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EFFECT OF PHYTOECDYSTEROID ON SILK PRODUCING POTENTIAL OF MULTIVOLTINE MULBERRY SILKWORM *BOMBYX MORI* LINN

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ABSTRACT

Effect of phytoecdysteroid on silk producing potential of multivoltine mulberry silkworm *Bombyx mori* was studied. Experiments were performed by 40, 50, 60 and 70% concentration of phytoecdysteroid obtained from *Achyranthes* leaf extract. A control set was always maintained with each set of experiment. Maximum shell ratio (15.45 $\pm$ 1.07) was noticed to be in case of the double treatment (IV and V instar larvae were treated) by 60% phytoecdysteroid and minimum (12.25 $\pm$ 0.831) was recorded in triple treatment (III, IV and V instar larvae were treated) by 70% phytoecdysteroid concentration. Phytoecdysteroid treatment,if applied tactfully,may be useful for boosting up the sericulture industry as well as the economy of silkworm rearing.

INTRODUCTION

The silk industry has developed as a popular cottage industry providing self employment to more than ten million rural persons in the unorganized sector. It is well known for its low investment and quick and high return which makes it an ideal industry fitting well in to the socio-economic frame of India. An analysis of the international trends in the silk production suggests that sericulture has better prospects for growth in the developing countries than in the developed countries. The efforts are being made to evolve new technologies that are cast effective, labour saving and ecofriendly. In order to increase the production of silk, efforts have been made to study the effect of ecological factors (Upadhyay *et al.,* 2004), relative humidity (Upadhyay and Mishra, 2002), refrigeration of eggs (Pandey and Upadhyay, 2000) and cocoon (Upadhyay *et al.,* 2006 and Upadhyay *et al.,* 2009), magnetization of eggs (Tripathi and Upadhyay, 2005a, 2005b and Upadhyay and Tripathi, 2006), cocoon (Upadhyay and Prasad, 2010a and 2010b) and larval performance (Prasad and Upadhyay, 2011). The ecdysone has been noticed to influence the reproductive potential of *Bombyx mori* (Pondeville *et al.,* 2008; Parlak *et al.,* 1992; Kawaguchi *et al.,* 1993 and Okuda *et al.,* 1993). Vitamins of B-complex group and certain essential sugars, proteins, amino acids, minerals etc. are responsible for the proper growth and development of the silkworm, *B. mori* (Faruki,1998). A number of researchers have worked on the effects of vitaminenriched food on the reproduction of *B. mori* females (Faruki *et al.,* 1992 and Saha and Khan, 1999) .In recent years, many attempts have been made to improve the quality and quantity of silk (Hiware, 2006), through enhancing the leaves with nutrients, spraying with antibiotics, juvenile hormone, plant products, with JH-mimic principles or using extracts of plants. Ecdysteroids play key role in moulting and metamorphosis in insects. Plant-produced insect smoulting 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 (Schmulz *et al.,* 2002).The plant like *Achyranthes aspera* (Lat jeera) and *Cassia tora* (Choti chakwar) have been identified to have phytoecdysteroids (Lafont and Horn, 2004). 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 and Chou and Lu, 1980). 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. Keeping this in view, an attempt has been made to investigate the influence of phytoecdysteroid on shell ratio of multivoltine mulberry silkworm (*B. mori*).

MATERIALS AND METHODS

Seed cocoon

The seed cocoons (pupa enclosed in silken case) of multivoltine mulberry silkworm (*Bombyx mori* nistari), a native of West Bengal in India, were obtained from the silkworm grainage Behraich, Directorate of Sericulture Uttar Pradesh and were maintained in the plywood trays (23 x 20 x 5cm) under the ideal rearing conditions (Krishnaswamy *et al.,* 1973) in the silkworm laboratory, department of Zoology, D. D. U.

Gorakhpur University, Gorakhpur. The temperature and relative humidity were maintained at 26 ± 1ºC and 80 ± 5 % RH respectively till the emergence of moths from the seed cocoons. The moth emerged generally in the morning. The newly emerged moths were picked up and kept sexwise in separate trays to avoid copulation. The male moths were smaller in size but more active than the female moths which were comparatively larger and less active.

Copulation

Moths have a tendency to pair immediately after the emergence and therefore, the female moths, required to copulate with the male moths, were allowed their mates for copulation. 360pairs, each containing one male and one female from newly emerged moths were allowed to mate at 26 ± 1ºC, 80 ± 5% RH and 12h / day dim light condition. After mating, the paired moths were decoupled and male moths were discarded while the female moths were allowed to egg lying.

