

Effect of Phytoecdysteroid on Pure Breed Performance of Silkworm *Bombyx mori* L.

Kanika Trivedy*, Anindita Dhar, S. Nirmal Kumar, K. Sashindran Nair, M. Ramesh and Nisha Gopal
Central Sericultural Research and Training Institute, Mysore-570008, Karnataka, India.

(Received 14 April 2003; Accepted 23 June 2003)

Phytoecdysteroids with moulting hormone (MH) activity induce different responses in silkworms when used on different day of final instar, which can be manipulated for maximum benefit like early and uniform spinning behaviour, reducing crop loss and to increase cocoon yield. The results showed that application of this hormone on seed crop viz., CSR2, CSR4 and BL44 and BL67 in early stage of 5th instar i.e., at 72 hrs and 96 hrs though induced early and uniform spinning behaviour, there was an adverse effect by 9–36% on the economic characters like cocoon yield, cocoon weight, cocoon shell weight and also on fecundity etc. Application of this hormone in late stage of 5th instar i.e., at the onset of spinning showed non-significant variations in some of the characters like cocoon weight, cocoon shell weight, cocoon shell ratio and fecundity. The physiological implications of phytoecdysteroid in hastening the maturation events and synchronization of spinning activities in different breeds are discussed.

Key words: *Bombyx mori*, Phytoecdysteroid, Maturation, Synchronization of spinning

Introduction

Antagonistic to the action of exogenous JH, administration of extra dose of ecdysone hastens the developmental process and reduces the larval duration. This physiological process is being exploited in sericulture to hasten the maturation events of the last larval instar and to synchro-

nize the spinning activities. By administering moulting hormone when the silkworms are about to spin, it was found that spinning was more or less simultaneous, enabling improved efficiency in mounting and uniformity in pupation (Chou and Lu 1980; Dai *et al.*, 1985; Sehnal and Akai 1990; Li *et al.*, 1992). Similar observations were made by using phytoecdysteroids on hybrids during initiation of spinning in Indian conditions (Shivakumar *et al.*, 1995, 1996; Anantharaman *et al.*, 1996). 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). At present, in countries like Japan, China and Korea, phytoecdysteroid is being used extensively in sericulture as reported by Ninagi and Maruyama (1996). Prakash and Ghosal (1979) identified ecdysteroid-containing plants in 33 families covering several hundred species. 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, presence of the hormone was confirmed through thin layer chromatography analysis and dose was quantified through HPLC (High Performance Liquid Chromatography). Hormone was successfully tested in laboratory and Regional Sericultural Research Centers of CSRTI along with Chinese hormone. The results were similar to that of Chinese moulting hormone. Many workers have investigated the effect of phytoecdysone on the growth of silkworms. It was found to increase cocoon yield (Kobayashi, 1978) and to enhance larval maturation (Ito *et al.*, 1970) without affecting the quality of cocoons. Sudo and Akai (1988) reported prolongation of larval duration and increased cocoon weight when 20 hydroxy ecdysone (20-OH) is administered at the beginning of 5th instar. It stimulated the silkworm larvae for tolerance to toxins and viral infection (Chernysh *et al.*, 1983)

*To whom correspondence should be addressed.

Silkworm Physiology, Central Sericultural Research and Training Institute, Mysore-570008, Karnataka, India. Tel: 091-821-362406; Fax: 091-821-362 845; E-mail: kantrivedy@rediff.com

enhanced the RNA synthesis in the silk gland (Dai *et al.*, 1985) and accelerated growth and development in silkworms, thus reducing larval duration. It is reported that moulting hormone reducing crop losses caused by diseases *viz.*, grasserie, flacherie and muscardine (Liu and Hou, 1985). They also observed that 20-OH inhibits the infection of nuclear polyhedrons virus after using Mayuran, an ecdysteroid containing agent. Hou and Yang (1990) tested the ability of 20-OH to suppress the infection of larvae using inoculation tests and by ELISA and found that the mortality was lowest when fifth instar larvae were injected with 20-OH either before or after inoculation and 20-OH treated larvae had lower ELISA values than the control. By the application of high dosage of juvenile hormones sometimes non-cocooning silkworms might occur, in which case, use of ecdysteroid will promote spinning (Kajiura and Yamashita, 1992). Thus a combination of juvenile and moulting hormones may prove to be more beneficial in increasing the productivity in sericulture. The occurrence of non-cocooning silkworms, due to high rearing temperature or feeding of tender leaves can be brought down to 9.6% by phytoecdysteroid application. Non-spinning larvae reared on artificial diet were found to spin after feeding on diet-containing ecdysterone (Sudo and Akai, 1988).

