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Influence of Different Treatment Regimes of Phytoecdysteroid on Silkworm, *Bombyx mori* L.

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The response of silkworm, *Bombyx mori* L. to phytoecdysteroid (PE) when administered at different ages of 5th instar was studied in the popular bivoltine (CSR2 × CSR4) and multi × bivoltine (PM × CSR2) silkworm hybrids, reared on the Victory-1 variety of mulberry leaves. PE was administered to 5th instar silkworm *per os at* a rate of 250 μg per 100 larvae to different batches of silkworm at 0, 24, 48, 72, 96, 120, 144 hrs and at the onset of cocoon spinning when a few larvae were ripe. The larval and mounting duration, cocoon yield and cocoon characters were influenced by PE. The intensity of influence was dependent on the time of application. The larvae treated at the beginning of the instar, improved the economic traits significantly with a marginal increase in larval duration. In the larvae treated at the middle of the instar, larval duration was shortened remarkably but the economic traits were adversely affected. This particular treatment can become a good management strategy in the case of mulberry leaf shortage or disease incidence. In the larvae treated at the onset of cocoon spinning, the mounting duration was substantially reduced without much effect on the cocoon traits which would be a big benefit in commercial sericulture. The physiological significance of varied response of silkworm to PE administration is discussed.

Key words: *Bombyx mori*, hastened maturation, phytoecdysteroid, silkworm, synchronized spinning

Introduction

One of the most cumbersome activities of the silkworm rearing is during the last phase of 5th larval instar which involves timely picking of ripe larvae and leaving them on the cocoon forming frames. If the ripe larvae are not picked up at the right time, silk waste will be remarkable. Usually, all the larvae in a population do not mature simultaneously and it takes 2-3 days for completion. This non-uniformity in maturation forces the farmers to employ additional skilled laborers to examine the larvae at definite intervals and to pick up the ripe ones. These problems lead to difficulties in mounting, management of labourers, harvesting of cocoons and their marketing.

This could be solved to a great extent by administering phytoecdysteroid (PE) which contains active insect moulting hormone to silkworm (Chow and Lu, 1980). The PE when applied at the onset of spinning hastens the maturation process and synchronizes cocoon spinning enabling the farmers to mount almost all the larvae in a period of 12 to 18 hrs and thus saving substantial amount of leaves and labour (Nair *et al.*, 2002; Trivedy *et al.*, 2003). In many parts of India, the use of PE has become quite ubiquitous. Our recent studies outlined time-bound application protocol of PE depending on the prevailing temperature ranges in different areas of silkworm rearing in India (Nirmal Kumar *et al.*, 2005, 2006).

It is known that apart from shortening the mounting duration to a considerable 12~18 hrs, the product can also be used to save the crop from an imminent loss. PE applied in the latter half of 5th instar shortens the larval duration considerably (Nair *et al.*, 2005). This enables the farmers to tide over the difficult situations of unforeseen shortage of mulberry leaves and crop loss due to sudden outbreak of diseases and save the crops to certain extent. This of course may adversely affect the cocoon quality. It has also been reported that PE applied early in the 5th instar can induce enhanced cocoon production (Chow and Lu, 1980). But a systematic study on the impact of PE administration on different days from first day of 5th instar has not been taken up in India yet.

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The present study is aimed at determining the impact of PE on larval and mounting duration when it is administered in different time period in 5th instar starting from resumption and finding out what could be its impact on cocoon yield and traits.

Materials and Methods

Silkworm rearing

Disease free eggs of popular bivoltine × bivoltine silkworm hybrid, CSR2 × CSR4 and multivoltine × bivoltine hybrid, PM × CSR2 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 variety were fed to silkworms three times a day.

