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Role of Phytoecdysteroid Treatment Time in the Maturation Process of Multi × Bivoltine (BL67 × CSR101) Hybrid Silkworm, *Bombyx mori* L. When Maintained at Low, Medium and High Temperature

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Use of products containing phytoecdysteroid (PE) as active principle has become popular in prominent sericultural areas of India for hastening larval maturation events and synchronizing cocoon spinning activities as an obvious advantage is assured. At times, the present recommendation of administering PE at the onset of spinning results in peak labour requirement at odd hrs. To enable making recommendation for the use of PE on multi × bivoltine silkworm hybrids based on the climatic conditions prevailing in different areas especially with regard to temperature, the experiment was taken up to determine proper treatment times so that the induced spinning will be more orderly and the labour can be leveraged more efficiently. Different brackets of low (18 – 22°C), medium (24 – 28°C) and high (29 – 32°C) temperature were simulated during the latter half of V larval instar and cocoon spinning. PE was administered to multi × bivoltine silkworm (BL67 × CSR101) hybrid batches as per the recommended dose at three different times *viz.*, 10 am, 4 pm and 10 pm. Three replicates of 100 larvae were maintained for each treatment. Absolute controls were also maintained in each temperature range to compare the results. Cumulative maturation percentage was recorded at 6 hrs interval to ascertain peak mounting span. The influence of the treatment on the cocoon traits also was studied. Based on the peak mounting span, it was evident that in low temperature 10 pm treatment would be better. In medium and high temperature, treatment at 4 pm proved to be a better option. The influence of the treatment times at different temperature range on labour management is discussed.

Key words: *Bombyx mori*, Hastened maturation, Phytoecdysteroid, Silkworm, Synchronized spinning

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Introduction

Use of phytoecdysteroid (PE) available in the brand name *Sampoorna* has become a routine practice among sericulturists. PE is administered to silkworm towards the commencement of maturation process at the end of the 5th larval instar so that all the larvae mature almost simultaneously and the spinning process is synchronized enabling the farmers to market the cocoon in a single lot, early compared to the general practice. In the usual practice, the product with PE as active principle is administered to silkworm *per os* at the onset of cocoon spinning and almost all the larvae in the population mature within 18 – 24 hrs. This helps the farmers in mounting the larvae together, cutting short the labour requirement and mulberry leaf remarkably (Nair *et al.*, 2002; Trivedy *et al.*, 2003). It was foreseen that in the Indian sericulture scenario the use of PE would prove useful and the reports of Chow and Lu (1980) gave the right fillip to initiate serious research. Earlier research reports (Shivakumar *et al.*, 1995, 1996) gave enough indication that the phytoextracts with ecdysteroid indeed would be useful in silkworm rearing management. But until a method for quantification of the ecdysteroid was introduced, the results were not consistent (Nair *et al.*, 2002). The studies made it clear that silkworms regardless of the hybrid combination respond to the treatment (Trivedy *et al.*, 2003) although there is a difference in the intensity of response between and bivoltine and multivoltine hybrids. Once the PE products became popular in the field, it was understood that the effect var-

ies as per the areas of use. This variation is mainly because of the temperature prevailing in different areas. It was also understood that the recommended time of administration does not go well always for labour management. Hence the present study was taken up to measure and compare the response of multi × bivoltine silkworm hybrid to PE under different rearing temperature and to come out with different treatment times for better labour management.

Materials and Methods

Silkworm rearing

Disease free eggs of multi × bivoltine silkworm hybrid, BL67 × CSR101 were used in this study. Silkworms were reared under standard recommended conditions at 25 ± 1°C temperature, 75 ± 5% relative humidity under 12:12 (light: dark) photoperiod. Mulberry leaves of Victory-1 genotype were fed to silkworms three times a day.

Maintenance of temperature and administration of ecdysteroid

After rearing the 5th instar silkworm for 4 days, the larvae were shifted to rearing enclosures specially arranged for different temperature brackets. Three different temperature ranges namely, low (18 – 22°C), medium (24 – 28°C) and high (29 – 32°C) were set up for maintaining the treated larvae. Once a few larvae showed the symptom of maturation, the larvae were fed with mulberry leaves treated with phytoecdysteroid 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 2.5 µg. The treatment was done to different such batches at different times of the day *viz.*, 10 am, 4 pm and 10 pm. For each treatment three replications of 100 larvae were maintained in ventilated plastic trays measuring 56 × 36 cm. Controls were maintained in parallel in the three temperature brackets to compare the results.

