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Synchronization of maturation in *Bombyx mori* L. (Lepidoptera: Bombycidae) utilizing phytoecdysteroids from local plants

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Abstract

Sericulture is a labour-intensive enterprise which involves establishment of mulberry plantation for mulberry leaves production, the sole food of silkworms, *Bombyx mori* L.; silkworm rearing and cocoon processing. Rigorous investigations included the utilization of phytoecdysteroids (PEs) in extremely low doses to enhance silkworm larval development. PEs is plant ecdysteroids considered as secondary metabolites used to protect plants from phytophagous insects. PEs in this study were prepared from local plants such as *Amaranthus spinosus* L., *Ipomea pes-caprae* L. and *Portulaca oleracea* L. and administered to mulberry leaves sprayed with 100% and 50% concentrations to 5th instar silkworms 116, 92, 76, 52 and 28 hours before spinning. It resulted to synchronized maturation of silkworms which took for 2-3 hours compared to control group which reached about 6-7 hours. Consequently, the utilization of PEs also reduced the time of mounting activities, saving approximately 72 - 77%. It also contributed to the regulated growth and development of the silkworm larvae which could consequently reduce the labor cost of cocoon production particularly in mounting. The findings demonstrate that the utilization of PEs is a potential valuable technology in sericulture which could direct a significant increase in efficiency in silkworm rearing for cocoon production.

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Introduction

Sericulture, the art and science of producing silk, the Queen of Textiles, is a laborious enterprise, commencing with the establishment of mulberry plantations as sources of mulberry leaves which are principally used as a source of nutrition for silkworms, *Bombyx mori* L. The growing or rearing of *B. mori* L. to produce cocoons that are processed into silk yarn and fashioned into clothing is another aspect of sericulture. Several efforts have been done in recent years to increase the quantity and quality of silk (Hiware, 2006 as cited in Srivastava & Upadhay, 2013), including nutrient augmentation of mulberry leaves, spraying with antibiotics, juvenile hormone, plant products, and ecdysteroids.

Interestingly, ecdysteroids, which are insect hormones involved in the development and molting process, are the subject of impressive and extensive research (Gorelick-Feldman, 2009 as cited in Srivastava & Upadhay, 2012). Insects' life cycles are regulated by a variety of endocrine hormones that are chemically distinct from one another. It is well known that two types of hormones influence insect molting and metamorphosis: ecdysteroids and juvenile hormones (JH). In most insect larvae, such as those of the *B. mori*, the prothoracic glands produce and secrete ecdysone, which is then hydroxylated by peripheral tissues to produce 20-hydroxyecdysone. The 20-hydroxyecdysone then exerts hormonal effects on target tissues, such as the epidermis. The corpora allata synthesizes and secretes JH, which is a sesquiterpene. The interaction of ecdysteroids and JH throughout insect postembryonic development orchestrates the transition from one developmental stage to the next. During their life cycle, ecdysteroids start the molting process, and JH controls the quality of the molt (Thakur, 2011). JH is also known to affect the larval period, according to Wigglesworth (1936, as cited in Thakur, 2011).

Nair *et al.* (2005) elucidated that exogenous administration of analogues or mimics of these hormones has the potential to disturb normal insect growth by disrupting metabolic functions. The balance between these two hormones, which is determined by a well-programmed interplay, determines the pattern of physiological activities in insects at any particular time.

Ecdysteroids in plants are called phytoecdysteroids (Pes) and are considered secondary metabolites. The ecdysteroids, 20-E hydroxyecdysone (ecdysone) and sesquiterpene are the two most prevalent hormones detected in insects, and juvenile hormone is typically accompanied by a variety of additional minor ecdysteroids (Al Naggar *et al.*, 2017). Ecdysteroid has a beneficial reaction to trace amounts of these hormones or its analogues. It has been observed to affect the reproductive potential of *Bombyx mori* (Pondeville *et al.*, 2008; Parlak *et al.*, 1992; Kawaguchi *et al.*, 1993; Okuda *et al.*, 1993 as cited in Srivastava & Upadhay, 2012).

