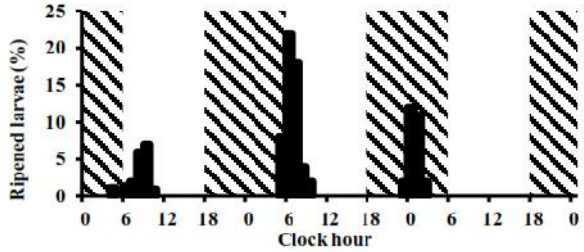


FIGURE-1

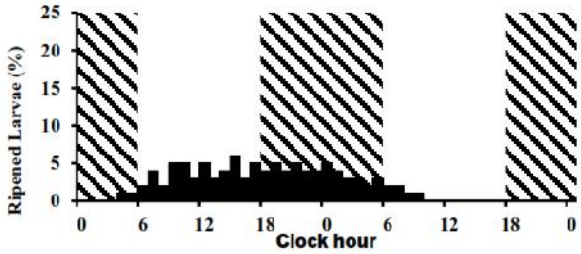


FIGURE-2

Distribution of ripening in CSR_2 (*Bombyx mori*) under LD 12:12 condition. Note occurrence of ripening for 3 consecutive days (gates). The rhythm was circadian. Cross-hatched area indicates the dark phase imposed as shown in fig. 1.

Implications of *Sampoorna* application on the distribution of ripening in CSR2 (*Bombyx mori*) under LD 12:12 condition. Note ripening initiated at 04.00 h and continued till 11.00 h on the next day, expressing a continuous (neither diurnal nor gating) phenomenon. The ripening completed in just 30 h. as shown in fig. 2.

II. Ripening patterns in CSR_4

A. Ripening patterns in CSR_4 under natural day conditions (LD 12:12): The ripening in CSR_4 under LD 12:12 (Fig. 4) conditions also occurred just after 'lights-on' phase of the imposed photoperiod occurring for three consecutive days, expressing diurnal phenomenon, as the peaks of ripening recorded in the light phase imposed. The interval between two peaks is ≈ 24 h and therefore circadian. Gating in ripening is also noticed.

Fig. 3: Cumulative ripening pattern in CSR_2 (*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* (ɵ) have followed an additive curvy pattern. The control batches completed ripening in 47 hours whereas the *Sampoorna* treated batches in just 30 h.

B. Impact of Sampoorna on ripening patterns in CSR_4: The response of ripening to *Sampoorna* application in CSR_4 (Fig. 5.) followed that of CSR_2. Ripening was completely continuous for 28 h. Further, circadian periodicity, diurnal expression and gating patterns were not observed.

C. Impact of Sampoorna on cumulative ripening patterns in: Data on the cumulative ripening patterns both in control and sampoorna treated batches of CSR_4 is presented Fig. 6. Ripening in control batch recorded a curve of step-wise increment, as that observed for CSR_2, because of the gating phenomenon, lasting for 47 hours. In the sampoorna treated batch, the curve was additive. Ripening duration was 28 hours only.

Fig. 4: Distribution of ripening in CSR_4 (*Bombyx mori*) under LD 12:12 condition. Note occurrence of ripening for 3 consecutive days with circadian and gated nature. Cross-hatched area indicates the dark phase imposed.

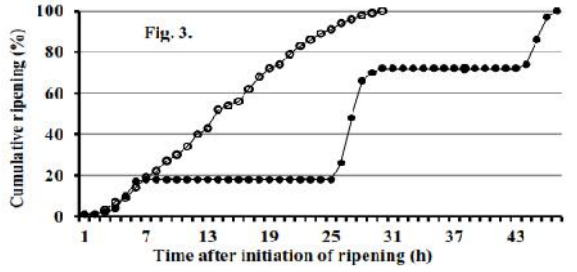
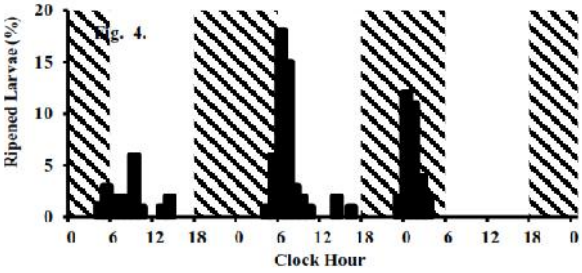


