Ecdysteroid extract from common catchfly, *Silene gallica* L. for rearing management of silkworm, *Bombyx mori* L. and stabilized cocoon crop

K. Sashindran Nair*, C. M. Babu, Kanika Trivedy and P. K.Chinya

ABSTRACT

The cocoon spinning process takes 24 to 72 h depending on the seasons as the maturation process in a silkworm colony is not uniform. To hasten the larval maturation process and to shorten the mounting duration, administration of phytoecdysteroid was planned. 20-hydoxyecdysone (20E) equivalents was extracted through a soxhlet apparatus from the dry powder of high altitude herb, *Silene gallica* known as common catchfly, partially purified through liquid partitioning and thin layer chromatography and the active principle was quantified through HPLC using pure 20E as reference. The active principle was made to a concentration of 25 ppm in water and administered to silkworm at the onset of maturation process. In winter months, about 80 % of the treated silkworms were ripe by 24 hours whereas it took 48 h for 80 % maturation in control. In rainy season > 80 % of treated larvae took about 18 hours for ripening while that in control took 30 h. In summer though, the threshold of 80 % maturation reached by 18 h in treated and by 36 h in control larvae. Though phytoecdysteroid is a known and potential biopesticide, it is used in the present work as a beneficial compound. The physiological and economical implications of such a use are discussed.

Key words: *Bombyx mori*, cocoon spinning, common catchfly, phytoecdysteroid, silkworm.

INTRODUCTION

Insect growth and development is largely under the control of circulating hormones *viz*., juvenile hormone (JH) and ecdysone. Exogenous application of the analogues or mimics of these hormones could induce derangement in the metabolic activities and create disruptions in the insect development. Plant-produced insect moulting hormones, known as phytoecdysteroids (PEs), assume the functions of defense against insects by acting either as feeding deterrents or as agents that induce developmental disruption (Schmelz *et al*., 2002). This realization was a formidable breakthrough which helped in working out various viable formulations of botanicals to combat insect pests. Phytoecdysteroids assume great importance as biopesticide of significant efficacy mainly because they are not compounds of potential health hazards. Quite interestingly, the response of silkworm, *Bombyx mori* L. to minute quantities of these hormones or its analogues is beneficial. In China, various plant sources were identified which contained moderate to high amounts of PE and used them in sericulture to manage the silkworm rearing during the last stage of larval development (Wong *et al*.,1979; Chow and Lu, 1980). Such a concept had been totally alien to Indian sericulture but serious efforts initiated in the past one decade led to a few studies to identify plant sources in India which are available in plenty, containing high amounts of PE and to develop a viable technique to extract the PE. In commercial silkworm rearing, PE is to be administered to silkworm at an appropriate time so that the management of silkworm rearing towards the end of larval period when they are to be transferred to the mounting device for cocoon building becomes easy and the labour involvement is reduced. This paper deals with the identification of such a plant source, the common catchfly for extraction of ecdysteroid, its processing and extraction of the PE, quantification of the 20E equivalent in the extract, development of a technique to apply it to the silkworms and the benefits arrived at. Plants belonging to the genus, *Silene* are known to be a good source of ecdysteroid and the extraction and purification processes were fine tuned and the comparative recovery of the active principle worked out (Zibareva, 2000; Mamadalieva, *et al*., 2004; Simon *et al*., 2009). The impact of PE on silkworm maturation has been established and the economic gain derived from the technology has been considered important for the viability of this technology (Nair *et al*., 2005; Trivedy *et al*., 2006; Dinan *et al*., 2009).

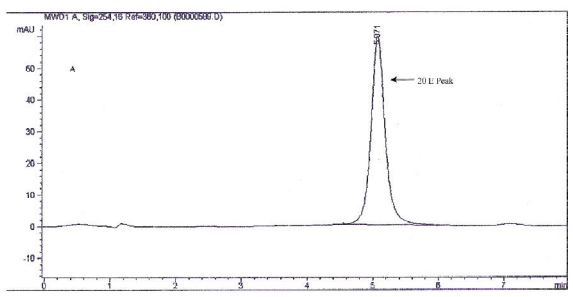
MATERIALS AND METHODS

The plant material

The mature weed, *Silene gallica* (Caryophillaceae) was collected from Uthakamandalam area of Nilgiri hills by cutting at ground level and the whole plant material was shade dried (Figure 1).

Figure 1. Common catchfly (*Silene gallica*)

The dried material of the whole plant was coarse-milled and the material was loaded in a Soxhlet apparatus. PE was extracted in MeOH for 6 h continuously. The extract was collected, concentrated in a water bath, dissolved in water and partitioned between 1-BuOH and water, with the ecdysteroid partitioning into BuOH phase. PE was then dissolved in known volume of MeOH after BuOH was evaporated and separated through TLC run on CHCl3-MeOH system against 20E as reference. PE was eluted in MeOH, centrifuged and the content was quantified in RP-HPLC using an Agilent 1100 HPLC system with 21 % acetonitrile mobile phase. The reference 20E was procured from Sigma, USA. The PE present in the extract was thus calculated from the chromatogram using the area of the peak against the concentration of the reference (Figure 2).



