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Early and Uniform Maturation in Silkworm *Bombyx mori* L. by Phytoecdysteroid Extracted from a Plant of Family Caryophyllaceae

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One of the biggest problems encountered during the last phase of silkworm rearing is non-uniform maturation of the silkworms especially during cooler months. Phytoecdysteroid (20-hydroxy ecdysone) was extracted in large-scale from a plant belongs to Caryophyllaceae and fed to silkworm larvae to test the effect of phytoecdysteroid. About 80% of the silkworms were ready for mounting by 18 hrs after treatment (when the treatment is done for uniform spinning), whereas in control batch only 37% worms were ready for mounting by the same time.

Key words: Early maturation, Plant extract, Phytoecdysteroid, Uniform maturation

Introduction

The maturation and spinning activities in a given silkworm population is not uniform. In normal practice of silkworm rearing, farmers are forced to pick and mount the silkworm as and when they mature and the mounting process extends up to 2–3 days and even more during cooler seasons. This involves lot of time, labour and extra mulberry leaf and also ends up in higher production cost besides some marketing difficulties or extended grainage operation where seeds are produced. These problems could be avoided or minimized to a great extent if ecdysteroid is administered to silkworm as it controls the maturation and spinning activities. Because of these specific advantages, the moulting hormone can be effectively used in Indian Sericultural Industry for early and uniform maturation. To combat leaf shortage, spinning activities can be advanced or synchronized by administering ecdysone. If administered when the silkworms are about to spin (Onset of spinning), it will introduce simultaneous and uniform spinning (Li *et al.*, 1992). In China, ecdysteroid is extracted from locally available plants and marketed extensively for early or uniform larval maturity. By inducing early spinning, total crop loss can be avoided. Phytoecdysteroids are also used commercially to increase productivity in sericulture (Chou and Lu, 1980). Shivkumar *et al.* (1995, 1996) made some preliminary efforts to use crude phyto extracts in sericulture. Simultaneously, Trivedy *et al.* (1998) also extracted ecdysteroid from different plants and the presence of hormone was confirmed through thin layer chromatography and dose was quantified through HPLC. Hormone was successfully tested in laboratory and Regional Sericultural Research Centers of CSRTI, Mysore. The results were similar to that of Chinese moulting hormone. Similar observations were reported by Anantharaman *et al.* (1996), Maribashetty *et al.* (1997) and Chandrakala *et al.* (1998). Various ecdysteroids with moulting hormone activities in insects were isolated from plant materials (Novak, 1975; Chou and Lu, 1980; Slama *et al.*, 1993). Ecdysterone or 20-hydroxy ecdysterone is the most widely occurring phytoecdysterone and many plants like *Achyranthus aspera* and *Trianthema portulacastrum* in south India have been identified to have this compound (Banerjii *et al.*, 1971). The present technology is developed to induce uniform spinning or to advance maturation events in silkworm by application of hormone extracted from the plant of family Caryophyllaceae.

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Material and Methods

To study the effect of Sampoorna on commercial crop and economic characters, CSR2×CSR5 (bivoltine×bivoltine) silkworm hybrid was utilized following the rearing method

of Krisnaswami (1998). Different batches were treated at the age of 72 hrs, 96 hrs, 120 hrs and 132 hrs (on set of spinning) of 5th instar. Same numbers of disease free layings (dfls) were treated for positive control (with 100 mg of purified 20 hydroxy ecdysone dissolved in 4 litre water for 100 dfls) at onset of spinning and same number of dfls kept as absolute control without any treatment. Three replications of 100 dfls each for all the treatments were maintained. For the uniform spinning "Sampoorna" was sprayed at (132 hrs treatment) onset of spinning, when 3–5% of the worms in a batch initiated spinning.

Process of application of phytoecdysteroid - "Sampoorna"

40 ml of "Sampoorna" was diluted with 4 liters of water to treat 100 Dfls *i.e.*, 40,000 larvae. Thin feeding was given to silkworms, which are to be treated with Sampoorna. Diluted solution was sprayed on the feed using a hand sprayer. After 6–8 hrs of treatment, one more feeding was given after complete consumption of treated leaf wherever required. Mountages were kept on the rearing beds at the same time to all the batches (treated and control) to observe crawling of the mature larvae *i.e.*, self-mounting procedure.

