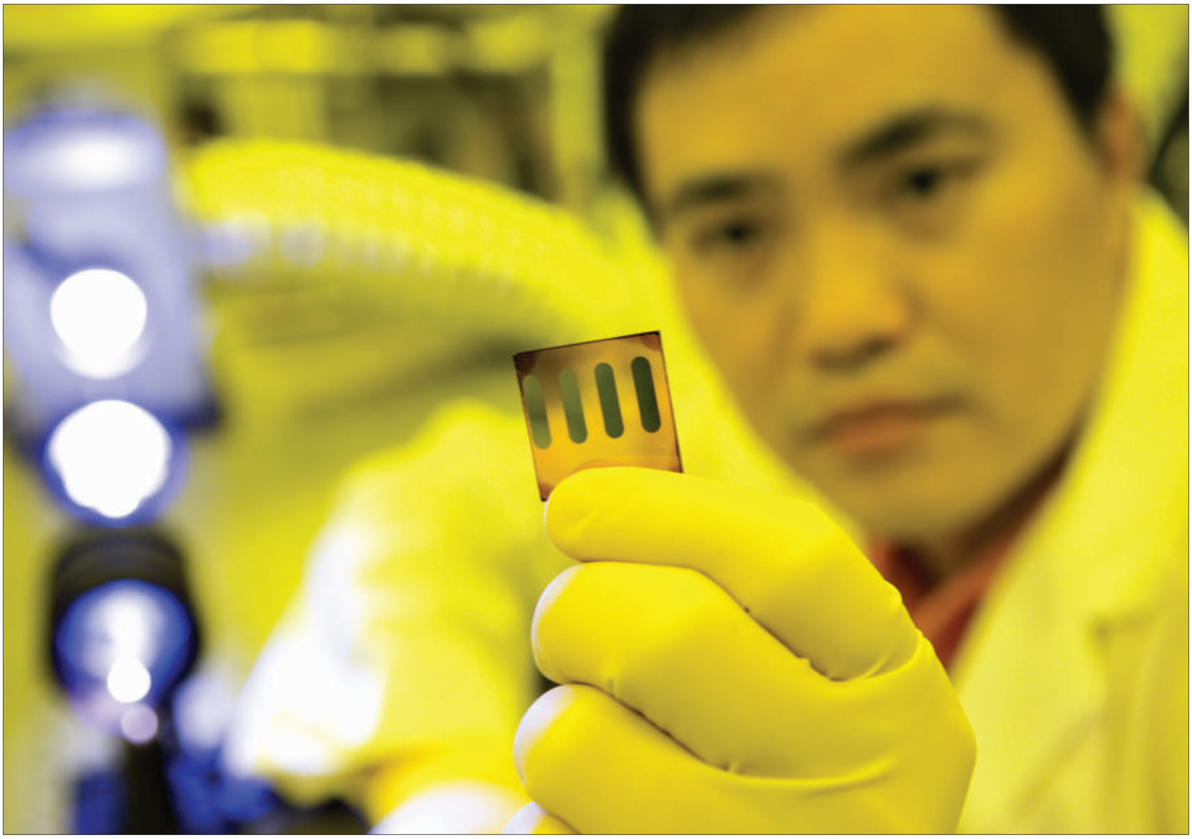

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Research Article
Phyto-ecdysteroids Modulated Synchronisation of Cocoon-spinning in Tasar Silkworm, *Antheraea mylitta* D

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Abstract

Background: Tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) is a polyphagous sericigenous insect, producing tasar silk of high commercial value. Generally, rearing of tasar silkworm (DABA ecorace) is conducted two to three times in a year. During second and third crop rearing sometimes during low temperature regimes cause prolongation of larval duration which causes unsynchronized cocoon-spinning leading to decreased production and productivity. **Methodology:** In the present study, impact of exogenous application of phyto-ecdysteroids has been evaluated on growth, development, moulting, spinning and cocoon-spinning synchronisation pattern of tasar silkworm *A. mylitta.* **Results:** The highest percentage of cocoon spinning was observed with treatment T5 (Sampoorna) which was followed by T4 (*Vitex negundo*). Although the cocoon weight, shell weight and silk ratio of treated and control lots were not varied significantly, marked improvement in cocoon-spinning synchronisation was observed when phyto-ecdysteroid T1 (*Achyranthus aspera*) and T5 (Sampoorna) treatment. **Conclusion:** It is expected that, an application of a refined dose of phyto-ecdysteroid will synchronise the spinning for enhanced production and productivity of the silkworm, *A. mylitta*.

