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PHYTOECDYSTEROID CHANGES AMINO ACID CONTENT IN THE LARVAE OF *BOMBYX MORI*

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ABSTRACT
Phytoecdysteroids affect the total amino acid content in the silkgland of fifth instar larvae of *Bombyx mori* at the initial stage ($P2 < 0.05$) and final stage of spinning. The amino acid content increased with increase in phytoecdysteroid concentration and number of larval treatment but with triple treatment of 70% concentration the amino acid content was decreased.The phytoecdysteroids may prove to be a boon for increased silk production.

INTRODUCTION

Sericulture plays an important role in transformation of the rural economy as it assures regular employment,income resource and provides return round the year. To increase the silk production efforts have been made to study the effect of ecological factors (Upadhyay *et al.*, 2004; Upadhyay and Mishra, 2002; Pandey and Upadhyay,2000; Upadhyay *et al.*, 2006 and 2009) and magnetization of eggs (Tripathi and Upadhyay, 2005a, b; Upadhyay and Tripathi, 2006) and cocoons (Upadhyay and Prasad, 2010a, b; Prasad and Upadhyay, 2011).The ecdysone is known to influence the reproductive potential and silk producing potential of *Bombyx mori* (Parlak *et al.*, 1992; Kawaguchi *et al.*, 1993, Okuda *et al.*, 1993,Srivastava and Upadhyay, 2012a and b,2013a and b) . Phytoecdysteroid changes the protein content in the larvae of *Bombyx mori*. Srivastava and Upadhyay, (2013c). Attempts have been made to improve the quality and quantity of silk, through enhancing the leaves with nutrients, spraying with antibiotics, juvenile hormone, plant products, with JH-mimics. Ecdysteroids play key role in moulting and metamorphosis in insects.Plant-produced insect moulting hormones, phytoecdysteroids (PEs), act either as feeding deterrents or agents that induce developmental disruption (Schmulz, *et al.*, 2002). The plant like *Achyranthes aspera* (Lat jeera) and *Cassia tora* (Choti chakwar) have been identified as source of phytoecdysteroids (Lafont and Horn, 2004). In China, various plant sources were identified to contain moderate to high amounts of PE and used in sericulture to manage the silkworm rearing (Wong *et al.*, 1979; Chou and Lu., 1980). In this study, an attempt was made to investigate the effect of phytoecdysteroid on amino acid content in the silkgland of the fifth instar of *B. mori* race *nistari* at the initial and final stages of spinning.

MATERIALS AND METHODS

The Insects

The seed cocoons of multivoltine silkworm (*Bombyx mori* Nistari) obtained from the silkworm grainage Behraich, Directorate of Sericulture Uttar Pradesh were maintained in the plywood trays (23×20×5cm) under laboratory in BOD incubator at 26 ± 1ºC and 80 ± 5 % RH until the emergence of moths. The newly emerged moths were sexd and separated (Krishnaswami *et al.*, 1973). They were paired and after four hour moths were decoupled. The females were allowed to lay eggs and after 24h females were individually examined for disease freeness. The formalin treated eggs were transferred to the incubator for hatching. After hatching, the larvae were reared on chopped mulberry leaves in the trays and III instar larvae were taken for experiment.

Experimental Design

The leaves of *Achyranthes aspera* were collected, washed with distilled water and dried in incubator at 37ºC for the extraction of phytoecdysteroid. The leaves were powdered and 50gm powder was subjected to extraction through soxlet apparatus with 250mL distilled water for 40 hours. The concentrated solution obtained was dried. The dried powder was then dissolved (5gm in 25mL water) in distilled water to obtain the solution (100% concentration). From which solutions of 40, 50, 60 and 70 % concentrations were prepared. Four phytoecdysteroid concentrations were applied by spraying (10mL/ 100g mulberry leaves) and the larvae were

Table 1(a): Effect of phytoecdysteroid treatment on the total free amino acids content (ìg/mg) in the silk gland of *Bombyx mori* larvae at the initial stage of spinning

Number of treatment	Phytoecdysteroid concentration (%)					F_1 ratio
(Larval instar)	Control (X_1)	40 (X_2)	50 (X_3)	60 (X_4)	70 (X_5)	n_1 = 4
Single (V)	31.65 ± 1.090	31.8 ± 0.722	32.85 ± 0.962	33.5 ± 1.05	31.55 ± 0.349	
Double (IV-V)	31.65 ± 1.090	32.06 ± 0.262	33 ± 1.020	34.8 ± 0.985	31.4 ± 0.421	1.63*
Triple (III-V)	31.65 ± 1.090	31.5 ± 0.685	31.47 ± 1.060	31.35 ± 0.347	31.12 ± 0.426	

F_2 ratio = 6.46** n_2 = 2; *Non-significant **P_2 < 0.05; Each value represents mean ± S.E.of six replicates; X_1, X_2, X_3, X_4 and X_5 are the mean values of the total free amino acids content (ìg/mg) in the silk gland in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

Table 2(a): Effect of phytoecdysteroid treatment on the total free amino acids content (ìg/mg) in the silk gland of *Bombyx mori* larvae at the final stage of spinning

Number of treatment	Phytoecdysteroid concentration (%)					F_1 ratio
(Larval instar)	Control (X_1)	40 (X_2)	50 (X_3)	60 (X_4)	70 (X_5)	n_1 = 4
Single (V)	28.1 ± 0.310	28.22 ± 0.098	28.3 ± 0.124	28.55 ± 0.242	27.95 ± 0.071	
Double (IV-V)	28.1 ± 0.310	28.37 ± 0.227	28.5 ± 0.221	28.68 ± 0.340	27.65 ± 0.310	0.66*
Triple (III-V)	28.1 ± 0.310	28 ± 0.221	27.9 ± 0.124	27.75 ± 0.189	27.48 ± 0.137	