Additionally, the use of extremely low doses has shown to be useful for enhancing the synchronization of development and yield of some commercially significant arthropods (Lafont *et al.*, 2021). The most well-researched examples involve silk moths (*B. mori* L.), where protocols have been developed for the preparation of ecdysteroid-containing extracts from native plants and application to the food plant (Morus spp) at specific developmental stages, which lead to more synchronous cocoon formation (Changrakala *et al.*, 1998 as cited in Al Naggar *et al.*, 2017), hence, this investigation to compare the synchronization of maturation in *B. mori* L. using PEs from various local plants.

Materials and methods

The leaves and branches of local plants, *Amaranthus spinosus* L, and *Portulaca oleracea* L. were collected, washed, and air-dried while seeds of *Ipomea pes-caprae* L. were gathered. The plant parts were pulverized and soaked in methanol and subjected to rotary evaporation to obtain the extract. High performance liquid chromatography (HPLC) confirmed the presence of PEs in these local plants.

The research design involved three-factor factorial experiment in Complete Randomized Design (CRD) replicated three times in which factor P represented the plant-source of PE; Dn corresponded the nth day of the 5th instar silkworms when the phytoecdysteroid-enriched mulberry leaves were fed to the silkworms and C represented the concentrations of PE.

Plant-source of PE (P)

P1 *Amaranthus spinosus*

P2 *Ipomea pes-caprae*

P3 *Portulaca oleracea*

Day or Hours of application of PE on 5th instar (Dn) before spinning

D1 1st day (116 hr)

D2 2nd day (92 hr)

D3 3rd day (76 hr)

D4 4th day (52 hr)

D5 5th day (28 hr)

Concentration

C1 100%

C2 50%

Application of phytoecdysteroids (PEs)

The methods of preparing the phytoecdysteroids and their applications by Rufaie *et al.* (2012) were adopted. The crude methanol plant extract was re-dissolved in methanol to prepare 10% stock solution (10 g in 100 ml). The solution was diluted further into 1:100 and 1:50 (v/v) concentrations of the extract in distilled water.

There were three replications during the conduct of the study and a single replication contained 50 silkworm larvae fed 4 times a day at 6:00 and 10:00 in the morning; and 2:00 in the afternoon and 6:00 in the evening with mulberry leaves var. Alfonso sprayed with PE concentration. The sets of replicates of the 5th instar silkworm larvae were prepared and fed with 150 g mulberry leaves with 7.5 ml each of the concentration of 1:100 and 1:50 (v/v) at 116 hr (Day 1), 92 hr (Day 2), 76 hr (Day 3), 52 hr (Day 4) and 28 hr (Day 5) before spinning their cocoons.

Silkworm Rearing

The rearing of silkworm was conducted at the Sericulture Research and Development Institute, Bacnotan, La Union, Philippines. A week prior to the rearing activity, the rearing house and paraphernalia (rearing trays, nets and cocooning frames) were thoroughly cleaned and sanitized with 5% hypochlorite solution.

Newly-hatched bivoltine silkworm larvae were reared following the standard silkworm rearing management implemented by SRDI. The temperature and relative humidity were maintained at 26 to 28^{o}C and 75 - 90%, respectively. Two rearing trials were conducted in 2021 covering the months of March-April and August–September.

The mulberry leaves enriched with phytoecdysteroids from the plant extracts with 1:100 and 1:50 concentrations were administered orally to 5th instar silkworms at 116 hr, 92 hr, 76 hr, 52 hr and 28 hr before spinning.

Data Gathering

The cumulative number of matured and mounted silkworms was gathered after every hour at the 6th day of the 5th instar, which was the expected day of spinning their cocoons.