Data collection

The time taken for the completion of maturation/mounting from the time of treatment was recorded for the each batch. For this, the larvae were examined every 6 hrs after the treatment, the ripe larvae were picked, counted and transferred to cocoon mounting frames. Progressive maturation percentage was calculated and plotted in line graph. After cocooning, cocoon yield, survival, cocoon weight, cocoon shell weight and shell percentage were recorded/worked out. The data were subjected to statistical analysis employing ANOVA to ascertain the significance of the result.

Results

The results with respect to the response of multi × bivoltine silkworm maintained at different brackets of temperature, to the administration of PE in terms of uniform larval maturation and peak mounting span when done at different times are presented below.

Low Temperature (18 – 22°C)

Although the maturation process initiated in the first 6 hrs of the treatment, the percentage was negligible in the larvae treated at 10 am and 4 pm (Fig. 1a). The total time period required for completing the mounting process though varied according to the time of treatment, it was not prominent. There was a moderate surge in the maturation percentage from about 38% to 67% between 24 and 30 hrs when treated at 10 am and it reached 80% by 36 hrs. Such a surge was also seen from 30 to 70% in 4 pm treated larvae, and from 53 to 99% in the 10 pm treated larvae. In the control silkworm, there was no sudden surge in the percentage of larvae matured, it gradually increased over the time, and the mounting was completed in 54 hrs. The control, in effect took 24 hrs more than the 10 pm treated larvae, 18 hrs more than the 4 pm treated larvae and 12 hrs more than the 10 am treated larvae. The time taken for the absolute maturation did not differ much because it was only 6 hrs between the treated and control in the case of 10 am and 4 pm treated batches and 12 hrs in the case of 10 pm treated and the control. But to reach about 80% maturation or close to that mark, the difference ranges from 12 to 18 hrs.

Medium temperature (24 – 28°C)

The silkworms treated and maintained at the medium temperature (Fig. 1b) showed quite interesting results and it was largely dependent on the time of treatment. In the case of the larvae treated at 10 am, the maturation percentage increased gradually and steadily and completed within 30 hrs of the treatment. But in the case of larvae treated at 4 pm, there was a sudden surge from 29 to 79% between 12 and 18 hrs and the mounting was completed by 24 hrs. A similar surge was also seen from 30 to 70% in the larvae treated at 4 pm but between 6 hrs and 12 hrs. Both 4 pm and 10 pm treated larvae completed maturation process by 24 hrs. The control took 36 hrs for completion without any prominent surge at any time interval between two larval picking.

High temperature (29 – 32°C)

The multi × bivoltine silkworm maintained at high temperature showed varied response to phytoecdysteroid treatment depending on the time of treatment (Fig. 1c).