In the present study, it has been planned to determine the effect of the phytoecdysteroid compound on seed crop performance of silkworm *Bombyx mori* L., and the effect on the most important economic characters like silkworm cocoon weight, silkworm shell weight and shell ratio. In fact, there is no literature at present, which can assure the safe use of phytoecdysteroid for seed crop performance and its grainage activity.

Materials and Methods

Two-bivoltine pure breeds *viz.*, CSR2 and CSR4 and two multivoltine pure breeds *viz.*, BL67 and BL44 were utilized for the study following the rearing method of Krishnaswami (1994).

Administration of phytoecdysteroid

To study the effect of phytoecdysteroid on seed crop and economic characters, different batches were treated at the age of 72 hrs, 96 hrs, 120 hrs and 132 hrs (on set of spinning) in the case of bivoltine and 72 hrs, 96 hrs and 120 hrs (on set of spinning) in the case of multivoltine of 5th instar larvae. Recommended dose of phytoecdysteroid *i.e.*, 25 mg/lit/10,000 worms was orally fed to silkworms by spraying topically to 10 kg of fresh mulberry leaves. Three replications of 250 larvae each were maintained for

each treatment. Three replications of 250 larvae each were also maintained as control for each breed.

Mounting of matured larvae

Fully matured larvae were picked-up and mounted one by one in separate tray and the number of matured larvae was periodically recorded in both the treated and control batches till the completion of mounting process. Data on maturation percentage, economic characters *viz.*, cocoon yield, cocoon weight, cocoon shell weight, shell ratio and fecundity were calculated and plotted on the graph. The experiment was conducted two times and the data were subjected to statistical analysis to find out the significant differences in the effect of hormone, if any.

Results

The results on the effect of phytoecdysteroid on various characters *viz.*, maturation percentage, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio and fecundity of the selected silkworm breeds, CSR2, CSR4, BL44 and BL67 of silkworm, *B. mori* L. are presented in Tables 1, 2 and Fig. 1, 2, 3, 4.

Cumulative maturation percentage in different ages (hrs)

Multivoltine breeds: Detailed results on cumulative maturation percentage of multivoltine races *viz.*, BL44 and BL67 treated with phytoecdysteroid at 72 hrs, 96 hrs and 120 hrs as compared to control are presented in Fig. 1 and 2, respectively. In both the races *viz.*, BL44 and BL67, it was observed that in 72 hrs treatment 13% and 17% respectively of larvae started maturing after 30 hrs of the treatment *i.e.*, at the 102 hrs age and progressively 100% larvae matured within 138 hrs in BL67 and 144 hrs in BL44 *i.e.*, 30–36 hrs earlier than their respective control. In 96 hrs treatment, 2% of the larvae started spinning in both the breeds *viz.*, BL44 and BL67 after 6 hrs of treatment (*i.e.*, at the age of 102 hrs) and progressively reached 100% at 144 hrs *i.e.*, on the same day as in 72 hrs treatment. Both breeds mature 30 hrs earlier than their respective control. In the case of 120 hrs treatment, which was on the day of the onset of spinning, it was observed that the larvae started maturing in a large number after 6 hrs of treatment time (126 hrs) and progressively the maturation was completed within 150 hrs *i.e.*, on the beginning of the 7th day of the 5th instar in both the breeds. It took 30 hrs for complete maturation. In BL44 and BL67, 80 and 92% larvae matured within 144 hrs age whereas in control at the same time, it was 66 and 68%, respectively. Both breeds mature 24 hrs earlier than their respective control.

Table 1. Effect of phytoecdysteroid on the economic characters of different multivoltine breeds of *Bombyx mori* L.

Treatment hours in 5 th instar	Cocoon yield/10,000 larvae (no.)	Cocoon yield/10,000 larvae (kg)	Single cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell ratio (%)	Fecundity (no.)
BL44						
72	8248	10.692*	1.288*	0.185*	14.38	484*
96	8476	11.772	1.386*	0.198*	14.26	508*
120	8840	13.246	1.499	0.225	15.01	538
Control	8649	13.039	1.560	0.255	16.31	548
CD at 5%	NS	1.34	0.147	0.056	NS	24.074
BL67						
72	9183	13.214*	1.439*	0.221*	15.33*	458*
96	8991*	13.765*	1.531	0.242*	15.80	502
120	9449	14.766	1.562	0.263	16.81	513
Control	9538	15.363	1.611	0.270	16.78	517
CD at 5%	529.89	0.984	0.092	0.025	1.191	26.136

*Significantly at 5% level.

NS, Non-significant.

Table 2. Effect of phytoecdysteroid on the economic characters of different bivoltine breeds of *Bombyx mori* L.