Experimental design and PE administration

Before resumption to 5th instar, 100 larvae each were counted and kept in ventilated plastic trays measuring 56 cm × 36 cm for various treatments. Different batches were treated at 0, 24, 48, 72, 96, 120 and 144 hrs of 5th instar. Phyto-extract containing 250 µg of ecdysteroid was then mixed in 10 ml of water and sprayed on 100 g fresh mulberry leaves and fed to 100 larvae in a tray so that each larva would get 2.5 µg of PE. Apart from this, when a few larvae were about to spin, one more dose was administered to synchronize the maturation. Absolute control was maintained in parallel to compare the results. The treatment and control were replicated thrice. A total of 24 trays for each hybrid covered the experiment. The weight of 10 larvae was recorded on reaching maximum growth.

Mounting of ripe larvae and cocoon assessment

Fully ripe larvae were picked up and mounted in separate trays maintained in parallel at an interval of 6 hrs from the time of treatment. The time taken to complete the mounting process in each treatment was calculated. The larval duration in the 5th instar from the time of resumption till completion of mounting also was recorded. Cocoons were harvested on 6th day from completion of mounting. The cocoons were sorted out from each replication and the number recorded. After recording the total weight, cocoons were cut open and the weight of cocoon and cocoon shell of 10 males and 10 females were recorded. Finally, traits such as survival, ERR by number and weight and shell percentage were calculated from the observations recorded.

The experiment was tried twice, once in September-October and once in January-February. The data were compiled and subjected to statistical analysis employing ANOVA to find out the significance in the difference found among the treatments and between the treatments and the control.

Results

PE is usually administered to silkworm to induce hastened larval maturation and synchronized cocoon spinning. In this work, PE was administered in two doses one on the designated day in the 5th instar and the other one at the onset of spinning. The results of these treatments on various economic traits are presented in Tables 1 and 2.

Effect of PE treatment on economic traits

Larval and mounting duration

In both the hybrids, larval duration was influenced by the treatment depending on the time of application. In the bivoltine hybrid, the control larvae took 178 hrs (7 days 10 hrs) to complete the fifth instar duration. Treatment at 0 hr took 168 hrs (7 days) which was 10 hrs less than that of the control. But 24 hrs treatment batch took 174 hrs (7 days 6 hrs) which was still less than that of the control by 4 hrs. Treatment at 48, 120 and 144 hrs had completed the fifth larval stage in 162-164 hrs which was 14-16 hrs less than that of the control. The treatments at 72 and 96 hrs responded quite significantly to the treatment and the larvae could be mounted for cocoon formation within 140 hrs (5 days 20 hrs).

In PM × CSR2 also the trend was almost similar. In this case, the control took 180 hrs to complete the fifth instar. Here, the silkworms treated at 0, 120 and 144 hrs had completed the 5th instar in 164 hrs. The 24 hrs treatment took 168 hrs to complete the 5th instar while 48 hrs treatment took only 160 hrs. But 72 and 96 hrs treatment commenced cocoon spinning after 144 hrs (Table 2).

The difference in the mounting duration among the treatment and that between the treatment and the control was not as prominent as in the case of larval duration. In bivoltine hybrid, the control took 33 hrs for completion of mounting. At the same time, in the case of the larvae treated with PE at 72, 96 and 120 hrs and at the onset of spinning, and the larvae treated at 144 hrs (only one treatment could be given) mounting could be completed within 18 hrs establishing a difference of 15 hrs in the mounting duration. Those treated at 0, 24 and 48 hrs and again at the onset of spinning, could be mounted within 20-23 hrs. Here the difference shrunk to 10-13 hrs compared to control (Table 1). In the multi × bivoltine hybrid the control took 32 hrs for completion of mounting. But when the larvae were treated at 144 hrs, a few larvae were almost ripe and the remaining larvae took only 12 hrs and the whole larvae could be

Table 1. Effect of administration of PE on larval and mounting duration and crop performance of bivoltine silkworm hybrid, CSR2 × CSR4