Time and percentage of maturation was recorded, cocoons were harvested, assessed and the data were subjected to statistical analysis (ANOVA) to find out the significant differences in the effect of hormone, if any.

Results and Discussion

In uniform spinning treatment, about 79.53% of the silkworms were ready for mounting by 18 hrs whereas in control batch only 36.82% silkworms were ready for mounting by that time (Fig. 1). About 93.98% larvae were crawled on to mountages and matured within 24 hrs after spraying "Sampoorna". Along with "Sampoorna", pure ecdysterone (positive control) also was used for comparison. Similar trend was observed in positive control batches (92.15%). Whereas in control batches, crawling percentage was around 42.39. Generally, when about 80% of the silkworms are ready, even the remaining silkworms are mounted at once. To reach 100% maturation the treated batches and control took 36 and 54 hrs respectively. There was no difference in maturation time between the "Sampoorna" and the positive control. The effect of treatment on the general economic parameters like cocoon weight, cocoon shell weight and shell ratio did not show significant difference when treated at the onset of spinning (Table 1). Therefore, it is clear that treatment at onset of spinning do not affect the economic traits of cocoons.

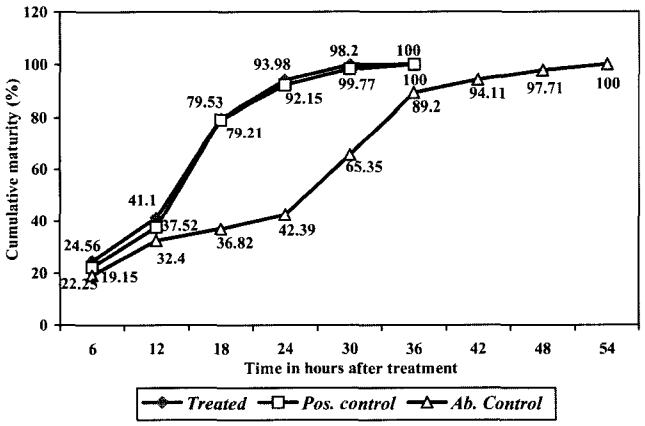

Fig. 1. Effect of "Sampoorna" treated at onset of spinning on maturation of silkworm, compared with positive control (Pos. control) *i.e.*, 20-hydroxy treated batch and absolute control (Ab. Control) *i.e.*, without treatment.

For advanced spinning (crop saving application) "Sampoorna" was administered after the completion of 72, 96 and 120 hrs in the 5th instar. Since spinning initiated at 132 hrs of 5th instar in control batches, treatment beyond 120 hrs has not been done. Control batches took another 54 hrs to achieve 100% maturation. 75.38 and 83.84% silkworms were matured after 24 hrs in 96 and 120 hrs treatment respectively and more than 90% silkworms matured by 38 hrs after treatment whereas only 43.18% in 72 hrs treatment at the same time. In 72 hrs treatment spinning did not initiate after 24 hrs of treatment.

As already mentioned, there is no significant difference in economic characters of cocoons *viz.*, cocoon weight, cocoon shell weight and shell ratio % between control batch and treatment at onset of spinning. Where as gradual decrease in economic characters of cocoons is recorded from 120 hrs treatment to 72 hrs treatment. This resulted in significant reduction in the cocoon weight and cocoon shell weight and not much variation in shell ratio

Table 1. Effect of "Sampoorna" treatment for uniform maturation at on-set of spinning (132 hrs of 5th instar) on the cocoon characters of CSR 2×CSR5 *Bombyx mori*

Treatment	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
Sampoorna	2.265	0.536	23.69
Positive control (20 hydroxy ecdysone)	2.262	0.522	23.06
Absolute control	2.346	0.555	23.64
C. D. at 5%	NS	NS	NS

NS, Non-significant.
C. D., Critical difference.