Results and discussion

The physiological function of an exogenous ecdysteroid in the insect development system is responsible for the variance in larval and mounting times. Ecdysone levels in feeding larvae are always low, but they rise to a pupation-inducing peak before pupation. By administering an additional dose of plant-based ecdysteroid at the crucial time, the pupation-triggering peak of ecdysteroid content in silkworms is advanced, altering the behavior of the larvae (Sehnal, 1989, as cited in Nair *et al.*, 2005). The primary goal of using PE in sericulture is to hasten larval development in the final larval instar and to synchronize the spinning of the cocoon so that the larvae can be transferred to the cocoon spinning apparatus early and collectively, and the larvae can produce cocoons virtually simultaneously.

Since the maturation and spinning activities of silkworms are not uniform, it is commonly practiced by farmers or sericulturists that during silkworm rearing, silkworms are forced to be picked and mounted in cocooning structures. Maturation and mounting may usually extend to 2- 3 days and even more during cooler seasons, this concern involves lot of time, labor and extra mulberry leaf and also ends up in higher production cost. It had been investigated that the administration of ecdysteroid, the problem could be avoided or minimized. The Chinese made used of PEs in sericulture to regulate silkworm rearing during the latter stage of larval development (Wong *et al.*, 1979; Chow & Lu, 1980, as cited in Nair *et al.*, 2005). Table 1 presents the cumulative average percentage of matured 5th instar silkworm (*B. mori*) larvae for mounting at specified time during the 6th day on which only a few silkworms (6.49%) under the control (no PE) treatment started to mature between 8:00 – 9:00 in the morning and reached complete maturation (100%) between 12:00 noon to 1:00 in the afternoon which was approximately six (6) hours. This entailed that a number of silkworms took time to mature, waited for every silkworm to reach maturation and prepared for mounting to their cocooning frames.

Table 1. Cumulative average percentage of matured 5th instar silkworm (*B. mori*) larvae for mounting at specified time during the 6th day.

Treatments	Time of Maturation						
	6:30- 7:59 AM	8:00- 8:59 AM	9:00- 9:59 AM	10:00- 10:59 AM	11:00- 11:59 AM	12:00- 12:59 AM	1:00 PM
Control	-	6.49	28.57	58.44	81.82	94.81	100.00
P1C1D1	41.86	100.00					
P1C2D1	43.02	100.00					
P2C1D1	44.59	78.38	100.00				
P2C2D1	29.58	80.28	100.00				
P3C1D1	49.41	100.00					
P3C2D1	41.98	100.00					
P1C1D2	48.75	100.00					
P1C2D2	48.05	100.00					
P2C1D2	56.06	98.48	100.00				
P2C2D2	53.62	92.75	100.00				
P3C1D2	48.15	100.00					
P3C2D2	49.40	100.00					
P1C1D3	44.83	100.00					
P1C2D3	42.70	100.00					
P2C1D3	62.50	100.00					
P2C2D3	49.18	100.00					
P3C1D3	45.88	100.00					
P3C2D3	46.34	100.00					
P1C1D4	42.35	100.00					
P1C2D4	45.78	100.00					
P2C1D4	47.62	100.00					
P2C2D4	43.53	100.00					
P3C1D4	45.88	100.00					
P3C2D4	44.83	100.00					
P1C1D5	45.68	100.00					
P1C2D5	53.16	100.00					
P2C1D5	48.10	100.00					
P2C2D5	47.22	100.00					
P3C1D5	49.41	100.00					
P3C2D5	44.83	100.00					

Note: P1= A. Spinosus, P2= I.pes-caprae, P3= *P. oleracea,* C1= 100%, C2= 50%, D= Day

The treated silkworms, regardless of time of administration and plant sources of PEs, however, reached complete maturation between 8:00 – 8:59 in the morning with approximately 50% of silkworms reaching maturity during the first hour on the expected day of spinning and completed their maturation the following hour except those treated with PE sourced from *I. pes-caprae* applied during the first and second day of the 5th instar stage. The results demonstrate synchronized maturation of 5th instar silkworms when applied with PEs from the identified plant sources. It also implies that the larval period of the treated silkworms was shortened by at least four (4) hours as compared to the negative control.