FIGURE-3



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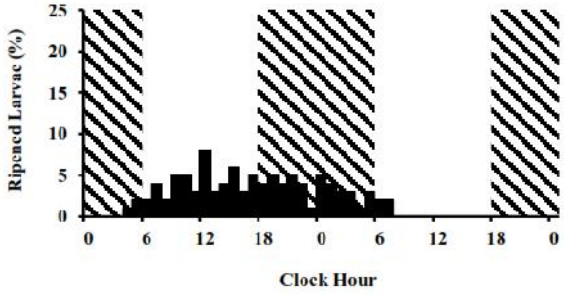

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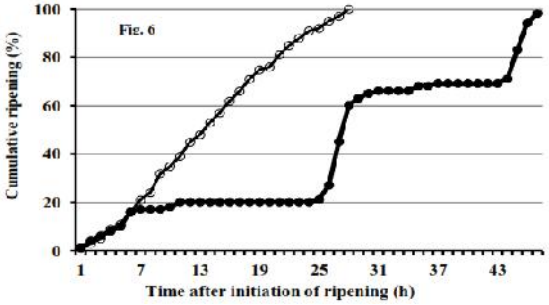

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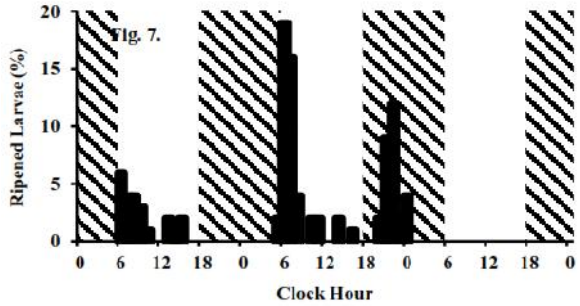

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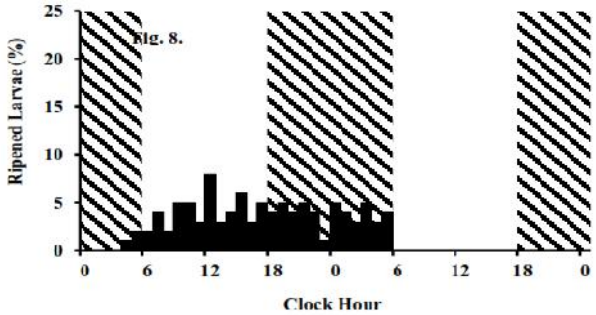

FIGURE-8

III. Ripening patterns in CSR_2 x CSR_4

A. Ripening patterns in CSR_2 x CSR_4: The ripening patterns in CSR_2 x CSR_4 under LD 12:12 conditions are depicted in Fig. 7. Ripening occurred after lights-on, occurring for three consecutive days. Occurrence of peak in the light phase imposed indicates diurnal pattern. Interval between two consecutive peaks is around 24 h and therefore circadian and gating phenomenon.

B. Impact of Sampoorna on ripening patterns in CSR_2 x CSR_4: Ripening, in response to Sampoorna application in CSR_2 x CSR_4 (Fig. 8) was completely continuous, restricting the entire activity duration to 26 h. Rhythmic patterns such as circadian periodicity, diurnal pattern and gating patterns were not observed.

Durations of ripening under control conditions: Comparison of ripening duration is more essential to determine the effectiveness of external phytoecdysone on the commercial silkworm ripening. Ripening duration of two breeds (CSR_2 and CSR_4) of the silkworm, *Bombyx mori* L. and their hybrid, CSR_2 x CSR_4 is presented in Fig. 10. It is observed that in all the breeds/hybrid, the ripening duration is more than 40 hours, as also seen in Figs. 1, 4 and 7. While the ripening durations for two breeds, CSR_2 and CSR_4 under control (without *Sampoorna*) are more (47 h), the same for the hybrid, CSR_2 x CSR_4 was significantly (5% level) with a ripening duration period of 43 h (Fig. 10.).

Fig. 5: Implications of *Sampoorna* application on distribution of ripening in CSR4 (*Bombyx mori*) under LD 12:12 condition. Note ripening initiated at 04.00 h, continued till 08.00 h on the next day, expressing diurnal predominance with no gating phenomenon. Ripening completed in just 28 h.

Fig. 6: Cumulative ripening in CSR_4 (*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* (ɵ) followed an additive curve. The control batches completed ripening in 47 hours where as the sampoorna treated batch in just 28 h.