Key words: Phyto-ecdysteroids, synchronisation, cocoon-spinning, tasar silkworm, *Antheraea mylitta*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insect's larval growth, development and metamorphosis are mainly regulated by the endogenous level of two hormones ecdysone (secreted by prothoracic gland) and juvenile hormone (from corpora allata). Their levels in the haemolymph increases due to endocrine signalling received from the brain under the influence of environmental token stimuli. Recently, Guo *et al.*[1] reported nuclear receptor ecdysone-induced protein 75 is required for larval-pupal metamorphosis in *Leptinotarsa decemlineata* and Uryu *et al.*[2] investigated the role of ecdysteroids in adult insects germline development and circadian clock. Ingestion of phytoecdysteroids at in appropriate time in the insects leads to dose-dependent growth and development disruption[3]. The growth and development pace have also been reported to be effected by the use of exogenous phytoecdysteroids resulting in synchronized moulting and in acceleration of maturation events leading to synchronized spinning activities in silkworm, *Bombyx mori*. It is reported that, phyto-ecdysteroid application on mulberry leaves speeding up the maturation events of silkworm to avoid the crop loss due to shortage of mulberry leaf and sudden outbreak of silkworm diseases[4].

Ecdysterone or 20-hydroxy ecdysterone is the most widely occurring phyto-ecdysterone and many plants like *Achyranthus aspera* and *Trianthema portulacastrum* have been identified to have this compound[5]. It is important to note that different plant parts contain different amounts of ecdysteroids and that ecdysteroid concentration varies with the season and location of the plant[6]. Phytoecdysteroids have been reported to occur in over 120 plant families[7]. Trivedy *et al.*[8] extracted ecdysteroid from different plants and a formulation like sampoorna was used commercially in *B. mori* rearing for synchronised maturation of larvae and spinning of cocoons. Availability of phyto-ecdysteroids in weeds and it extraction and purification has been reported by several researchers[5,9-11] in addition[12] isolated ecdysteroids from *Vitex strickeri* using RLCC and recycling HPLC. Therefore, plant based ecdysteroid need to be utilised for synchronisation of spinning.

Rearing of DABA ecorace of tasar silkworm is generally conducted two to three times in a year to produce the tasar silk cocoons. During second and third crop rearing sometimes low temperature cause prolongation of larval duration which cause unsynchronized cocoon-spinning leading to decreased production and productivity. Perusal of earlier research suggests that, al though several phyto-ecdysteroid have been extracted and the formulations were made by several investigators but its impact on *A. mylitta* cocoon-spinning synchronisation was not evaluated. Therefore, present study was undertaken to see the impact of phyto-ecdysteroid on cocoon-spinning synchronisation of tasar silkworm, *A. mylitta* which is one of the economically important sericigenous insects.

MATERIALS AND METHODS

Culturing of the insect: Rearing of tropical tasar silkworm, *A. mylitta* Drury (Lepidoptera: Saturniidae) DABA ecorace was conducted in outdoor conditions of its primary food plants *T. tomentosa* by using standard rearing protocol[13].

Extraction of physot-ecdysteroid: For phyto-ecdysteroid extraction, plant materials (mostly weeds) were collected from the vicinity of Ranchi region. Plants having good quantity of ecdysteroids were subjected to extraction of phyto-ecdysteroids. Plant extracts were prepared using *Achyranthes aspera* (T1), *Trianthema monogyna* (T2), *Chenopodium album* (T3) and *Vitex negundo* (T4). In addition, commercially available formulation of sampoorna, (T5) and Control-DD water (T6) was also used for comparison.