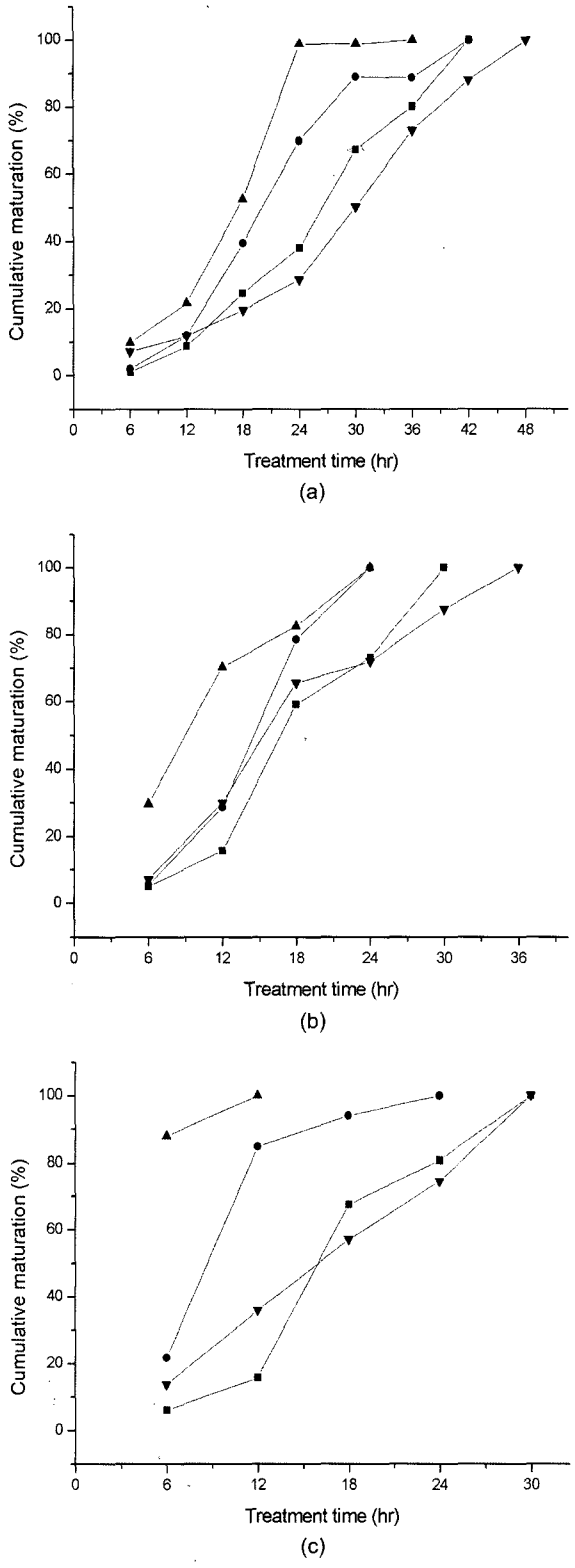

Fig. 1. Effect of phytoecdysteroid on maturation and of silkworm (Hybrid: BL67 × CSR101) at (a) low (18 – 22°C), (b) medium (24 – 28°C), and (c) high (29 – 32°C) temperature ranges at different times of treatments: (■): 10.00 am, (●): 4.00 pm, (▲): 10.00 pm, (▼) control.

Influence on crop performance

The larvae treated at 10 am took 30 hrs for complete maturation with a steep surge between 12 and 18 hrs from 16 to 68%. More than 80% maturation was obtained in 24 hrs. In the larvae treated at 4 pm, the rise was huge from 22 to 85% between 6 to 12 hrs. In the 10 pm treated batch, about 88% larvae matured within the first 6 hrs and the remaining in the next 6 hrs. In control, it took 30 hrs for complete maturation without any major surge in between.

Influence on crop performance

The PE treatment did not have any significant influence on the yield parameters of multi × bivoltine silkworm hybrid when maintained at low temperature regardless of the time of the treatment (Table 1). The cocoon yield by number and weight increased marginally in the treated batches in almost all the cases but statistically not significant.

When the silkworm larvae were maintained at low temperature treatment with PE affected the cocoon traits such as cocoon weight, cocoon shell weight and shell percentage compared to the control maintained at the same temperature. The cocoon weight was affected significantly when treated at 10 am and it was to the tune of 5% (Table 1). A similar statistically significant reduction of about 3.5% was also seen in the shell weight in the case of treatment at 10 pm. The shell percentage was significantly affected as well in the case of treatment at 10 pm. Although the treatment generally exerted a declining effect on the cocoon characters in the other treatments as well, the reduction was not statistically significant.

As seen in the case of low temperature, the yield by number was not negatively affected by the treatment at medium temperature (Table 2). In fact, there was a slight but statistically non-significant increase in the yield by number. Such was the case with yield by weight also. Although, there was a slight reduction in the cocoon traits on PE treatment, it was negligible and statistically non-significant.

In the case of treatment at high temperature, the increase in cocoon yield by number is quite consistent regardless of the time of the treatment. But these improvements did not turn out to be statistically significant (Table 3). Although, cocoon weight, cocoon shell weight and shell percentage got affected by the treatment compared to the control regardless of the treatment time the statistical significance was obvious was only with the shell percentage of the batch treated at 10 pm.