Treatment hours in 5 th instar	Cocoon yield/10,000 larvae (no.)	Cocoon yield/10,000 larvae (kg)	Single cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell ratio (%)	Fecundity (no.)
R2						
72	9378*	12.848*	1.336*	0.246*	18.40*	445*
96	9551*	14.628	1.531*	0.289*	18.75*	500
120	9237	14.883	1.614	0.340	21.09	500
132	8861	13.994	1.628	0.360	22.11	528
Control	8474	14.879	1.756	0.390	22.21	539
CD at 5%	843.81	1.930	0.158	0.065	3.104	48.166
CSR4						
72	9617	15.708*	1.634*	0.298*	18.20*	485*
96	9042*	14.035*	1.552*	0.272*	17.53*	530
120	9466	15.554*	1.772	0.369	20.82	531
132	9410	17.054	1.818	0.378	20.79	563
Control	9547	18.221	1.909	0.400	21.00	570
CD at 5%	491.50	1.524	0.137	0.032	1.772	44.636

*Significantly at 5% level.

NS, Non-significant.

Control batch started spinning at 120 hrs and completed 100% within 174 hrs from the resumption time of 5th instar. It took 54 hrs to complete its maturation. Though the maturation process started with 120 hrs treatment, the maturation in control batches prolonged 24 hrs more than the 120 hrs treatment. In other word treated batches at 72, 96 and 120 hrs were mature 30–36, 30 and 24 hrs earlier respectively than controls of multivoltine breeds.

Bivoltine breeds: Detailed results on cumulative matu-

ration percentage of multivoltine races viz., CSR2 and CSR4 treated with phytoecdysteroid at 72 hrs, 96 hrs and 120 hrs as compared to control are presented in Figs. 3 and 4 respectively. In 72 hrs treatment, the larvae started maturing after 18 hrs of the treatment in CSR2 race whereas, CSR4 took 24 hrs from the treatment time. But in both the cases, maturation was completed within 132 hrs of the resumption of 5th instar. Both the breeds mature 42 hrs earlier than their respective controls. In 96 hrs of

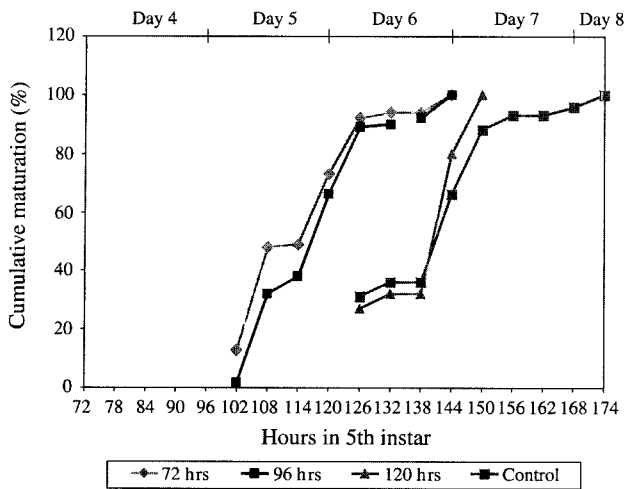


Fig. 1. Effect of Phytoecdysteroid on the maturation of multivoltine silkworm, *Bombyx mori* L, BL44 treated at 72, 96 and 120 hrs of 5th instar and compared with untreated control.

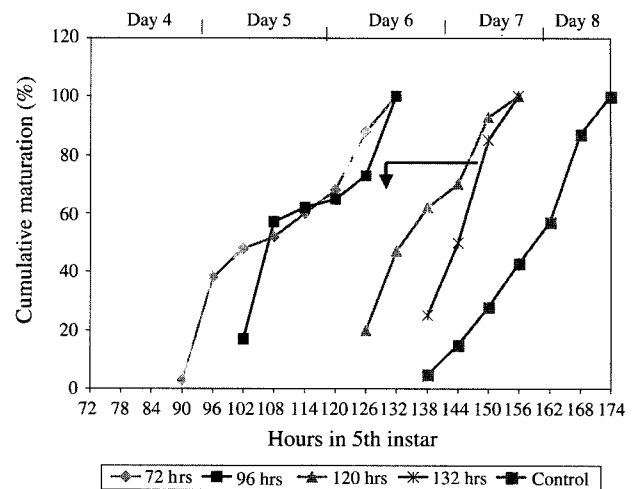


Fig. 3. Effect of Phytoecdysteroid on the maturation of bivoltine silkworm, *Bombyx mori* L, CSR2 treated at 72, 96, 120 and 132 hrs of 5th instar and compared with untreated control.