Treatment time in V instar	V instar duration (h)	Mounting duration (h)	Larval weight (g)	Survival (%)	ERR by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage
0 h	168*	20*	43.68	82.69*	19.12	1.971*	0.432*	21.89
	(−10)	(−13)	(1.37)	(−4.34)	(7.53)	(7.70)	(8.82)	(0.97)
24 h	174	21*	40.39	83.95	17.88	1.845	0.399	21.64
	(−4)	(−12)	(−6.27)	(−2.88)	(0.56)	(0.82)	(0.50)	(−0.18)
48 h	162*	23*	39.64*	87.07	14.21*	1.614*	0.348*	21.59
	(−16)	(10)	(−8.00)	(0.73)	(−20.08)	(−11.80)	(−12.34)	(−0.42)
72 h	140*	18*	36.34*	94.38*	15.63	1.591*	0.329*	20.67*
	(−38)	(−15)	(−15.66)	(9.19)	(−12.09)	(−13.06)	(−17.13)	(−4.66)
96 h	140*	18*	35.55*	87.93	15.65	1.668*	0.351*	21.07
	(−38)	(−15)	(−17.49)	(1.73)	(−11.98)	(−8.85)	(−11.59)	(−2.81)
120 h	164*	16*	40.49	88.56	18.63	1.847	0.381	20.62*
	(−14)	(−17)	(−6.03)	(2.45)	(4.78)	(0.92)	(−4.03)	(−4.89)
144 h	164*	18*	42.49	90.86*	18.99	1.867	0.396	21.21
	(−14)	(15)	(−1.39)	(5.11)	(6.80)	(2.02)	(−0.25)	(−2.17)
Control	178	33	43.09	86.44	17.78	1.830	0.397	21.68
SE ±	1.59	1.30	0.96	1.19	0.79	0.020	0.010	0.021
CD at 5%	4.80	3.90	2.93	3.60	2.40	0.058	0.018	0.640

Values in parentheses are percentage difference from the control except in the case of larval and mounting period which are actual difference.
*Significant at $P<0.05$

mounted within 12 hrs. There was a difference of 20 hrs between the control and the treated which was significant. All other batches which were given two treatments, took 16~19 hrs for completion of mounting with a difference of 13~16 hrs compared to the control (Table 2).

Larval weight and survival

The results showed that larval weight was greatly influenced by the administration of PE depending on the time of application. In the bivoltine hybrid, there was no much difference in the larval weight between the control and the larvae treated at 0 h of 5th instar. But this gradually declined as the days of treatment progressed in the 5th instar and bottomed out in the case of 96 hrs treatment. But there was a clear increase in the case of 120 and 144 hrs treated silkworm compared to 72 and 96 hrs treatment although they were still less than that of the control (Table 1). Similar was the case with the multi × bivoltine hybrid, PM × CSR2. The larval weight of the control and that treated at 0 hr was almost same and the one which was treated at 144 hrs had marginal difference. All other treatments had significantly low larval weight compared to the control, the lowest being 72 and 96 hrs treated batches. These two batches had about 16% decline in the larval weight compared to the control. The batches treated at 24, 48 and 120 hrs showed a decline ranging from 8 to 9% (Table 2).

Survival is an important parameter in silkworm rearing as it has a direct impact on the yield level. In this experiment the larvae responded quite interestingly to the treatment in terms of survival. In the bivoltine hybrid, the control registered a survival of 86%. The batch treated almost after the completion of the feeding period *i.e.*, at 144 hrs showed the survival, a little above the control with almost 91%. But the treatment made at 72 hrs and then at the onset of spinning had a telling effect on the survival with 94%, which was 8% more than that of the control. The treatment made at 48, 96 and 120 hrs did not show any significant difference in the survival compared to the control but that treated at 0 and 24 hrs showed a clear decline with 83 and 84%, respectively (Table 1).

In the multi × bivoltine hybrid, the variation in the survival due to the treatment was almost nil. Almost all treatments except the one treated at 0 hr showed the survival a shade above the control which showed 89%. The 0 hr treated batch had a slightly low survival (88%). These minor differences from the control were of no statistical significance.