Fig. 7: Distribution of ripening in CSR_2 x CSR_4 (*Bombyx mori*) under LD 12:12 condition. Note occurrence of ripening for 3 consecutive days. The rhythm was diurnal, circadian and gated. Cross-hatched area indicates the dark phase imposed.

C. Impact of Sampoorna application on cumulative ripening patterns in CSR_2 x CSR_4: Data on the cumulative ripening patterns both in control and *Sampoorna* treated batches of CSR_2 x CSR_4 are presented in Fig. 9. The cumulative ripening in control batch was a curve that resembled a step-wise increase, may be due to the gating phenomenon. The ripening duration was 42h. The curve in the *Sampoorna* treated batches was additive one. Ripening continued for only 25 hours.

Fig. 8: Implications of *Sampoorna* application on distribution of ripening in CSR_2 x CSR_4 (*Bombyx mori*) LD 12:12 condition. Note ripening initiated at 04.00 h and it continued till 06.00 h on the next day with no circadian nature, diurnal expression and gating phenomenon. Ripening lasted for just 26 h.

Fig. 9: Cumulative ripening in CSR_2 x CSR_4 (*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* (ɵ) followed an additive curvy pattern. The control batches completed ripening in 43 hours where as the sampoorna treated batches in 25 h.

Durations of ripening with Sampoorna application: *Sampoorna* application greatly reduced the ripening period (Fig. 11) to more than 17 h. In pure silkworm bivoltine breeds, CSR_2 and CSR_4, the ripening durations were 30 and 28 h respectively and that for CSR_2 x CSR_4 was only 26h. The differences in ripening durations between breeds/hybrid are statistically significant (1% level). In addition, when the ripening durations between the treated batches of pure breeds/hybrid and controlled batches (without *Sampoorna* treatment) are compared, the differences are statistically highly significant at 1% level.

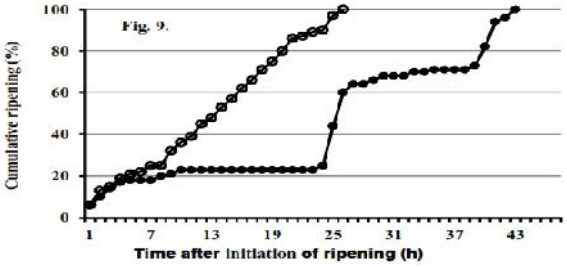

FIGURE-9

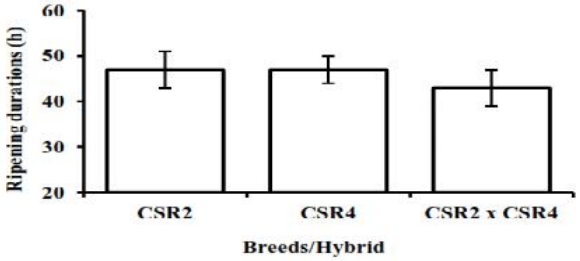

FIGURE-10

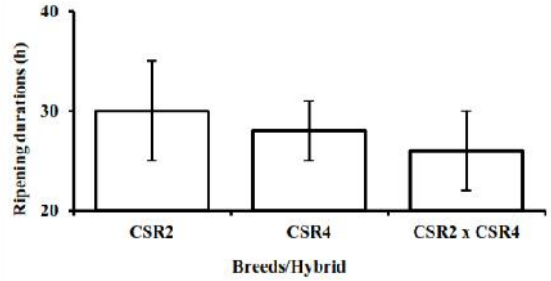

FIGURE-11

DISCUSSION

Behavioral phases between eating period (larval) and that of pre-pupal are very distinct in *Bombyx mori*. The transitional phase between larval and pre-pupal period can be determined by the cessation of feeding, change in larval colour and wandering of larvae in search of an appropriate site for cocooning. This transitional stage has been recognized as 'wandering stage' by many workers (Piepho *et al*., 1960; Lounibos, 1976; Riddiford, 1980; Truman and Taghert, 1981; de Wilde *et al*., 1980). However, in the present study, this stage is coined as 'ripening stage', since this name is broadly used in contemporary Indian sericulture (Krishnaswami *et al*., 1973; Krishnaswami, 1986; Sivarami Reddy, 1993; Sivarami Reddy *et al*., 1993a, Srinath, 2014). Although insects are extremely diverse group of organisms, the endocrine control of growth, moulting and metamorphosis, within this group are remarkably similar (Riddiford, 1980). It is well established that the major features of moulting cycles are regulated by a sequence of three hormones; PTTH, ecdysone and JH (Riddiford, 1980; Reynolds, 1980; Truman and Taghert, 1981; Happ, 1984). In the presence of high haemolymph titer of JH, the moulting cycle produces an additional larval or nymphal stage (Williams, 1961; Riddiford, 1980; Happ, 1984). On the other hand, if the JH titer is very low or virtually absent, morphogenesis is initiated leading to the production of pupal or adult stage. The JH, responsible for the maintenance of larval characters (Riddiford, 1980) remains high, up to ultimate larval-to-larval moult, and drastically decreases to plateau level when the final instar larva attains its absolute size. At this 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.