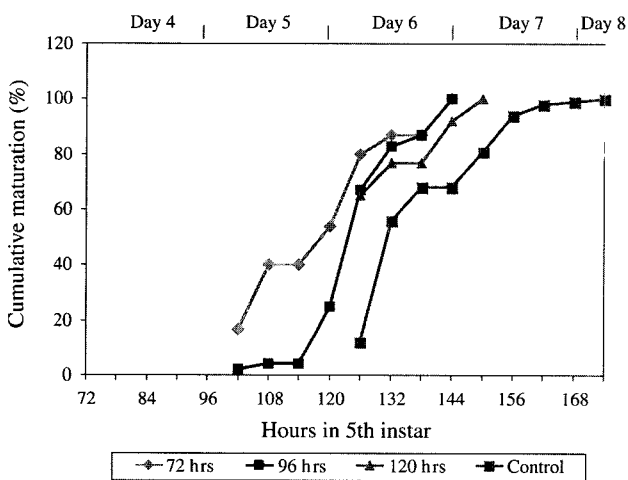


Fig. 2. Effect of Phytoecdysteroid on the maturation of multivoltine silkworm, *Bombyx mori* L, BL67 treated at 72, 96 and 120 hrs of 5th instar and compared with untreated control.

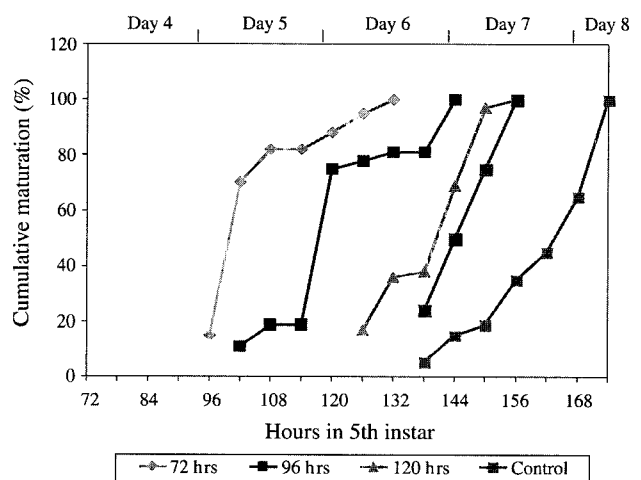


Fig. 4. Effect of Phytoecdysteroid on the maturation of bivoltine silkworm, *Bombyx mori* L, CSR4 treated at 72, 96, 120 and 132 hrs of 5th instar and compared with untreated control.

treatment, 17 and 11% maturation started just after 6 hrs of treatment in CSR2 and CSR4, respectively and maturation completed in CSR2 within 132 hrs, it means it took 30 hrs to complete 100% maturation, whereas, in CSR4 it took 42 hrs to complete 100% maturation. In this treatment CSR4 and CSR2 matured completely 30 and 42 hrs earlier than their respective control respectively. In both the races *viz.*, CSR2 and CSR4, it was observed that in 120 hrs treatment, 20 and 17%, respectively of larvae started maturing after 6 hrs of the treatment *i.e.*, at the 126 hrs age and progressively 100% larvae matured within 156 hrs. It took 30 hrs to complete it. Both the breeds matured 18 hr before their respective control. In the case of the treatment at the onset of spinning *i.e.*, at 132 hrs it was observed that the larvae started ripening after 6 hrs of

treatment (138 hrs) with 25 and 24% maturation, respectively in CSR2 and CSR4, whereas, in both the control batches it was 5%. In treated batches, the larvae matured completely within 24 hrs after the treatment in both CSR2 and CSR4 *i.e.*, at 156 hrs of age of 5th instar. At 150 hrs age 85 and 75% larvae matured in CSR2 and CSR4, respectively whereas, in control, it was 28 and 19%, respectively. Both the breeds matured 18 hrs before their respective control.

In control batch the maturation started along with that of 132 hrs treatment but 100% maturation time prolonged up to 174 hrs. It took 42 hrs to complete maturation process, which is 18 hrs more than that 120 hrs and the onset of spinning treatments (132 hrs) and 42 hrs more than 72 and 96 hrs treatments.

Effect of phytoecdysteroid on the yield of silkworm

In this study, the yield by number, which may otherwise be understood as survival in a general entomological arena, was not largely affected although a marginal change was noticed in one or two cases. In sericulture, yield by number is considered as one of the important characters and this largely reflects the management capacity of the person who oversees the silkworm rearing rather than the effect of the compound administered. In all the races, there was non-significant difference in yield by number at 72 hrs treatments as compared to control but yield by weight was reduced significantly. But in CSR2, both number and weight were significantly reduced (Table 2). At onset of spinning treatment in all the breeds, there were no significant differences in yield both by number and weight as compared to control.