Cocoon yield

The cocoon yield was calculated as the effective rate of rearing per 10000 larvae. The influence of PE on the sur-

Table 2. Effect of administration of PE on larval and mounting duration and crop performance of multi × bivoltine silkworm hybrid, PM × CSR2

Treatment time in V instar	V instar duration (h)	Mounting duration (h)	Larval weight (g)	Survival (%)	ERR by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell percen tage
0 h	164*	18*	39.19	87.68	18.46*	1.978*	0.370*	18.71
	(−16)	(−14)	(−1.18)	(−2.58)	(10.07)	(8.68)	(9.47)	(0.81)
24 h	168*	19*	36.55	89.02	16.93	1.932*	0.366	18.92
	(−12)	(−13)	(−7.84)	(−1.09)	(0.95)	(6.15)	(8.28)	(1.94)
48 h	160*	19*	35.66*	90.72	15.98	1.837	0.337	18.32
	(−20)	(13)	(−10.08)	(0.78)	(−4.71)	(0.93)	(−0.29)	(−1.29)
72 h	144*	16*	34.06*	89.08	14.90*	1.530*	0.275*	17.97
	(−36)	(−16)	(−14.12)	(0.36)	(−11.15)	(−15.93)	(−18.63)	(−3.17)
96 h	144*	18*	34.04*	89.83	15.30*	1.592*	0.286*	17.96
	(−36)	(−14)	(−14.17)	(−0.19)	(−8.76)	(−12.52)	(−15.38)	(−3.23)
120 h	164*	16*	36.01*	89.80	16.77	1.716*	0.314	18.28
	(−16)	(−16)	(−9.20)	(1.17)	(0.00)	(−5.71)	(−7.10)	(−1.51)
144 h	164*	12*	38.47	90.01	17.90	1.815	0.337	18.59
	(−16)	(20)	(−3.00)	(1.41)	(6.74)	(−0.27)	(−0.29)	(0.16)
Control	180	32	39.66	88.76	16.77	1.820	0.338	18.56
SE±	1.46	0.64	1.09	2.99	0.43	0.030	0.010	0.42
CD at 5%	4.40	1.90	3.29	NS	1.30	0.082	0.029	NS

Values in parentheses are percentage difference from the control except in the case of larval and mounting period which are actual difference.
*Significant at $P<0.05$
NS - Non-significant

vival and larval duration reflected on this parameter as well to a certain extent. In the bivoltine hybrid maximum yield was obtained in the batch treated at 0 hr. This was almost 8% more when compared to the control. The batches treated at 120 hrs and 144 hrs showed a significantly better ERR with 5 and 7% increases over the control. The treatment made at 24 hrs did not have any significant effect on the ERR but that made at 48, 72 and 96 hrs had an adverse effect. A significant decline of 20 and 12% was noticed in the case of these treatments (Table 1).

Although similar effect was noticed in the case of PM × CSR2 hybrid as well, the intensity of change was relatively low at least in the case of decline. The larvae treated at 0 hr showed a significant improvement of 10%. Treatments made at 144 hrs showed a relatively better yield with an increase of 6.7% but at the same time the treatment at 24 hrs showed a marginal increase of 1.6%. Treatments made at 48, 72 and 96 hrs induced a clear negative effect on the survival with 4% in the case of 48 hrs, 11% in the case of 72 hrs and almost 9% in the case of 96 hrs.

Cocoon traits

Cocoon traits, which constitute cocoon and cocoon shell weight and shell percentage are important traits to evaluate the effect of any physiological treatment. In the case of bivoltine hybrid, in the control the cocoon weight was 1.830 g. Compared to this the larvae treated at 0 hr showed an improvement of 7. 7% (1.971 g). The treatments made at 24, 120 and 144 hrs showed marginal increase at 1-2%. At the same time, the treatments at 48, 72 and 96 hrs have registered considerable decline in the cocoon weight. They were down by 12, 13 and 9%, respectively compared to the control.