The findings are similar with that of Trivedy *et al.* (2003) who noted that the application of PE on late-age silkworms made 80% ready for mounting 18 hours after treatment and only 37% were ready for mounting under the control group. Other investigation involved the use of Sampoorna, a commercial brand of PE which resulted to hastened maturation of silkworms, synchronized spinning and shortened mounting period.

Table 2. Time saved in mounting matured silkworms with the utilization of Phytoecdysteroids (PEs).

Treatments	Time Saved (hr)	Average Time Saved (hr)	Time Saved (%)	Average Time Saved (%)
P1C1D1	5.02	4.69	77.18	72.05
P1C2D1	5.02		77.18	
P2C1D1	4.02		61.79	
P2C2D1	4.02		61.79	
P3C1D1	5.02		77.18	
P3C2D1	5.02		77.18	
P1C1D2	5.02	4.69	77.18	72.05
P1C2D2	5.02		77.18	
P2C1D2	4.02		61.79	
P2C2D2	4.02		61.79	
P3C1D2	5.02		77.18	
P3C2D2	5.02		77.18	
P1C1D3	5.02	5.02	77.18	77.18
P1C2D3	5.02		77.18	
P2C1D3	5.02		77.18	
P2C2D3	5.02		77.18	
P3C1D3	5.02		77.18	
P3C2D3	5.02		77.18	
P1C1D4	5.02	5.02	77.18	77.18
P1C2D4	5.02		77.18	
P2C1D4	5.02		77.18	
P2C2D4	5.02		77.18	
P3C1D4	5.02		77.18	
P3C2D4	5.02		77.18	
P1C1D5	5.02	5.02	77.18	77.18
P1C2D5	5.02		77.18	
P2C1D5	5.02		77.18	
P2C2D5	5.02		77.18	
P3C1D5	5.02		77.18	
P3C2D5	5.02		77.18	

Note: P1=*A. spinosus,* P2=*I.pes-caprae,* P3=*P. oleracea,* C1=100%, C2=50%, D=Day

The study of Rufaie (2012) also disclosed that different concentrations of PE were sprayed on mulberry leaves and fed to 5th instar larvae and resulted synchronized maturation. Moreover, the aforementioned results corroborated with Nair *et al.* (2005) that the primary goal of utilizing PE in sericulture was to accelerate larval development in the final larval instar and synchronized spinning process. A similar study was conducted by Jat *et al.* (2010) who made used of *O. sanctum* on silkworms which contained PE that contributed to high percentage of maturation of the 4th instar larvae.

The time saved in mounting matured silkworms for cocoon production is also presented in Table 2. Results show that there was an average of 4.69 (4 hr 41 min) to 5.02 (5 hr 1 min) hours of time saved, which is equivalent to 72 - 77% time reduction in mounting due to the earlier and synchronized maturation of silkworms in the PE-treated groups as compared to the control group.

It can be implied then that the utilization of PEs commonly given at the final instar, can save a lot of skilled work that would otherwise be necessary to pick up only the matured silkworms and a large number of mulberry leaves in a timely manner.

Conclusion

PEs were prepared from local plants such as *Amaranthus spinosus* L., *Ipomea pes-caprae* L. and *Portulaca oleracea* L. and administered to mulberry leaves sprayed with 100% and 50% concentrations to 5th instar silkworms 116, 92, 76, 52 and 28 hours before spinning and resulted to synchronized maturation of silkworms which took for 2-3 hours compared to control group which reached about 6-7 hours. Consequently, the utilization of PEs also reduced the time of mounting activities, saving approximately 72 - 77%.

It also contributed to the regulated growth and development of the silkworm larvae which could consequently reduce the labor cost of cocoon production particularly in mounting. The findings demonstrate that the utilization of PE is a potential valuable technology in sericulture which could direct a significant increase in efficiency in silkworm rearing for cocoon production and contribute to improved productivity in sericulture.

Recommendations

1. In order to achieve refined results, it is recommended to standardize the utilization of PEs to be administered in silkworm rearing.
2. There is a need to undergo verification trial at the farmer's level.

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