Effect of phytoecdysteroid on the cocoon weight of silkworm

Exogenous administration of phytoecdysteroids during early stage of the last instar induces uniform spinning and increases yield by number but decrease in cocoon weight and shell weight was observed mainly in the early hours of treatment. This is due to shortening of feeding period of the last instar. But in later hours of treatment, that is, on onset of spinning, the cocoon weight and shell weight were not adversely affected in both bivoltine and multivoltine races.

In BL44 race, the cocoon weight was significantly less by 17.45% in 72 hrs treatment (1.228 g) and by 11.16% in 96 hrs treatment (1.386 g) as compared to control (1.560 g). It is seen that there was marginal difference (3.91%) with 120 hrs (onset of spinning) treated and control batch which was statistically non significant. Similarly, in BL67, the cocoon weight in the treated batch was reduced significantly by 10.67% in the early hour treatment *i.e.*, at 72 hrs (1.439 g) as compared to control (1.611 g). But in the onset of spinning (120 hrs) batch, the difference was minimum in comparison to control (3.04%), which was non-significant. In CSR2, the cocoon weight of batch treated at 72 hrs was significantly less by 23.95% (1.336 g), but gradually increased in other treated batches *viz.*, 96 hrs, 120 hrs and 132 hrs (on set of spinning). At 96 hrs, it was 1.531 g (12.79% less), which was significantly lower than control batch, where, cocoon weight was highest (1.756 g) among all the other treated batches. In treated batches at 120 hrs and 132 hrs there was no significant difference in cocoon weights (1.614 and 1.628 g, respectively) as compared to control. Similar trend as in CSR2 was observed in CSR4. The cocoon weights in batches treated early (72 hrs and 96 hrs) were (1.634 and 1.552 g) significantly less (14.39 and 18.71%) than control. The

other treated batches *viz.*, 120 hrs and 132 hrs have shown no significant difference when compared to control. (1.909 g).

Effect of phytoecdysteroid on the cocoon shell weight of silkworm

Cocoon shell weight along with cocoon weight and cocoon shell ratio is considered to be the most important economic characters in silkworm. In BL44 race, the cocoon shell weight was significantly reduced by 27.47 and 22.50% in 72 hrs (0.185 g) and 96 hrs treatment (0.198 g), respectively whereas at on-set of spinning, *i.e.*, at 120 hrs (0.225 g) there was no significant difference as compared to control batch (0.255 g). In BL67, the same result was observed. The cocoon shell weight was significantly reduced by 18.36 and 10.52 % in 72 hrs (0.221 g) and 96 hrs treatment (0.242 g) respectively whereas at onset of spinning, *i.e.*, at 120 hrs (0.263 g), there was no significant difference as compared to control batch (0.270 g). Similar trend was observed in CSR2 as in multivoltine races, the shell weight was significantly lower in 72 hrs (0.246 g) by 36.80%, 96 hrs (0.289 g) by 26.01%, whereas, in 120 hrs and 132 hrs (on set of spinning) treated batches, there were no significant differences as compared to control (0.390 g) (Tables 1 and 2). In CSR4, the cocoon shell weight was significantly reduced in early hours treated batches *i.e.*, 72 and 96 hrs (25.51 and 31.99% less) as compared to control (0.400 g), whereas, no significant difference was noticed in the other batches.

Effect of phytoecdysteroid on cocoon shell ratio of silkworm

Cocoon shell ratio is considered as one of the most important characters because it decides even the price of cocoons in the market. As such, the physiological treatments usually do not induce much change in silk ratio. But in the present work, in multivoltine breeds, there was no significant difference but there was significant difference in bivoltine breeds. In BL44, the shell ratio percentage obtained at 72 hrs, 96 hrs and at 120 hrs (onset of spinning) were 14.38, 14.26 and 15.01%, respectively the differences of which were statistically non-significant as compared to control (16.31%). In BL67, the cocoon shell ratio obtained at 72 hrs treatment (15.33%) was significantly less by 8.62% in comparison with control but it gradually improved at 96 hrs (15.80%), 120 hrs (16.81%), the difference of which were statistically non-significant when compared to control. It was noticed that there was no significant effect at on set of spinning treatment. In CSR2, the cocoon shell ratio at 72 hrs and 96 hrs treatment was significantly reduced by 17.16 and 15.57%, respectively. In other treated batches at 120 hrs (5.06%),

132 hrs (0.450%), there was no significant difference when compared to control (22.21%). In CSR4, the cocoon shell ratio reduced significantly (by 13.32 and 16.55%) in both the early treated batches *i.e.*, at 72 and 96 hrs. It had no significant effect in the other batches *viz.*, 120 hrs and 132 hrs, respectively as compared to control (21.00%).