Oviposition

Just after separation, the gravid females laid eggs on the sheet of paper in dark condition at 26 ± 1ºC and 75 ± 5% RH. The egg laying moths were covered by open plastic cellules to prevent intermixing of egg masses deposited by different moths. After 24h of egg lying, the female moths were individually examined for their disease freeness. The disease free layings (D. F. L'[s]) were treated with 2% formalin for 15min to increase the adhesiveness of egg of the paper sheet and surface disinfection. Thereafter, the egg sheets with egg laid on were thoroughly washed with running water to remove formalin and eggs were dried in shade. The dried eggs, thus obtained, were taken for phytoecdysteroid treatment under various experimental conditions.

Experimental design

To observe the influence of bioactive phytoecdysteroid hormone on the performance of *Bombyx mori*, the experiments were performed with different concentrations of phytoecdysteroid hormone with respect to the treatment of III, IV and V instar larvae.For extraction of phytoecdysteroid, the leaves of *Achyranthes aspera* were collected, washed thoroughly with distilled water and dried in incubator at 37ºC. The dried leaves were powdered with the help of mechanical device. Further, 50gm powder, thus obtained was subjected to extraction through soxlet apparatus with 250mL distilled water for 40h. After 40h of extraction a little amount of concentrated solution was obtained which was dried and 6.75g powdered material was obtained. The dried powder was dissolved in distilled water as 5gm in 25mL water and used this solution for further experiment as 100% concentration of phytoecdysteroid. For further experiment the suitable narrow range of *Achyranthes* phytoecdysteroid concentration viz; 40, 50, 60 and 70 % were taken.Thus, four phytoecdysteroid concentrations were applied topically by spraying as 10mL on 100g mulberry leaves and the larvae were fed on the treated leaves. Three set of experiments were designed viz single, double and triple treatment of larvae.

Single treatment

Single treatment of larvae was performed with the V instar larvae just before two days of the beginning of larval spinning. 100 larvae were taken out from the BOD incubator and the mulberry leaf, treated with 40% concentration of *Achyranthes* leaf extract, was given as food. Further, the treated larvae were given normal mulberry leaves as food.

Double treatment

Double treatment of larvae was started from the final stage of IV instar larvae**.** In the first treatment, 100 larvae of IV instar were treated just before two days of IV moulting, by providing treated mulberry leaf with 40% solution of *Achyranthes* leaf extract. The treated larvae were then transferred in BOD incubator for further rearing and development. Further, second treatment for the same larvae was given at the final stage of V instar *i.e.* just before two days of spinning.

Triple treatment

For triple treatment, the third instar larvae just before III moulting were separated from BOD incubator. In the first treatment 100 larvae of III instar were treated by providing extract treated mulberry leaf and kept in BOD incubator for rearing. The second treatment of same larvae was done just before two days of IV moulting ie at the final stage of IV instar larvae and transferred in BOD incubator for rearing. Third treatment was given to V instar larvae, two days before the start of spinning. Thus, in the triple treatment III, IV and V instar larvae were treated.

Similar experiments were performed by 50, 60 and 70% concentration of phytoecdysteroid obtained from *Achyranthes* leaf extract. A control set was always maintained with each set of experiment.

Shell ratio

To estimate the shell ratio, the weight of 20 cocoons and 20 shells from each batch were recorded separately on the fifth day of spinning. Three replicates of each experiment were made. The shell ratio percentage was calculated based on 20 cocoons and 20 cocoon shells taken at random from the good cocoon lot. Shell ratio was calculated as given below based on following formula (Ghosh, 1987)

$$\text{Shell ratio (\%)} = \frac{\text{Weight of cocoon shells}}{\text{Weight of cocoon}} \times 100$$

RESULTS

Shell ratio

The data presented in table 1a indicates that change in the phytoecdysteroid concentration and the number of larval treatment infruenced the shell ratio. With the increasing number of larval treatment from one to two times, the shell ratio increased in case of 40, 50 and 60% phytoecdysteroid treatment but triple treatment caused notable decline in the shell ratio in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the shell ratio with increase in the number of treatment from single to triple. The trend of increase in the shell ratio with increasing number of treatment has been recorded to be almost same in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum shell ratio was noticed to be 15.45 ± 1.07 in case of the double treatment by 60% phytoecdysteroid concentration and the minimum 12.25 ± 0.831 was recorded

Table 1a: Effect of phytoecdysteroid treatment on the shell ratio of *Bombyx mori*

Number oftreatment (Larval instar)	Phytoecdysteroid concentration (%)					F_1 ration$_1$ = 4
	Control(X1)	40(X2)	50(X3)	60(X4)	70(X5)	
Single (V)	13.28=0.526	13.600.937	14.000.630	14.300.701	13.220.902	4.13*
Double (IV-V)	13.28=0.526	13.920.581	14.500.480	15.451.07	12.580.462	
Triple (III-V)	13.28=0.526	13.150.750	13.020.725	12.750.920	12.250.831	

F_2 ratio =13.26**; n_2 = 2; **P1 <0.05; **P2 <0.01; Each value represents mean + S. E. of three replicates; X1, X2, X3, X4 and X5 are the mean values of the shell ratio in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively