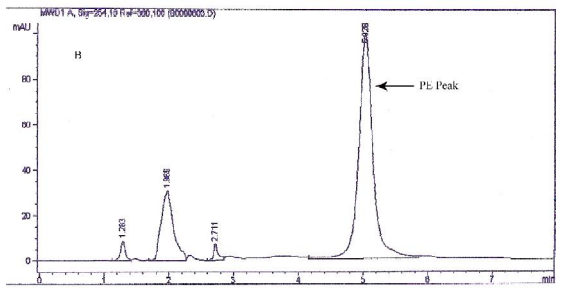


Figure 2. High performance liquid chromatogram showing 20E and PE peaks. A- 20E peak at a retention time of 5.071 minutes in the reference sample. B- PE (20E equivalent) peak at a retention time of 5.028 minutes.

Administration of PE to silkworm

The extract containing PE was diluted to a concentration of 25 µg/ml using distilled water. Popular bivoltine silkworm hybrid, CSR2 x CSR4 was used for the study in three different seasons *viz.*, winter, rainy and summer since the silkworm rearing conditions are known to affect the time required for the larval maturation process as observed by Kumar *et al.* (2006). When the silkworms were fully grown and maturation just set in, mulberry leaves was spread as a thin layer. The diluted extract was sprayed on to the leaves at a rate of 10 ml (250µg PE)/100 g leaves/100 larvae. The larvae were allowed to consume the treated leaves and fed again with normal leaves whenever required. Control larvae were maintained in parallel without any treatment, for comparison. The control larvae were not sprayed with distilled water since water treatment did not have any effect on silkworm maturation as reported earlier (Nair *et al.*, 2005). The treatments were replicated 5 times with 250 larvae per replication.

Data collection and analysis

When the larvae started ripening, the rearing beds were examined at 6 hours interval, the ripe worms were collected and transferred to larval mounting frames. Progressive maturation percentage was calculated. After cocooning, survival of the pupae, cocoon weight, cocoon shell weight and shell percentage were recorded/worked out. The data were subjected to 't' test to ascertain the significance of the result using 'Analyse It' statistical package.

RESULTS AND DISCUSSION

The results of the present study indicate that the problem of non-uniform maturation in silkworm could be solved to a great extent by administering PE extracted from *S. gallica*

Table 1. Effect of PE on the cumulative maturation percentage of silkworm (Hybrid: CSR2 x CSR4) through different seasons (PE: Phytoecdysteroid; C: Control)

Treatment	Cumulative maturation % at										
	6 h	12 h	18 h	24 h	30 h	36 h	42 h	48 h	54 h	60 h	72 *H*
	WINTER										
PE	10.99	26.31	65.22	80.65	98.77	100.00	-	-	-	-	
C	4.06	4.06	11.13	23.13	36.43	57.86	75.86	82.52	90.81	96.98	100.00
t value	P < 0.05	P < 0.01	P < 0.01	P < 0.01	P < 0.01	P < 0.01	-	-	-	-	
RAINY											
PE	33.03	46.83	95.25	96.83	100.00	-	-	-	-	-	
C	19.15	32.41	36.82	42.39	89.20	94.11	97.71	97.71	100.00	-	
t value	P < 0.05	P < 0.05	P < 0.01	P < 0.01	P < 0.05	-	-	-	-	-	
SUMMER											
PE	1.96	70.26	81.37	100	-	-	-	-	-	-	
C	2.02	56.23	65.99	66.33	74.75	97.31	99.33	100.00	-	-	
t value	P > 0.05	P < 0.05	P < 0.05	P < 0.01	-	-	-	-	-	-	

to fifth instar silkworm. One of the most important prerequisites in administering PE to silkworm is precise quantification of the active principle. In the present work, the 20E equivalents present in the extract was partially purified through TLC and quantified through HPLC. The chromatograms showed in Figure 2 facilitated quantification of PE with reference to 20E from the peaks shown in same retention time.

The PE extract when applied to the silkworm larvae in fifth instar when a few larvae were ripe, hastened the maturation process facilitating advanced and synchronized cocoon spinning process. This effect was however dependent on the seasons. Table 1 shows that in winter, in the treated larvae, 80.65 % was ready for mounting by 24 h whereas only 23.13 % was ready in the control. The control took 72 h for 100 % maturation but in the treated, complete maturation was attained by 36 h. In effect, the treated batch took 24 h less than the control for 80 % maturation and 36 h for 100 % maturation. In rainy season, 100 % larval maturation was observed in the PE treated larvae by 30 h whereas in the control, it was by 54 h. Here again, there was a difference of 24 h for complete maturation between the treated and the control. Although in summer as well, the difference was 24 h between the treated and the control for complete maturation, the larvae in the treated batch was mounted fully by 24 h and that in control by 48 h. The results in general corroborates the earlier reports in this line. Chow and Lu (1980) reported synchronization of spinning in Chinese silkworm varieties when PE was administered *per os* at the rate of 2.2 µg/ larva when 5-10% larvae had reached maturity. The mounting process could be completed within 12 h. Shivakumar *et al*. (1995) reported an accelerated maturation in silkworm and mounting within 24 h of treatment when phytoecdysteroid was administered. Similar results were also reported when ecdysteroid extracted from plant sources was administered to silkworm leading to advanced and synchronized maturation (Nair *et al*., 2005; 2008; Trivedy *et al*., 2006; Dinan *et al*., 2009)