Effect of phytoecdysteroid treatment at different ages on the fecundity (fitness character) of seed crop

The present study is based on the performance of the seed crop under the influence of phytoecdysteroid administration. In seed crop, the effect on fecundity is considered as one of the most crucial and important factors. It is a fitness character. Here, the fecundity was significantly reduced in early hours treatment *i.e.*, at 72 hrs treatment in both multivoltine and bivoltine races but there was no adverse effect on the larvae, which were treated at onset of spinning stages. The effect on fecundity on administration of phytoecdysteroid was determined at different ages treatment, which are presented in Tables 1 and 2. In the case of BL44, the fecundity recorded at 72 hrs (484) and 96 hrs (508) treatment were significantly less by 11.62 and 7.24% than control batch (548) but there was no significant difference between 120 hrs (538) and control. In BL67, treatment of phytoecdysteroid at 72 hrs had significantly negative effect (reduced by 11.38%) on the fecundity (458) as compared to control (517) but in the other treatments *viz.*, at 96 hrs (502) and 120 hrs (513), there were no significant differences as compared to control. The bivoltine breeds also behaved similarly to multivoltine. In early hours of treatment (at 72 hrs), the fecundity was significantly low but in the other treatments, there was no significant difference in fecundity. In CSR2, the fecundity was reduced by 17.33% at 72 hrs treatment (445) as compared to control (539). In CSR4, the fecundity was significantly lower by 14.88% at 72 hrs treatment (485) as compared to control (570) but progressive increase was noticed at 96 hrs (530), 120 hrs (531) and 132 hrs (onset of spinning) (563).

Discussion

With the elucidation of the role of ecdysteroid in the development of silkworm, attempts were made to control silkworm growth and development. This opened up a new route in manipulation of larval duration. Larval duration is one of the most important characters that decide the quantity of the cocoon crop. 20-hydroxy ecdysone is also referred to as ecdysterone. The active ecdysterone present in the phytoecdysteroid induces certain physiological manifestations upon exogenous administration as done by

the innate ecdysterone. Early maturity and high fecundity are desirable characters in insects for efficient mass production. In the present study, the responsiveness of the larvae to the administration of phytoecdysteroid with regard to developmental events particularly with reference to the hastening of the maturation process was investigated on pure silkworm breeds. In the course of larval pupal metamorphosis of lepidopteron insects, visible developmental events sequentially occur in the order of spinneret pigmentation, onset of spinning and gut purge. These sequential events have been assumed to be under the control of ecdysteroid. Responsiveness of the larvae to exogenous ecdysterone was markedly higher when the treatment was carried out at the onset of spinning (120 hrs) in multivoltine. Among the multivoltines, BL67 recorded the best response, which started off with high 65% maturity, and the remaining larvae matured in just 24 hrs time. Further, the observation on the quantitative characters depicts non-significant difference when compared to the control. Similar trend was observed in the fecundity. However, the responsiveness of the larvae for the maturation process in the early treated batches although is encouraging it has been found to have a negative correlation with the quantitative and the fecundity as fitness character.

When a general comparison is made, it is obvious that, as in the case of multivoltine, the responsiveness of larvae to ecdysterone was prominently higher when the treatment was carried out at the onset of spinning (132 hrs) in bivoltine. A comparison between CSR2 and CSR4 in terms of hastened maturity in response to the treatment shows that CSR2 is marginally better in responding to treatment. Although 100% worms matured by 156 hrs, there was a difference of about 10% when it reached 150 hrs *i.e.*, the cumulative maturity was attained. The larvae treated at 120 hrs also completed mounting at 156 hrs but took a longer time as it started at 126 hrs itself. The observations on the quantitative characters make it amply clear that the treatments at the onset of spinning did not affect it significantly. The trend was not different in the fitness character either. But as stated in the case of multivoltines although the larvae responded well to the treatments made at 72 and 96 hrs it manifests a rather strong adverse effect on the quantitative characters. The fitness character was negatively affected at a significant level only in the case of 72 hrs treatment.

A close examination and comparison between the response of multivoltine and bivoltine breeds show that in bivoltine, the period taken to complete maturation and mounting is shorter than in multivoltine. When treated at the onset of spinning (132 hrs), the mounting of larvae was completed within a span of 18 hrs in bivoltine whereas it took 24 hrs in the case of multivoltine when

treated at the onset of spinning. But this shorter span in bivoltine hybrids compared to multivoltine cannot be fully attributed to the phytoecdysteroid treatment because even in the control, the total mounting span is more in multivoltine than in bivoltine. The mounting span was only 30 hrs in bivoltine control and it was 48 hrs in multivoltine control. So the inherent breed dependent difference in the mounting duration is prominent even when phytoecdysteroid was administered but the total period was brought down from 36 hrs to 18 hrs in multivoltine and from 48 hrs to 24 hrs in bivoltine. So it is clear that when silkworms are treated at the onset of spinning with phytoecdysteroid, the mounting period can be shortened almost by 50%.