F_2 ratio = 1.82** n_2 = 2; *Non-significant; Each value represents mean ± S.E.of six replicates; X_1, X_2, X_3, X_4 and X_5 are the mean values of the total free amino acids content (ìg/mg) in the silk gland in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

fed on the treated leaves. In single treatment V instar larvae before two days of the commencement of spinning were taken out from the BOD incubator and provided with 40% concentration treated leaves and then normal leaves were provided as food. In double treatment IV instars were, provided 60% solution treated mulberry leaves before two days of IV moulting. Second treatment was given at the V instar two days before spinning. For triple treatment, 100 III instars (before III moulting) were treated by providing 40% extract treated mulberry leaves. The second treatment was done two days before IV moulting and the third treatment at V instar two days before spinning. Similar treatments were performed using 50, 60 and 70% phytoecdysteroid with respective controls. The amino acid contents in the silk gland of *B. mori* larvae was estimated at the beginning and final stages of spinning following Spies (1957) method as modified by Singh and Agrawal (1989) in six replicates. Values were expressed as ìg/ mg amino acid.

RESULTS

The data in Table 1 and 2 shows that different phytoecdysteroid concentration and the number of larval treatments influenced the amino acid content in the silk gland of *B. mori* larvae at the initial stage of spinning. The amino acid content increased in 40, 50 and 60% phytoecdysteroid treatment but triple treatment caused notable decline in all the above concentrations. However, 70% treatment caused decline in the amino acid content with increase in the number of treatment. The maximum free amino acids content in the silk gland of larvae at the initial stage of spinning has been noticed to be 34.80 ± 0.985 μg/mg and at the final stage of spinning was recorded to be 28.68 ± 0.340 μg/mg in double treatment of larvae by 60% phytoecdysteroid concentration and that was minimum 31.12 ± 0.426 μg/mg (initial stage) & 27.48 ± 0.137 μg/mg (final stage) in case of triple treatment by 70% phytoecdysteroid concentration. Two-way ANOVA indicates that the number of larval treatment significantly (P_2 < 0.05) influenced the amino acid content in the silk gland of larvae at the initial stage of spinning treatment and the variation in the phytoecdysteroid concentration did not cause significant effect on the free amino acids content in the silk gland of larvae at the final stage of spinning.

DISCUSSION

Changes in the amino acid spectrum, net synthesis of protein and kinetics of certain enzymes associated with the synthesis and degradation of amino acid and protein occur during insect development (Digley and Smith, 1969), which is directly related to the spinning process in silkworms (Beament *et al.,* 1995, Terra *et al.,* 1975). Increase in the strength of static magnetic field up to 3000 gauss caused an increase in the total free amino acids level in different tissues of larvae of *Bombyx mori* (Upadhyay *et al.,* 2006). *Bombyx mori* larvae when exposed to lethal and sublethal doses of fenitrothion and ethion, the protein content indicated a depletion followed by contamitant increase in the accumulation of free amino acids (Nath *et al.,* 1981). The amino acids like aspartic acid, methionine, hdroxyproline, isoleucine, cystein and tryptophane are instantly utilized for the synthesis of silk (Hsu and Wang, 1964). The increase in the amino acids utilization for the formation of fibroin in the posterior silk gland of *Bombyx mori* larvae may be due to direct influence of the presence of free amino acids in the tender leaves of mulberry (Qader and Haque, 1997). Exposure of larvae to low magnetic field resulted in increased cellular activity and protein content (Tripathi *et al.,* 2012). The magnetization of silkworm eggs triggers certain physiological and biochemical events affecting the accumulation of total free amino acids in the silk gland of V instar *Bombyx mori* larvae (Upadhyay *et al.,* 2006). Variation in the temperature significantly influenced the total free amino acids content of silk gland (Gaur and Upadhyay, 2003). Maximum level of total free amino acids in the *Bombyx mori* was recorded at 10ºC (Gupta *et al.,* 2005). Administration of JHA Bemchi (*Psoralea corylifolia* L.) to the V instar larvae of *Bombyx mori* influence the free amino acids content in all the tissues (Nair *et al.,* 2009). Phytoecdysteroid treatment increased the protein content in *Bombyx mori* (Srivastava and Upadhyay, 2013c).

Thus it may be concluded that the treatment of *Bombyx mori* larvae with different concentration of phytoecdysteroid may cause ultra structural changes in the cells during larval and pupal stages. Due to increased metabolic rate of treated larvae more food was consumed by the larvae and more free amino acids were liberated which increased the free amino acids content in silk gland. The higher concentration of phytoecdysteroid may cause adverse effect, causing decline in the consumption of food by the larvae resulting in the decrease of total free amino acids content in different tissues of silkworm larvae.

CONCLUSION

The variation in the number of larval treatment significantly ($P_2 < 0.05$) influenced the free amino acids content in the silk gland of *Bombyx mori* larvae at the initial stage of spinning while the free amino acids content in the silk gland of larvae at the final stage of spinning was not significantly influenced due to number of larval treatment and change in phytoecdysteroid concentration. The maximum free amino acids content in the silk gland of larvae at the initial and final stage of spinning was recorded in double treatment by 60% phytoecdysteroid concentration.

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