In the multi × bivoltine hybrid, the treatment at 0 and 24 hrs improved the cocoon weight by about 9 and 5% respective compared to the control. 48 and 144 hrs treatment did not induce any change in the cocoon weight while those treated at 72, 96 and 120 hrs showed significantly negative change. The 72 hrs treatment reduced the cocoon weight by 16%, 96 hrs treatment by about 13% and 120 hrs treatment by about 6%.

The changes found in cocoon shell weight in response to the PE administration at various hours of 5th instar was almost corresponding to that found in the cocoon weight. The shell weight in the bivoltine hybrid was 0.397 g in the case of control. As against this the treatment made at 0 hr of 5th instar improved the shell weight by about 9% (0.432 g). The treatments made at 24 and 144 hrs did not show any change in the shell weight. But all other treat-

ments significantly reduced the shell weight, the maximum being 72 hrs treatment with about 17% reduction. Treatments at 48, 96 and 120 hrs reduced the cocoon weight by 12, 12 and 6.6%, respectively.

The picture was not altogether different in the case of multi × bivoltine hybrid. In this hybrid, the treatments at 0 and 24 hrs improved the shell weight by 9.46 and 8.28%, respectively. On the contrary, the 72 and 96 hrs treatments had a significant negative influence on the shell weight, reducing it by about 19 and 15% respectively. The reduction in shell weight in the 120 hrs treatment was 7%.

The shell percentage changed corresponding to the intensity of changes in the cocoon and shell weight. In the bivoltine hybrid, the shell percentage of 0 hr treatment was better compared to the control, whereas in 24 and 48 hrs treatments the shell percentage was almost at par with that of the control. Shell percentage of 96 and 144 hrs treatment was slightly on the lower side but the treatment at 120 hrs was down by more than a percent and half.

The shell percentage of the multi × bivoltine hybrid is as such much less than that of the bivoltine hybrid and the same varied prominently according to the PE administration albeit in line with the changes in the cocoon weight and cocoon shell weight. Here again the treatment at 0 and 24 hrs showed a slightly better shell percentage compared to control. 48, 120 and 144 hrs treatment did not show much variation in the shell percentage. But the treatment at 72 and 96 hrs had a marginal negative impact on shell percentage reducing it by less than a percent.

Discussion

In the commercial silkworm rearing, the physiological processes happening during every stage have got direct or indirect relation with the crop performance. The fifth instar in silkworm could be considered as a physiological open field because crop quality and quantity can be tailor-made by some treatments during this period. This includes exogenous administration of insect hormone mimics which may either prolong the larval period or shorten it. A prolonged period would make the larvae consume more and produce heavier and bigger cocoons and the shortened period would naturally result in smaller and lighter cocoons. There are definite advantages in both the cases and selection of the technique is need-based. Non-uniform silkworm maturation and staggered cocoon spinning is another serious problem the farmers face. Induced synchronization in larval maturity followed by uniform cocoon spinning is now a popular technique at farmers' level silkworm rearing by using the formulation of phytoextracts containing ecdysteroid as active principle.

Silkworms are rather sensitive to exogenous dose of ecdysterone administered at different days of 5th instar (Sehnal and Akai, 1990). Dai *et al.* (1985) indicated that ecdysone plays a significant role in nucleic acid metabolism and the related protein synthesis in silkworm. Although it induces growth and silk production depending on the age of administration, the intensity of manifestation is different (Nair *et al.*, 2002). "If administered when a few larvae are ready for spinning, the manifestation in the silkworm development is in the form of hastened maturation and synchronized cocoon spinning." The results of the present study make it amply clear that PE administered on different days of 5th instar induces varied responses in silkworm.