Application of phyto-ecdysteroid: Extracted phyto-ecdysteroid were sprayed on leaves of *Terminalia tomentosa* (food plants) in different concentrations i.e., dose 1 (1.0%), dose 2 (1.5%) and dose 3 (2.0%). Prior to spinning stage, 30 *A. mylitta* larvae of each treatment group were released for feeding on phyto-ecdysteroid sprayed leaves.

Evaluation of phyto-ecdysteroid impact: Impact of various doses of phyto-ecdysteroid was evaluated. To study the cocoon-spinning synchronisation, cocoons and larvae were counted daily. In addition, moth emergence behaviour of treated lots was also recorded.

Statistical analysis: Analysis of variance of cocoon spinning percentage of larvae after application of different doses of plant extract at different duration was also carried out in a randomized block design for three factors namely treatments (6), doses (3) and duration (3) with five replications. Data were statistically analyzed using Microsoft excel software for ANOVA.

RESULTS AND DISCUSSION

Marked differences in growth and development of tasar silkworm were found when larvae were fed with exogenous phyto-ecdysteroids sprayed leaves. Phyto-ecdysteroid caused synchronized moulting and acceleration of maturation events leading to synchronized spinning activities of tasar silkworm. Analysis of variance of cocoon spinning percentage of larvae after application of different doses of plant extract at different duration was carried out in randomized block design for three factors namely treatments (6), doses (3) and duration (3) with five replication. Cumulative effect of duration and treatment is shown in Table 1, which revealed that the cocoon spinning percentage of plant extract T1-T5 varied from 31.53-32.04%, whereas, T6 (Control) was observed to be 4.47 %. The duration means (DR1 to DR3) of cocoon spinning ranged from 21.32-28.86% and interaction between duration and treatments means varied from 11.60-55.33% except with T6 (DR1 to DR2) which varied from 1.60-10.20%. The results indicated that mean difference of different plant extracts and duration were significantly different at $p<0.05$. Phyto-ecdysteroid significantly affected spinning duration.

Dose vs duration means (Table 2 and 3) were significantly different at $p<0.01$ and $p<0.05$ which varied from 11.53-35.63%. However, highest variability was recorded in DS3 vs DR1 followed by DS3 vs DR2 and DS2 vs DR1, respectively. However, the level of durations was highly significant at $p<0.01$ and significant difference were not found among different levels of doses, which varied from 27.13-27.40%.

Three factor interaction mean values of cocoon spinning percentage for dose vs duration vs treatment was observed to be lowest in T5 (DS3×DR3) at 8.00 and 70.00% highest at T1 (DS3×DR1). Dose and duration vs T6 (control) mean values varied from 1.60-10.20%. Overall it was found that level of doses and level of durations with different plant extracts are significantly different from each other at $p<0.05$ (Table 4).

The calculated F-test estimates for treatment, dose, duration, treatment vs dose, treatment vs duration, dose vs duration and treatment vs dose vs duration were 965.35**, 0.33^{NS}, 0.54^{NS}, 434.22**, 348.84**, 564.07** and 84.39**, respectively. Except dose mean, other mean differences of single factor, two factor and three factor interactions were highly significant at $p<0.01$ (Table 5).

Table 1: Cocoon spinning percentage of larvae of *A. mylitta* after application of different doses of plant extracts

	Doses (Mean±SE)			
Treatments	DS1	DS2	DS3	Treatment mean over doses
T1	32.53±3.89	31.20±1.55	31.73±4.28	31.82±1.95
T2	32.20±3.88	31.07±1.76	31.80±4.05	31.69±1.92
T3	32.27±2.91	31.87±1.36	31.40±3.86	31.84±1.64
T4	31.67±1.09	32.33±3.94	32.13±4.17	32.04±1.90
T5	31.27±2.84	31.87±6.29	31.47±7.35	31.53±3.28
T6	4.47±1.12	4.47±1.12	4.47±1.12	4.47±0.63
Dose mean over treatments	27.40±1.57	27.13±1.68	27.17±2.0	
CD at 5%	Treatment	1.001		
	Dose	0.708		
	Treatment×dose	1.734		