It is amply clear from the results that the phytoecdysteroid can be recommended as a useful package depending on the needs. If the mounting period is to be shortened without an adverse effect on the cocoon and fitness characters, the safe period for such a treatment is the onset of spinning. But on exigencies when an imminent crop loss is feared on account of an unforeseen shortage of mulberry leaf or a disease outbreak, the crop can be saved by administering the phytoecdysteroid well before the onset of spinning, with a compromise on the cocoon and fitness characters

The observation made in the present work is not much different from earlier reports in the same line. It has now become a common endocrinological phenomenon that the response of silkworm in terms of maturation events and cocoon yield depends largely on the time of application, provided the threshold concentration of the exogenous ecdysteroid is met with. Phytoecdysteroid serves as a tool by which the last phase of rearing can be effectively managed which leads to labour and leaf saving without any adverse effect on other important characters. Such an exploitation is possible only because silkworm is highly sensitive to the administration of the exogenous ecdysteroid as pointed out by Dai *et al.* (1985).

The physiological basis of the action of exogenous ecdysone on hastening the maturation events and its effect on silk production has become quite clear now. The interaction of exogenous ecdysteroid with the circulating hormones determines the maturation sequence and spinning behaviour (Sehnal, 1989). Ecdysteroid released from the prothoracic gland (PG) is controlled by PTTH released from the brain neurosecretory cells. Shirai *et al.* (1992) reported 5 peaks of haemolymph PTTH titre in the 5th instar silkworm spreading from the early 5th instar to the pre-pupal stage. The exogenous administration of ecdysteroid for uniform maturity is generally done after the third release of PTTH, which is added to the innate ecdysteroid, released in response to the third PTTH release.

In normal cases, the feeding larvae contain very low level of ecdysteroid that may be indispensable for the development (Sehnal, 1989). Once the body reaches the critical weight, the level of innate ecdysteroid increases gradually. The dependence of silk production on ecdysteroid possibly reflects a general tissue requirement for a low level of ecdysteroid concentration. A rise of ecdysteroid titre apparently terminates feeding and in the last instar, stimulates cocoon spinning. For proper course of these developmental events, it is probably significant that the ecdysteroid level is slightly elevated for 1–2 days before rising to the moult inducing height. The elevated titre of ecdysteroid apparently shifts silk glands to their regression phase when they reach the maximum protein synthesis. This gives an explanation as to why the silk production is not affected when ecdysteroid is administered at the onset of spinning. By this time, the maximum protein synthesis is attained and a switch over to the silk gland regression starts only after this point. On the contrary, when the ecdysteroid is administered earlier to the onset of spinning, the silk gland switches over to the regression phase before reaching the optimum point of silk production and naturally affects the quantitative characters.

The same point may not hold good for the effect of ecdysteroid on the fecundity because the development of ovarioles and the formation of oocytes is not completed in the 5th instar. It starts in the 3rd instar and continues through the 4th and 5th larval instars, pupal stage and the process is complete in the adult stage. Such being the scenario, it evolves that the ecdysteroid treatment at 72 hrs of 5th instar has an effect on the early programming of ovarioles. The synthesis and release of ecdysone by silkworm ovary during pupal and adult stage gives an indication that ovarian tissue is prone to ecdysteroid effect. But the timing of such an effect is programmed such a way that the major ecdysteroid releasing organ, the prothoracic gland has already disappeared from the system. The increased ecdysteroid titre towards the middle of the 5th instar will not support in this case, the programmed early developmental sequence of the ovary. The above revealed physiological sequence of developmental events explains the role of exogenous ecdysteroid on the maturation events, silk production and also on the fitness characters.

So it can be concluded that farmers can use phytoecdysteroid safely in pure breeds rearing at onset of spinning for uniform maturation as there is no significant effect on the economic parameters of the cocoons, whereas, application of phytoecdysteroid hormone at early stages of 5th instar for early maturation *i.e.*, from 72 hrs of 5th instar to day before onset of spinning showed significant reduction in economic characters like cocoon weight, shell weight,

shell ratio percentage and fecundity although, it reduces larval duration, but should be used when there is leaf/labour shortage or disease break.