Discussion

The progressive and informed sericulturists take extreme

Table 1. Effect of administration of phytoecdysteroid on the crop performance of bivoltine silkworm hybrid when maintained at low temperature (18 – 22°C)

Treatment time	Yield by number	Yield by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage
10 am	9530 (1.34)	16.98 (1.85)	1.823* (-4.94)	0.382* (-2.05)	20.95 (-1.40)
4 pm	9471 (0.71)	17.00 (1.98)	1.869 (-1.16)	0.384 (-1.54)	20.54 (-0.58)
10 pm	9556 (1.61)	16.85 (1.07)	1.872 (-1.00)	0.376* (-3.59)	20.08* (-2.80)
Control	9404	16.67	1.891	0.390	20.66
SE ±	125.75	0.370	0.020	0.010	0.190
CD at 5%	NS	NS	0.065	0.013	0.550

Values in parentheses are percentage difference from the control.
*Significant at $P < 0.05$
NS – Non-significant

Table 2. Effect of administration of phytoecdysteroid on the crop performance of bivoltine silkworm hybrid when maintained at medium temperature (24 – 28°C)

Treatment time	Yield by number	Yield by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage
10 am	9745 (0.73)	17.22 (2.50)	1.47 (-1.44)	0.382 (-1.54)	20.68 (-0.09)
4 pm	9758 (0.86)	16.93 (0.77)	1.867 (-0.37)	0.386 (-0.52)	20.67 (-0.14)
10 pm	9790 (1.19)	16.88 (0.47)	1.862 (-0.64)	0.380 (-2.06)	20.40 (-1.44)
Control	9674	16.80	1.874	0.388	20.70
SE ±	95.92	0.180	0.020	0.010	0.230
CD at 5%	NS	NS	NS	NS	NS

Values in parentheses are percentage difference from the control.
*Significant at $P < 0.05$
NS – Non-significant

Table 3. Effect of administration of phytoecdysteroid on the crop performance of bivoltine silkworm hybrid when maintained at high temperature (29 – 32°C)

Treatment time	Yield by number	Yield by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage
10 am	9717 (7.49)	16.93 (2.08)	1.779 (-1.07)	0.353 (-1.67)	19.84 (-1.04)
4 pm	9756 (7.93)	16.85 (1.60)	1.767 (-1.70)	0.351 (-2.22)	19.86 (-0.94)
10 pm	9638 (6.62)	17.23 (3.85)	1.809 (0.62)	0.348 (-3.06)	19.23* (-4.09)
Control	9040	16.59	1.798	0.359	20.05
SE ±	149.34	0.280	0.020	0.010	0.200
CD at 5%	NS	NS	NS	NS	0.570

Values in parentheses are percentage difference from the control.
*Significant at $P < 0.05$
NS – Non-significant

care in handling silkworms until cocooning and more so in the last 6 – 7 days of larval period. They adopt almost all new technologies which largely contribute to the sustained higher cocoon productivity and handsome returns. Adoption of proper rearing methods, timely harvesting and marketing have their own significance in ensuring better quality of the crop and thereby better returns to the farmers. The product in the brand name *Sampoorna* containing phytogenous ecdysteroid as active principle is being widely used nowadays for hastening larval maturation events and synchronized cocoon spinning. The use of these products as per the recommendation ensures maturation of the larvae in a relatively shorter span compared to the control enabling the farmers to mount the ripe larvae in about 18 – 24 hrs. This not only saves labour but quite a good amount of mulberry leaf. But the present

blanket recommendation indicates that the products be administrated in the recommended dose when about 5% of the larvae in a population are ripe (Nair *et al.*, 2002; 2005; Trivedy *et al.*, 2003a, b). It was revealed that the intensity of the response of silkworm to PE is not same in all the areas where the product is being used and variation is mainly because of the temperature prevailing in the area.

Since the product has become almost ubiquitous among the seri-farmers, it has become the responsibility of the scientists to decipher the varied response of silkworm at varied temperature range. This was examined in the light of labour management problems risen once the whole lot of silkworm matured simultaneously in the odd hrs when the farmers followed the initial recommendation. Such problems were not foreseen initially and naturally when they cropped up, it warranted a serious and prompt address.

Scientifically, the larvae which are not prepared to go for forming the cocoon, receives an additional amount of ecdysteroid from outside and thus those larvae also reach the pupation inducing peak earlier than usual (Nair *et al.*, 2005; Trivedy *et al.*, 2004). It is evident in practical sericulture that a higher temperature induces the silkworm to develop faster, mature early and spin cocoon quickly. The opposite happens when temperature falls below the normal range. But to develop a package of recommendation it is imperative to determine how silkworm behaved in each temperature range. There is no much support in the form of earlier works in this line as this is almost a new area of work.