When Chou and Lu (1980) isolated ecdysteroids from many plants and administered to silkworm *per os* through mulberry leaves, the results varied according to the time of treatment in the 5th instar. It indicated that when the compound was administered at the onset of spinning, the mounting process could be completed within 12 hrs and when administered in the early 5th instar, the silk out put was more. These results are of highest relevance to Indian sericulture and a lot of work has already been carried out to find out ways and means to hasten/synchronize maturation process mainly with an intention to shorten the mounting period.

An equally important fall out of this great idea could be shortening the larval duration as such in the fifth instar with manifold benefits. Although not very common, crop failures due to sudden outbreak of some diseases or unforeseen shortage of mulberry leaves during the last phase of rearing is not uncommon. This technology could come really handy as the reduction in the period can range from one to one and a half day if applied at a certain time of the fifth instar. At the same time the compound at the same dose at the early days could act as juvenile hormone and increase the cocoon weight and the silk yield notably.

PE as a yield enhancer

It is quite interesting to note that how a biological system like silkworm respond differently to a single compound when applied at different days of 5th instar. The response of silkworm to the administration can easily be demarcated based on the time application. Application on all the early days of 5th instar did not induce the same type or intensity of response. When applied on the first day or day 0, the compound literally acted like a JH mimic. This treatment induced a slightly prolonged feeding period and in turn got converted into higher cocoon weight and shell weight in both the hybrids. This gives a primary indication that the same compound can even be used for purpose of yield improvement by managing the time of applica-

tion. The second treatment given at the onset of spinning however did not have much difference in its response from that of the other treatments. The second treatment was primarily given to synchronize the maturation and spinning and is largely time dependent. The result makes it clear that regardless of the larval feeding period, mounting duration can be shortened to the beneficial period by the second treatment at the onset of spinning. The appreciable point is increase in the yield level and cocoon characters without a serious effect on the survival.

The treatment at 24 hrs also had a similar impact but the intensity was less compared to that of the treatment at 0 hr with regard to prolongation in the larval period and improvement in other economic traits. Although the earlier contention was that the treatment given before 72 or 48 hrs of fifth instar would be of similar effect, the present study makes it rather clear that even the treatment given during different days of the former half of the 5th instar could be of different intensity. The exogenous PE affected most of the economic traits like larval weight, cocoon weight, survival and yield level apart from the cocoon traits such as average cocoon and shell weight and shell percentage.

PE as a crop saver

The effect of the administration from 72 hrs onwards was most remarkable. Those done at 72, 96 and 120 hrs had a clear impact on the larval duration and obviously on other related characters such as larval weight and cocoon traits. Quite interestingly, survival was maximum in these batches, 72 and 96 hrs treated larvae being the best. This is understandable because, the larval duration and the survival are inversely related.

The most important effects in these treatments are reduced larval duration. The reduction was almost one and a half day in both the hybrids. This means a quantum reduction in the leaf consumption. The reduction in the duration and the resultant less consumption of mulberry leaves clearly reflected in the yield and cocoon traits. The farmer would opt for such a reduced yield only to a much bigger crop loss.

PE for synchronized cocoon spinning

PE is most commonly used to hasten the maturation and to induce simultaneous cocoon spinning. The skilled labour requirement for picking up ripe larvae is reduced remarkably by this technology. Once PE is administered after 2-3% larvae are ripe, within next 12-18 hrs, all the larvae could be transferred to the mountages. This brings in a lot of relief to the farmers apart form saving mulberry leaves and labour. The present study showed clear difference in mounting duration between the control and treated ones. The gap would be much more in the field conditions. It is also important that the compound works equally well in both the popular hybrids without affecting the cocoon quality.

It can be summarized that PE is an effective bioactive compound which elicits responses in silkworm depending on the time of administration. Silk quantity can be increased by applying PE in the early days 5th instar. The larval duration can be reduced at least by a day to save a crop from imminent loss by applying PE at about 72 hrs. The mounting duration can be reduced to about 24 hrs as against the regular 2-3 days and thereby labour involvement and leaf consumption. This study thus recognizes PE as one of the most important components in the silkworm rearing management.

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