Table 2. Effect of "Sampoorna" for early maturation treatment (Crop saving treatment) on the maturation percentage and cocoon characters of CSR 2×CSR5 *Bombyx mori*

Treatment at	Maturation after 24 hrs of treatment (%)	Maturation after 38 hrs of treatment (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
5th instar 72 hrs	–	43.18	1.900	0.450	23.68
5th instar 96 hrs	75.38	93.85	2.000	0.476	23.80
5th instar 120 hrs	83.84	90.63	2.140	0.490	22.90
5th instar 132 hrs (Control without treatment)	*	*	2.346	0.555	23.64
C. D. at 5%			0.052	0.011	0.146

*, Spinning initiated at 132 hrs of 5th instar and 100% completion of spinning took another 54 hrs.
C. D., Critical difference.

% between control and treated batches (Table 2).

The results from the farmer's field were highly satisfactory. In normal rearing condition, the maturation process and the mounting activity were completed within 24 hrs, when "Sampoorna" was administered. There were incidences where the mounting process was completed within 18 hrs when the temperature was high. Interaction with farmers revealed that much to their relief, the mounting process gets over quickly, which has been accepted widely by them. The farmer's response to this technology proves that there is something beyond the amount they save. The relief with quick mounting is the well-appreciated factor, which supports extensive use of "Sampoorna" for synchronizing spinning activities and quick mounting.

On "Sampoorna" application for uniform spinning, the duration of maturation activity and mounting process gets shortened to about 24–30 hrs from about 48–72 hrs depending on the season without affecting the cocoon quality. This helps in synchronizing all post cocoon activities and saving labour and mulberry leaf. On the contrary, when it is used for advanced spinning, the larval feeding period itself is shortened from about 6–7 days to about 5–6 days. But the shortage in larval feeding period resulted in marginal reduction in the cocoon characters. The advantage being the cocoon crop can be saved to certain extent, which would otherwise been lost due to disease or leaf shortage. On achieving uniform cocoon spinning, the mounting process can be completed within a period of 18–24 hrs instead of 2–3 days. The results are similar to that of 20-hydroxy ecdysone. So far, the hormone has been tested on more than 1 lakh disease free layings.

Plant sterol is converted to animal sterol in phytophagous species like *B. mori*. In other words we can say that the silkworm cannot synthesize precursors of moulting hormone *de novo* and therefore it depends on dietary sterol present in its food (Hoffmann and Hetru, 1983). The moulting hormones are hydrophilic in nature because of a number of hydroxy groups present in the molecule. So analogues of these hormones cannot penetrate the insect cuticle. Hence in present work per oral application of phytoecdysteroid has been done. The neurohormone of the brain or prothoracicotropic hormone (PTTH) enter into the haemolymph through the retrocerebral complex Corpus cardiacum (CC) and Corpus allatum (CA) and stimulates the prothoracic glands to synthesize and secrete ecdysone, a prohormone (Shirai *et al.*, 1992). At peripheral sites, ecdysone is converted into a moulting hormone, 20-hydroxy ecdysone (ecdysterone). When this hormone reaches a critical concentration, the moulting/spinning process is initiated (Senhal, 1989; Grieneisen, 1994). In a population, each larva has different endogenous ecdysteroid titre and will reach threshold level of ecdysteroid required for spinning at different time. Per oral application of ecdysteroid at the onset of spinning will enhance the ecdysteroid titre to reach quickly to its threshold level required for spinning.

The bioassay trials conducted at CSR & TI, Mysore revealed that "Sampoorna" is effective in inducing advanced maturation when used for early or advanced maturation. One can administer phytoecdysteroid any time after 72–75 hrs in fifth instar. For uniform or synchronized maturation, the administration is to be done on the on set of spinning *i.e.*, when 3–5% silkworms start spinning. In brief "Sampoorna" can be used for two different proposes, to synchronize the maturation activity and to achieve uniform spinning (regular use) and to advance the maturation activities to hasten the cocoon spinning process especially when crop loss is predicted either due to leaf shortage or due to disease out break (crop saving use).

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