Table 2: Cocoon spinning percentage of larvae of *A. mylitta* after application of different plant extracts at different durations

	Durations (Mean±SE)			
Treatments	DR1	DR2	DR3	Treatment mean across durations
T1	24.67±2.11	41.13±2.27	29.67±4.02	31.82±1.95
T2	24.40±2.07	40.00±2.13	30.67±4.12	31.69±1.92
T3	28.47±1.77	38.73±1.92	28.33±3.66	31.84±1.64
T4	38.67±2.12	40.00±1.21	17.47±2.31	32.04±1.90
T5	55.33±4.47	27.67±3.15	11.60±1.33	31.53±3.28
T6	1.60±0.13	1.60±0.13	10.20±0.46	4.47±0.63
Duration mean across treatments	28.86±1.98	31.52±1.70	21.32±1.50	
CD at 5%	Treatment	1.001		
	Duration	1.734		
	Treatment×duration	0.708		

Table 3: Cocoon spinning percentage of larvae of *A. mylitta* after application of different doses of plant extract at different durations (Interactive effect)

	Durations (Mean±SE)			
Doses	DR1	DR2	DR3	Dose mean across durations
DS1	18.63±1.90	29.67±2.49	33.90±2.95	27.40±1.57
DS2	32.30±3.47	30.57±2.74	18.53±1.61	27.13±1.68
DS3	35.63±3.84	34.33±3.54	11.53±0.48	27.17±2.09
Duration mean across doses	28.86±1.98	31.52±1.70	21.32±1.50	-
CD at 5%	Dose	0.708		
	Duration	1.734		
	Dose×duration	1.734		

Table 4: Cocoon spinning percentage of larvae of *A. mylitta* after application of different doses of plant extracts at different durations

	Treatment (Mean±SE)					
Dose×duration	T1	T2	T3	T4	T5	T6
DS1						
DR1	14.40±1.36	15.20±1.39	19.60±0.40	29.00±0.71	32.00±0.45	1.60±0.24
DR2	33.60±0.93	31.00±0.71	31.60±1.21	36.60±1.21	43.60±1.44	1.60±0.24
DR3	49.60±1.03	50.40±0.68	45.60±1.57	29.40±1.21	18.20±1.28	10.20±0.86
DS2						
DR1	30.60±2.16	25.80±2.08	32.40±1.21	39.40±1.25	64.00±0.71	1.60±0.24
DR2	37.40±1.36	39.00±1.14	36.80±1.16	45.60±1.03	23.00±0.89	1.60±0.24
DR3	25.60±1.08	28.40±1.50	26.40±1.72	12.00±0.71	8.60±0.68	10.20±0.86
DS3						
DR1	29.00±0.71	32.20±1.32	33.40±1.29	47.60±1.33	70.00±0.71	1.60±0.24
DR2	52.40±1.33	50.00±0.71	47.80±1.24	37.80±0.97	16.40±0.81	1.60±0.24
DR3	13.80±1.07	13.20±0.86	13.00±0.84	11.00±0.63	8.00±0.45	10.20±0.86
CD at 5%	Treatment×dose×duration	1.734				

Table 5: Analysis of variance of cocoon spinning percentage (Mean±SE) of larvae of *A. mylitta* after application of different doses of plant extract at different durations

Source of variation	Degree of freedom	Sum of squares	Mean squares	F-value
Replicates	4	2.76	0.69	0.12NS
Treatment	5	27995.86	5599.17	965.35**
Dose	2	3.80	1.90	0.33NS
Treatment×dose	10	31.58	3.16	0.54**
Duration	2	5037.07	2518.53	434.22**
Treatment×duration	10	20233.11	2023.31	348.84**
Dose×duration	4	13086.87	3271.72	564.07**
Treatment×dose×duration	20	9789.62	489.48	84.39**
Error (C)	212	1229.63	5.80	
Total	269	77410.30	287.77	

**Significant p<0.01, NS: Non significant

The trend of commercial parameters of cocoons after application of different plant extracts prior to spinning (Table 6 and Fig. 1). Mean cocoon weight, shell weight and silk ratio of treated and control lots did not vary significantly whereas some improvement was seen with T1 and T5 over control. No significant difference was observed on moth emergence and fecundity of pupae obtained after application of different plant extracts prior to spinning (Table 6).