The method adopted in this study to determine the cumulative maturation percentage at 6 hrs intervals as done in our earlier studies (Nair *et al.*, 2002; Trivedy *et al.*, 2003 a, b) was suitable to reflect the effect of different temperature on the maturation activities. The main idea behind administering the PE at different hrs was to find out which treatment time would be the most suitable one to the farmers to manage his labour force effectively for picking up the larvae when they are ready for mounting.

In the case of low temperature, although all the treatments made at different times had better impact on maturation compared to the control, the treatment at 4 pm had a clear advantage. As the control clearly lacked any peak maturation, the good old method of picking up the larvae as when they matured, continues. But treating the larvae at 10 in the morning may not be a good idea because about 13% of the larvae would need to be mounted the next night as seen in Fig. 1. Of course, about 29% must have been ready during the day time by 4 pm which would be a rather difficult time for the workers to get involved. The treatment at 10 pm also has a similar problem. About 31% of larvae would be ready for mounting the next day 4 pm and another 46% by the same night. It would be really difficult for the farmers to manage such a situation. Instead of gaining an advantage by the usage of the product, they would be disappointed. But, 4 pm would be comparatively a better time for treatment at low temperature. About 43% of the larvae will be ready for mounting by the next day 4 pm. By this time, 29% would have already been mounted. In such condition, the remaining 30% also could be mounted because by night 10 pm another 19% could be ready. Although by no means this could be considered the best option, among the three treatment times, 4 pm could be comparatively better.

If the multi × bivoltine silkworm hybrids are reared at a medium or normal temperature, it is clear that there is an advantage of about 12 hrs by treating the larvae with PE at night time. 10 am may not be the suitable time to advocate the treatment because about 43% of the larvae will be ready for mounting the next day 6 am. But if treated at 4 pm, a moderate amount of 23% is to be picked at 6 am next day but the whole lot could be mounted by 4 pm because by noon about 50% of the total larvae and 4 pm the remaining 21% will be ready. If treated at 10 pm, about 30% will be ready for mounting at 6 am next day, about 40% by 12 noon and about 12% by 4 pm. Although the remaining 18% could also be mounted at 4 pm, they will not be fully ripe. In such situation, the 4 pm treatment would be the best.

In the higher temperature, maximum advantage of PE treatment will be when treated at either 4 pm or 10 pm. By treating the silkworms at 4 pm, almost 85% will be over by 12 noon next day. If required, the remaining 15% also could be mounted at that time. Otherwise, by 4 pm the process could be over. In the case of treatment at 10 pm, quite a huge percentage will be ready for mounting at the early morning. Many farmers may like to avoid such early morning mayhem especially when the treatment at 4 pm assures a fairly comfortable situation. Much difference cannot be seen between the treatment at 10 am and the control.

Influence of the treatment on the cocoon characters

In silkworm, cocoon quality is largely dependent on the maturation process of larvae and its timely mounting. Delayed or early mounting could affect the quality of the cocoon to certain extent. When the larvae take longer time to mature, the feeding period gets extended and the quantity of leaf consumed will be more resulting in bigger and heavier cocoons. On the contrary, if feeding period is shortened, by any physiological manipulation, the leaf consumption will be reduced resulting in comparatively smaller and lighter cocoons. PE plays a crucial role here,

especially when applied at the onset of spinning. As the larvae have already consumed the optimum amount of leaf, the cocoon quality is not affected although maturation process is hastened and spinning activities are synchronized. In the present experiment, majority of the treatments did not affect the cocoon characters significantly as the primary objective of the treatments was to synchronize cocoon spinning and not to advance it as reported in the earlier works (Nair *et al.*, 2005; Trivedy *et al.*, 2003 b). Probably, the only exception was the treatment at 10 am at low temperature which resulted in a significant negative impact. This may be because in the low temperature, 10 am was too early for the treatment.

It can be concluded that temperature played a vital role in hastening the larval maturation events and synchronizing the cocoon spinning when PE was administered. Based on the peak mounting span it has become evident from the study that in low temperature 10 pm treatment would be better. In medium and higher temperature, treatment at 4 pm could be better for effective management of labour force.

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