Table 1b: Post-hoc test showing group difference in the effect of phytoecdysteroid treatment on the shell ratio of *Bombyx mori*

Mean differencein betweengroups	Number of treatment		
	Single	Double	Triple
X1 ~ X2	0.32	0.64	0.13
X1 ~ X3	0.72	*1.22	0.26
X1 ~ X4	1.02	*2.17	0.53
X1 ~ X5	0.06	0.43	1.03
X2 ~ X3	0.40	0.58	0.13
X2 ~ X4	0.70	*1.53	0.40
X2 ~ X5	0.38	1.07	0.90
X3 ~ X4	0.30	0.95	0.27
X3 ~ X5	0.78	*1.65	0.77
X4 ~ X5	1.08	*2.60	0.50

Honestly significant difference (HSD) = q" MS within / n; = 5.05" 0.1508/ 3 = 1.13; MS = Mean square values of ANOVA Table; q = Studentized range static; n = Number of replicates; * Showing significant group difference; X1, X2, X3, X4 and X5 are the mean values of the shell ratio in control,40%,50%,60% and 70% phytoecdysteroid concentration respectively

in case of triple treatment by 70% phytoecdysteroid concentration.

Two-way ANOVA indicates that number of larval treatment (P_2 < 0.01) and variation in phytoecdysteroid concentration (P_1 < 0.05) significantly influenced the shell ratio. The Post-hoc test (table 1b) indicates significant group difference in the shell ratio in between control and 50%, control and 60%, 40 and 60%, 50 and 70%, and 60 and 70% concentration in the double treatment of phytoecdysteroid.

DISCUSSION

Shell ratio

The variation in the phytoecdysteroid concentration and the number of larval treatment influenced the shell ratio. Maximum shell ratio was noticed in case of double treatment by 60% phytoecdysteroid concentration. Results indicated positive results in larval and shell weights, and filament length upon feeding larvae on mulberry leaves treated with *Nux vomica* (Hiware, 2006). Administration of a JH analogue resulted an enhanced yield of 20 - 35 % in cocoon and pupal weight (Murakoshiet *al.,* 1972). Considerable increase in the cocoon and shell weight on administration of minute quantities of a strong JH mimics (Trivedy *et al.,* 1997). The response of silkworm in terms of improvement in economic traits varies with the compounds used, silkworm races and geographical region (Nair *et al.,* 2003) .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 and Dinan *et al.,* 2009) .Synchronization of spinning in Chinese silkworm varieties when PE was administered *per os* at the rate of 2.2 ìg/larva when 5-10% larvae had reached maturity (Chou and Lu,1980). An accelerated maturation in silkworm and mounting within 24h of treatment when phytoecdysteroid was administered (Shivakumar *et al.,* 2005). Similar results were also reported when ecdysteroid extracted from plant sources was administered to silkworm leading to advanced and synchronized maturation (Trivedy *et al.,* 2006; Dinan *et al.,* 2009 and Nair *et al.,* 2008). It is reported that silkworm larvae are sensitive to exogenous ecdysteroid when administered at different h (Sehnal *et al.,* 1990 and Dai *et al.,* 1985) indicated that ecdysone plays a significant role in nucleic acid metabolism and the related protein synthesis in silkworm.PE is effective on silkworm for inducing advanced and synchronized maturation in the form of 12~24h reduction for 80% maturation and 24~36h reduction for 100 % maturation which was season dependent (Nair *et al.,* 2010). 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. The increase of silk gland function during feeding period of the last larval instar is due to stimulation by ecdysteroid (Fukuda, 1942).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).

Thus, it may be concluded that treatment of *Bombyx mori* at different concentration of phytoecdysteroid and number of larval treatment may influence the economic traits of *Bombyx mori*. The administration of considerable amount of ecdysone on V instar larvae influenced the silk gland cells towards the histolysis producing numerous lysosomes (Akai and Kiuchi, 1988). At several h after ecdysone administration, the granular endoplasmic reticulum is concentrated everywhere in the cytoplasm and then, they are enclosed by separate membrane with some mitochondria and ground matrix (Akai and Kiuchi, 1988). Similar ultrastructural changes in the gland cell occur during larval and pupal moulting stages (Akai and Kiuchi, 1988). During the larval and pupal metamorphosis the silkgland cells are histolysed completely (Akai and Kiuchi, 1988) .The ecdysones are very important because they control most of the physiological activities in the silkworm. Silkworm maturation for spinning depends on the time and dose of application of ecdysteroid. The treatment of *B. mori* larvae with low concentration of phytoecdysteroid caused beneficial effect on the silk yield, whereas, the higher concentration of phytoecdysteroid caused adverse effect. These changes may occur due to some ultrastructral changes in the silk gland and fibroin content of *Bombyx mori* treated with phytoecdysteroid.

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