Ecdysterone or 20-hydroxy ecdysterone is the most widely occurring phyto-ecdysterone and many plants like *Achyranthus aspera* and *Trianthema portulacastrum* have been identified to have this compound[5]. It is important to note that different plant parts contain different amounts of ecdysteroids and that ecdysteroid concentration varies with the season and location of the plant[6]. Phytoecdysteroids have been reported to occur in over 120 plant families[7]. Our results corroborate the finding of Shivakumar *et al.*[14] where phyto-ecdysteroids affected the larval maturation and economic parameters of *B. mori*. Similarly, Trivedy *et al.*[8] also reported impact of "Sampoorna" a combination of phytogenous ecdysteroids which induces the synchronization of spinning. Our finding indicates that after refinements on phyto-ecdysteroids concentration and dose, it can be used for synchronization of spinning. It will help in shortening and uniform maturation period of larvae to minimise losses. interestingly, the highest moulting percentage and cocoon

Fig. 1(a-c): (a) Mature larvae, (b) Spinning age larvae and (c) Cocoons of tasar silkworn, *A. Mylitta* used in the present study

Table 6: Impact of plant extracts application prior to spinning on cocoon characters, moth emergence and fecundity

Treatments	Cocoon weight (g)±SE	Shell weight (g)±SE	Silk ratio (%)±SE	Moth emergence (%)±SE	Fecundity (No)±SE
T1	12.316±0.325	2.087±0.036	17.013±0.385	86.400±0.991	216.500±1.098
T2	12.149±0.622	1.987±0.075	16.567±0.685	85.200±0.940	218.100±2.126
T3	12.260±0.702	1.951±0.066	16.182±0.665	85.700±1.620	218.000±0.471
T4	12.350±0.552	2.029±0.072	16.618±0.674	85.900±1.703	215.500±1.003
T5	12.495±0.602	2.022±0.051	16.376±0.497	86.800±1.638	219.400±1.661
T6	12.002±0.574	1.951±0.076	16.407±0.545	86.700±0.790	220.200±1.143
F value	0.0879	0.6593	0.2349	0.2671	2.0354

**Significant p<0.01, NS: Non significant

spinning were experienced with the treatment T5 (Sampoorna) followed T4 (*Vitex negundo*) without affecting the cocoon weight, shell weight and silk ratio significantly. The improvements were seen with T1 (*Achyranthus aspera*) and T5 (Sampoorna) over the control. Ingestion of phytoecdysteroids inappropriate time of the insect's cycle can lead to dose-dependent growth and development disruption[3]. Recently, Guo *et al.*[1] reported that nuclear receptor ecdysone-induced protein 75 is required for larval-pupal metamorphosis in *Leptinotarsa decemlineata.* Uryu *et al.*[2] investigated the role of ecdysteroids in adult insects germline development and circadian clock. The growth and development pace have been also reported to be affected by the use of exogenous phytoecdysteroids resulting into synchronized moulting and in acceleration of maturation events leading to synchronized spinning activities in silkworm, *Bombyx mori*. Problem of unforeseen shortage of mulberry leaf occasional crop losses due to or sudden outbreaks of diseases in the last phase of rearing has been deciphered by speeding up maturation events with similar chemicals[4]. Therefore, it is expected that the plant based phyto-ecdysteroid is having enormous scope for the benefit of tasar silkworm rearing, maturation and synchronisation.

CONCLUSION

Tropical tasar silkworm, *A. mylitta* rearing is undertaken two to three times in a year to produce the tasar silk cocoons. During the second and third crop rearing sometimes low temperature cause prolongation of larval duration which causes unsynchronized cocoon-spinning leading to reduction in productions and productivity. Our findings indicates that, after refinement of phyto-ecdysteroids concentration and dose, it can be used for synchronization of spinning. In addition, it will help in shortening and uniform maturation period of larvae to minimise the losses.

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