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Effect of Phytoecdysteroid on Fecundity of Multivoltine Mulberry Silkworm *Bombyx Mori* Linn.

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ABSTRACT

Effect of phytoecdysteroid on fecundity and hatchability 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.The parameter selected for observation was fecundity of *Bombyx mori*. Maximum fecundity (423.33 ± 4.67) was noticed to be in case of the double treatment (IV and V instar larvae were treated) by 60% phytoecdysteroid concentration. Phytoecdysteroid treatment,if applied tactfully,may be useful for boosting up the sericulture industry as well as the economy of silkworm rearing.

Key words : Bioactive hormone**,** Larvae**,** Moulting**,** Egg**,** Phytoecdysteroids**,** Fecundity.

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 & Mishra, 2002; Pandey & Upadhyay, 2000; Upadhyay et al., 2006 & 2009) and magnetization of eggs (Tripathi & Upadhyay, 2005a, b; Upadhyay &Tripathi, 2006), and cocoons (Upadhyay & Prasad, 2010a,b; Prasad & Upadhyay, 2011). The ecdysone is known to influence the reproductive potential,& silk producing potential of *Bombyx mori* (Parlak et al., 1992; Kawaguchi et al., 1993 a&b, Okuda et al., 1993, Srivastava & Upadhyay, 2012a & b, 2013a & b). Phytoecdysteroid changes the protein content in the larvae of *Bombyx mori*. Srivastava & 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 & 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 &Lu., 1980). In this study, an attempt was made to investigate the effect of phytoecdysteroid on egg laying capacity of *Bombyx mori*.

MATERIALS & 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 (23x20x5cm) under laboratory in BOD incubator at 26 ± 10C and 80 ± 5 % RH until the emergence of moths. The newly emerged moths were sexed 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 24 h 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 3rd instar larvae were taken for experiment.

Experimental Design

The leaves of *Achyranthes aspera* were collected, washed with distilled water and dried in incubator at 370C for the extraction of phytoecdysteroid. The leaves were powdered and 50gm powder was subjected to extraction through Soxhlet apparatus with 250 ml 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 fed on the treated leaves. In single treatment Vth 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 IVth instars were, provided 40% 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.

Fecundity

To observe the effect of phytoecdysteroid on the egg laying ability of moths obtained from the experimental larvae, the early emerged moths were kept sex-wise in separate trays to avoid copulation. Three batches of five males and five females were prepared and they were allowed to mate. After mating, the paired moths were decoupled manually. Further, female moths were allowed to lay eggs on the sheet of paper. The egg laying moths were covered by open plastic cellules to prevent the intermixing of egg masses deposited by different female moths and after

Table 1(a) - Effect of phytoecdysteroid treatment on the fecundity of *Bombyx mori.*

Number of treatment (Larval instar)		Phytoecdysteroid concentration (%)				F_1 ratio $n_1 = 4$
	Control (X_1)	40 (X_2)	50 (X_3)	60 (X_4)	70 (X_5)	
Single (V)	378.33 ±2.88	384.00 ±3.78	388.00 ±2.84	396.33 ±3.67	369.00 ±4.55	2.49*
Double (IV-V)	378.33 ±2.88	390.00 ±2.84	401.66 ±5.34	423.33 ±4.67	350.66 ±3.65	
Triple (III-V)	378.33 ±2.88	372.12 ±3.75	365.00 ±2.59	357.33 ±4.69	338.33 ±3.65	

F_2 ratio = 7.65** $n_2 = 2$

*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 fecundity in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

proper processing the eggs were transferred chronically to BOD incubator. For determining the fecundity, 15 layings (three batches of five layings in each batch) were taken. Thus, average of five layings was taken as representative number of eggs per laying, laid by female moth in each experiment.

RESULTS

The data presented in table 1a shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the fecundity. With the increasing number of larval treatment by phytoecdysteroid from one to two times, the fecundity increased in case of 40, 50 and 60% phytoecdysteriod treatment but triple treatment caused decline in the fecundity in all the above concentrations.