The present study also examined the effect of PE administration on the cocoon characters. This is extremely relevant and important because the benefit of hastened and synchronized maturation will be annulled if it adversely affects the cocoon traits beyond a certain limit. Table 2 shows that the cocoon traits *viz*., cocoon weight, cocoon shell weight and shell percentage was not affected

Table 2. Effect of PE on survival and cocoon traits of silkworm (Hybrid: CSR2 x CSR4) through different seasons (PE: Phytoecdysteroid; C: Control)

Treatment	Survival (%)	Cocoon weight (g)	Shell weight(g)	Shell percentage
Winter				
PE	*96.58*	*2.034*	*0.452*	*22.22*
Control	*93.75*	*2.040*	*0.463*	*22.69*
% difference	+3.01	-0.29	-2.37	-2.07
t value	P < 0.05	P > 0.05	P > 0.05	P > 0.05
Rainy				
PE	94.50	2.122	0.448	21.11
Control	95.20	2.015	0.439	21.78
% difference	-0.74	+5.30	+2.05	-3.07
t value	P > 0.05	P < 0.05	P > 0.05	P > 0.05
Summer				
PE	98.65	1.988	0.420	21.12
Control	94.40	1.935	0.409	21.13
% difference	+4.50	+2.74	+2.69	-0.05
t value	P < 0.05	P > 0.05	P > 0.05	P > 0.05

by the treatment. These results are in agreement with the earlier reports that the PE administration to silkworm for uniform maturation does not adversely affect the cocoon traits (Trivedy *et al*., 2006; Kumar *et al*., 2006). In fact, in rainy season, there was a significant increase in the cocoon weight in the PE treated silkworm. It was also observed that PE had a significant positive effect on survival in the larvae treated in winter and summer.

It is reported that silkworm larvae are sensitive to exogenous ecdysteroid when administered at different hours (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 will be different. Probably, onset of spinning is the most suitable period of application without any adverse effect on the cocoon quality especially because the feeding period is not shortened unlike in the case of administration at an age prior to that. As per Chow and Lu (1980) when phytoecdysteroid was applied at the rate of 4 µg/larva, 10 h before maturation, fifth instar larval period was shortened by 14 h and the mounting period was shortened by 39-14 h. This obviously resulted in adverse effect on the cocoon characters unlike what was observed in the present study.

The major thrust in this study was the difference in the maturation time recorded when PE from common catchfly was administered to the bivoltine silkworm depending on the seasons. It is clear from the result that PE is effective on silkworm for inducing advanced and synchronized maturation in the form of 12~24 h reduction for 80% maturation and 24~36 h reduction for 100 % maturation which was season dependent. These results are in line with that of Trivedy *et al*. (2006) and our own earlier works (Nair *et al*., 2005; 2008). The season depended response of silkworm to PE administration as seen in this work also vindicates the results of Kumar *et al*. (2006) when the experiment was conducted on different temperature regimes, on multibivoltine silkworm hybrid.

Physiological implications

Fukuda (1942) was the first to propose that the increase of silk gland function during feeding period of the last larval instar is due to stimulation by ecdysteroid. For this, the origin of ecdysteroid need not necessarily be endogenous. It was also understood that the response of the silk gland to exogenous ecdysteroid depends on the developmental stage of the silkworms (Akai and Kiuchi, 1988). Ecdysteroid represents a stimulator of silk gland. Feeding larvae always contain low level of ecdysteroid that may be indispensable for development (Sehnal, 1989). The dependence of silk production on ecdysteroid possibly reflects a general tissue requirement for such a low level of ecdysteroid concentration. A rise of ecdysteroid titre apparently terminates feeding and in last instar stimulates cocoon spinning. For proper course of these developmental events, it is 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 maximum protein synthesis. Since the time of application is just at the onset of spinning it could be comprehended that the maximum protein synthesis is attained and a switch to silk gland regression starts only after this point. This must be the reason why the cocoon characters are not adversely affected when the treatment is done at the onset of spinning. Induction of advanced maturation and hastened cocoon spinning observed in this work indicate that PE administration contributed to the increase in the haemolymph ecdysteroid titre leading to the physiological manifestation.

It can be concluded that the PE extracted from common catchfly can be effectively used in sericulture for hastening larval maturation events and synchronizing spinning activities and thereby shortening the mounting period by 12~24 h for 80 % maturation and 24~36 h for 100 % maturation depending on the seasons.

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K. Sashindran Nair*, C. M. Babu, Kanika Trivedy and P. K. Chinya

Central Sericultural Research and Training Institute, Manandavadi Road, Mysore – 570008, Karnataka, India.
*National Silkworm Seed Organization, Central Silk Board, BTM Lay out, Madivala, Bangalore-560068, Karnataka, India, Phone: 080-26282432, E-mail: nairjula@yahoo.com