Fig. 10: Ripening durations in bivoltine breeds (CSR_2 and CSR_4) and their hybrid (CSR_2 x CSR_4) (*Bombyx mori*) larvae under natural day (LD 12:12) conditions. Note the ripening duration for two pure breeds is identical; measuring 47 h. Ripening duration of the hybrid is less (43 h) compared to pure breeds. The values are the mean of 5 replications (± SD). Ripening duration between two breeds is not significant. However, ripening durations are significant (5% levels) compared to two breeds and hybrid.

Fig. 11: Impact of *Sampoorna* treatment on the ripening durations in two bivoltine breeds (CSR_2 and CSR_4) and their hybrid (CSR_2 x CSR_4) of *Bombyx mori* larvae under natural day (LD 12:12) conditions. Ripening durations were 30 h for CSR_2 and 28 h for CSR_4 while that for CSR_2 x CSR_4 was 26 h. The values are mean of 5 replications (± SD). Ripening durations are significantly (5% level) different between breeds and hybrid.

The second release of PTTH, approximately 1.5 to 2 days before the pupal ecdysis, as in *M. sexta* (Riddiford, 1980; Truman and Taghert, 1981) and in *Bombyx mori* (Shimada, 1989) in turn releases that second installment of ecdysone that is 5 to 8 times more than the first installment (Riddiford, 1980), and this hormone executes the larval-to-pupal ecdysial process, 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).

In *Samia cynthia recini*, the time lags between the release of PTTH and completion of larval-to-larval ecdysis was 40h (Fujishita and Ishizaki, 1981) with a similarity in *Bombyx mori* for the duration between settling for moult (one of the morphological marker event stages in ecdysial sequence, occurring after the release of PTTH/ecdysone) to completion of moulting around 24 h (Sivarami Reddy *et al*., 1990a, Sivarami Reddy, 1993). 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). With the release of second installment of PTTH/ecdysone at high concentrations, the insect is committed to apolysis (Hinton, 1973) causing pharate-pupal formation and consequently pupal ecdysis. The time between the first release of PTTH and appearance of wandering stage (as in *M. sexta*) or ripening (as in *Bombyx mori*, in the present study) should be apparently more over that between the second installment release of PTTH and the pharate-pupal formation.

Comparing the timings in ecdysis and eclosion, Truman and Taghert (1981) viewed that ecdysis itself is not a gated phenomenon. But ecdysis and eclosion 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).

The results in the present study are well supported by the fact that at 5% larval ripening stage (at which point, the sampoorna treatment is administered), all the larvae in the mixed age population of *Bombyx* silkworm are committed for ripening as all the larvae had virtually got the PTTH released. 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 internal clock. When the phytoecdysone (*Sampoorna*) is administered at 5% ripening level, the larvae did not wait for internal secretion/supply of ecdysone as they 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. 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. IV. 3, 6 and 9. And therefore, they have revealed an additive curvy-linear pattern in ripening process indicating that external source of *Sampoorna* delinked the larvae from direct control (?) of brain, indirect control of either PTTH and internal secretion of ecdysone. However, further decisive studies are required to prove the hypothesis. Thus, a lengthy ripening period of 47 h in CSR2/CSR4, 43 h in CSR2 x CSR4 under controlled conditions (without *Sampoorna*) has been condensed to 30 h for CSR_2, 28 h for CSR_4 and 26 h for CSR_2 x CSR_4.

Comparing the ripening durations (Fig. IV. 10 and IV. 11), it is inferred that more ripening durations (over 40 h) in control batches has been reduced to 30 h or even less (26 h for the hybrid). The differences in ripening durations between control and treated batches are, thus, highly (< 0.01) significant, indicating the most effectiveness of the *Sampoorna* in reducing ripening durations. Further, *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, 07; Srinath *et al*., 2009).

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