Table 1 (b) - Post-hoc test showing group difference in the effect of phytoecdysteroid treatment on the fecundity of *Bombyx mori.*

Mean difference in between groups	Number of treatment		
	Single	Double	Triple
X_1~X_2	5.67	11.67	6.21
X_1~X_3	9.67	23.33	13.33
X_1~X_4	18.00	*45.00	21.00
X_1~X_5	9.33	27.67	40.00
X_2~X_3	4.00	11.66	7.12
X_2~X_4	12.33	33.33	14.79
X_2~X_5	15.00	39.34	33.79
X_3~X_4	8.33	21.67	7.67
X_3~X_5	19.00	*51.00	26.67
X_4~X_5	27.33	*72.67	19.00

Honestly significant difference (HSD) = $q \sqrt{MS\ within / n}$

= $5.05 \sqrt{197.0685 / 3}$

= 40.93

MS = Mean square values of ANOVA table
q = Studentized range static
n = Number of replicates
* Showing significant group difference

X_1, X_2, X_3, X_4 and X_5 are the mean values of the fecundity in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

70% phytoecdysteriod treatment caused notable decline in the fecundity with increase in the number of treatment from single to triple. The maximum fecundity was noticed to be 423.33 ± 4.67 in case of the double treatment by 60% phytoecdysteroid concentration and the minimum 338.33 ± 3.65 was recorded in case of triple treatment by 70% phytoecdysteroid concentration. Two-way ANOVA indicates that number of larval treatment significantly ($P_2 < 0.05$) influenced the fecundity. The Post-hoc test (table-1b) indicates significant group difference in double treatment of larvae in between control and 60%, 50 and 70%, and 60 and 70% concentration of phytoecdysteroid treatment. In single and triple treatment of larvae no significant group difference was noticed in any combination of groups.

DISCUSSION

Variation in the phytoecdysteroid concentration and number of larval treatment caused considerable influence on the fecundity of moth. The egg laying capacity of *Bombyx mori* increased with the increasing number of larval treatment from one to two times in case of 40, 50 and 60% phytoecdysteroid concentration. The highest egg laying ability of moth was recorded in case of double treatment of larvae by 60% phytoecdysteroid concentration, whiles the egg laying capacity of moths, treated with 70% phytoecdysteroid concentration, declined sharply. The exposure of gamma radiation of *Bombyx mori* eggs caused an increase in the fecundity (Salam & Mahoud, 1995). In female insects, the steroid hormone 20-hydroxyecdysone (20 E) plays a major role in activating vitellogenesis, a process required for egg development (Pondeville et al.,2008). The PES are not hypersensitive, androgenic, oestrogenic, or anti oestrogenic, and do not induce virilisation (Dinan et al., 2009). Antijuvenile hormone is generally known to prevent egg maturation when applied to feeding adults (Hurkadli, 1998). Rearing of silkworm larvae at lower levels of RH resulted in lower fecundity, hatchability (Hussain et al., 2011). Generally mutants are inferior to the normal in several characters such as fecundity and hatching (Rajanna et al., 2012). Insect reproductive activity is controlled by juvenile hormone (Doane, 1973) and ecdysone hormone (Parlak,et al 1992). Corpus allatum (CA), the source of JH regulated egg formation and the presence of an active corpus allatum is necessary for successful yolk deposition and egg maturation (Doane ,1973). JH plays a key role in the ovariole development, oocyte maturation etc; with an equally important role by ecdysone released from prothoracic gland (PG) in silkworm, *Bombyx mori* (Kawaguchi,1993a). The egg laying capability of the *Bombyx mori* reduced when silkworm were treated by an antijuvenile hormone agent, KK-42 (Nair et al., 1998). The activity of juvenile hormones considerably influenced the reproductive potential of *B. mori* (Ghosh, 1987). Effect of 20- hydroxyecdysone on egg production of silkworm resulted into more large eggs in addition to normal eggs (Kawaguchi, 1993b). Addition of feed additives was effective in increasing the fecundity (Nagesh & Reddy, 1995). Variation in the 20-hydroxyecdysone concentration significantly ($P < 0.05$) influenced the reproductive potential of *B. mori* in terms of fecundity and hatchability of eggs (Prasad & Upadhyay, 2012).

Thus, it is concluded that the fecundity of moths obtained from the larvae treated with low phytoecdysteroid concentration may be due to ultrastructural changes in the cell contents and enzyme activity during larval and pupal stages caused positive effect on the reproductive traits resulting increase in the fecundity and hatchability of *Bombyx mori* eggs. The higher concentration of phytoecdysteroid caused adverse effect on the cellular level, therefore, reproductive potential declined.

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