References

- Anantharaman, K. V., G. R. Shivakumar and R. K. Datta (1996) Phytoecdysteroid effect on maturation in silkworm, *Bombyx mori* L. *Insect Evt.* **2**, 21-22.
- Chernysh, S. I., V. L. Lukhtanov and N. P. Simonenko (1983) Adoption to damage in the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) 2. Effect of ecdysterone and some other adoptogens on the tolerance of the caterpillars to entobacterin. *Entomol. Obozr.* **62**, 665-672.
- Chou, W. S. and H. S. Lu (1980) Growth regulation and silk production in *Bombyx mori* L., from Phylogenous ecdysteroids; in *Progress in ecdysone research*. Hoffmann, J. A. (ed.) pp. 281-297, Elsevier/North Holland Biomedical Press, New York.
- Dai, Y. J., R. Li and Y. Q. Zhang (1985) Moulting hormone and silk protein synthesis: Effect of ecdysone on nucleic acid metabolism in the posterior portion of silk gland in *Bombyx mori*. *Acta. Entomol. Sinica.* **28**, 8-14.
- Hou, R. F. and J. C. Yang (1990) Suppression of nuclear polyhedrosis virus infection by β ecdysone in the silkworm. *Bombyx mori* L. *Academia Sinica.* **29**, 21-27.
- Ito, T., Y. Horie and K. Watanabe (1970) Effect of phytoecdysone on the length of the 5th instar and the quality of cocoon in silkworm. *Bombyx mori*. *Ann. Zool. Jpn.* **43**, 175-181.
- Kajiura, Z. and O. Yamashita (1992) Induction of metamorphosis of the methoprene induced dauer larvae of silkworm *Bombyx mori* by ecdysteroids. *Comp. Biochem. Physiol.* **101A**, 277-280.
- Kobayashi, M. (1978) Insect Endocrinology; in *The Silkworm: An Important Laboratory Tool*. Tazima, Y. (ed.) pp. 159-187, Kodansha Ltd., Tokyo.
- Krishnaswami, S. (1994) A practical guide to mulberry silk cocoon production in tropics. Srirampala Sericulture Consultants, Bangalore. pp. 47-68.
- Li, X., J. Zhu and S. Hui (1992) Studies on improving the mounting rate of partitioner cocooning frame by using ecdysone. *Bull. Seric.* **23**, 23-26.
- Liu, H. S. and R. F. Hou (1985) Studies on inhibition of infection with nuclear polyhedrosis virus by an ecdysteroid containing agent in the silkworm, *Bombyx mori* L. *Chinese J. Entomol.* **5**, 23-30.
- Ninagi, O. and M. Maruyama (1996) Utilization of 20-hydroxyecdysone extracted from a plant in sericulture. *JARQ* **30**, 123-128.
- Prakash, A. and S. Ghosal (1979) Phytoecdysones. *J. Sci. Indust. Res.* **38**, 632-647.
- Sehnal, F. (1989) Hormonal role of ecdysteroids in insect larvae and during metamorphosis; in *Ecdysone: From Chemistry to Mode of Action*. Koolman, J. (ed.) pp. 271-278, Thieme Medical Publishers, New York.
- Shirai, Y., Y. Aizono, T. Iwasaki, A. Yanagida, H. Mori, M. Sumida and F. Matsubara (1992) Prothoracicotropic hormone is released five times in 5th larval instar of silkworm, *Bombyx mori*. *J. Insect Physiol.* **39**, 83-88.
- Sehnal, F. and H. Akai (1990) Insect silk glands: Their types, development and function and effects of environmental factors and morphogenetic hormones on them. *Int. J. Insect Morphol. Embryol.* **19**, 79-132.
- Shivkumar, G. R., K. V. Anantharaman, S. B. Magadum, R. K. Datta, S. S. Hussain, A. Banerji and S. K. Chowdhary (1995) Effect of phytoecdysteroid on larval maturation and economic parameters of the silkworm, *Bombyx mori* L. *Indian J. Seric.* **34**, 46-49.
- Shivkumar, G. R., K. V. Anantharaman, S. B. Magadum, R. K. Datta, S. S. Hussain, A. Banerji and S. K. Chowdhary (1996) Effect of phytoecdysteroids on the spinning, cocoon and reeling parameters of the silkworm, *Bombyx mori* L. *Allelopathy J.* **3**, 71-76.
- Sudo, M and H. Akai (1988) Induction of spinning from non-spinning larvae reared with an artificial diet by administration of molting hormone. *J. Seric. Sci. Jpn.* **57**, 405-408.
- Trivedy, K., S. Nair and P. K. Chinya, (1998) Annual Report, CSRTI, Mysore, India, pp. 76.