European Journal of Applied Sciences 4 (3): 117-122, 2012
ISSN 2079-2077

DOI: 10.5829/idosi.ejas.2012.4.3.6542

Influence of Phytoecdysteroid on Weight of Ovary and Weight of Egg of Multivoltine Mulberry Silkworm *Bombyx mori* Linn

K. Srivastava and V.B. Upadhyay

Silkworm laboratory, Department of Zoology
D.D.U. Gorakhpur University, Gorakhpur-273009, India

Abstract: Effect of phytoecdysteroid on weight of ovary and weight of egg of multivoltine mulberry silkworm *Bombyx mori* was studied. Variation in the phytoecdysteroid concentration and number of larval treatment (treated the larvae) influenced the weight of ovary and weight of *B. mori* eggs. 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. Number of larval treatment significantly ($P_2 < 0.05$) influenced the weight of ovary and weight of egg. Maximum weight of ovary (0.330±0.067 gm) and weight of egg (0.398±0.092 mg) was noticed to be in case of double treatment (IV^{th} and V^{th} instar larvae were treated) by 60% phytoecdysteroid and minimum was recorded in triple treatment (III^{rd}, IV^{th} and V^{th} 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.

Key words: Bioactive hormone · Larvae · Moulting

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 cost effective, labour saving and ecofriendly. In order to increase the production of silk, efforts have been made to study the effect of ecological factors [1], relative humidity [2], refrigeration of eggs [3] and cocoon [4, 5], magnetization of eggs [6-8], cocoon [9, 10] and larval performance [11]. The ecdysone has been noticed to influence the reproductive potential of *Bombyx mori* [12-15]. It is hypothesized that the *B. mori* larvae treated with phytoecdysteroid may cause some beneficial effect on the life pattern and reproductive potential. Ecdysteroids play a key role in moulting and metamorphosis in insects. Many plants are known to contain ecdysteroids with high moulting hormone activity. The plant like *Achyranthes aspera* (Lat jeera) and *Cassia tora* (Choti chakwar) have been identified to have phytoecdysteroids [16]. Keeping this in view, an attempt has been made to investigate the Influence of phytoecdysteroid on weight of ovary and weight of egg 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 5 cm) under the ideal rearing conditions [17] in the silkworm laboratory, department of Zoology, D.D.U. Gorakhpur University, Gorakhpur. The temperature and relative humidity were maintained at 26±1 °C and 80±5 % RH, respectively till the emergence of moths from the seed cocoons. The moth emerged generally in the morning at around 4 am. The newly

Corresponding Author: K. Srivastava, Silkworm laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273009, India.

emerged moths from seed cocoon were quickly picked up and kept 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. Therefore, the female moths, required to copulate with the male moths, were allowed their mates for copulation. A total of 360 pairs, each containing one male and one female from newly emerged moths were allowed to mate at 26±1°C , 80±5% RH and 12 h / day dim light condition The male moths were discarded while the female moths were allowed to lay egg.

Oviposition: Just after separation, the gravid females laid eggs on the sheet of paper in dark condition at 26±1°C 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 24 h of egg laying, the female moths were individually examined for their disease freeness. The disease free layings (eggs laid by disease free female moths) thus prepared, were treated with 2% formalin for 15 min to increase the adhesiveness of egg of the paper sheet and surface disinfection. Thereafter, the egg sheets with egg laid 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 *B. mori*, the experiments were performed with different concentrations of phytoecdysteroid hormone with respect to the treatment of IIIrd, IVth and Vth instar larvae. For extraction of phytoecdysteroid, the leaves of *Achyranthes aspera* were collected, washed thoroughly with distilled water and dried in incubator at 37°C. The dried leaves were powdered with the help of mechanical device. Further, 50 gm powder, thus obtained was subjected to extraction through soxlet apparatus with 250 ml distilled water for 40 h. After 40 h of extraction, a little amount of concentrated solution was obtained which was dried and 6.75 g powdered material was obtained. The dried powder was dissolved in distilled water as 5 gm in 25 ml 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 10 ml on 100 g 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 Vth instar larvae just before two days of the beginning of larval spinning. A total of 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 IVth instar larvae**.** In the first treatment, 100 larvae of IVth instar were treated just before two days of IVth 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 Vth instar just before two days of spinning.

Triple Treatment: For triple treatment, the third instar larvae just before moulting were separated from BOD incubator. In the first treatment, 100 larvae of IIIrd instar larvae 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 IVth moulting at the final stage of IVth instar larvae and transferred in BOD incubator for rearing. Third treatment was given to Vth instar larvae, two days before the start of spinning. Thus, in the triple treatment IIIrd, IVth and Vth 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.

Weight of Ovary: For determining the weight of ovary, the female moths were dissected in distilled water just after emergence and complete ovary was taken out. The ovary was cleaned and soaked with filter paper to remove the adhered water on it. For the weight of ovary, 15 ovaries (three batches of five ovaries in each batch) were weighed.

Weight of Egg: In order to observe the effect of phytoecdysteroid on the weight of egg, the average of 100 eggs were taken as representative no. of eggs per laying (eggs laid by moths that emerged from treated larvae) in case of each set of experiment. Three replicates of each experiment were made.

Wt.of egg = Average wt.of 100 eggs / 100

RESULTS

Weight of ovary: The data presented in Table 1a shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the weight of ovary of *Bombyx mori*. With the increasing number of larval treatment from one to two times the weight of ovary increased in case of 40, 50 and 60% phytoecdysteroid treatment but triple treatment caused notable decline in the weight of ovary in all the above concentrations. Seventy percentage of phytoecdysteroid treatment caused notable decline in the weight of ovary with the increase in number of treatment from single to triple. The trend of increase in the weight of ovary with increasing number of treatment has been recorded to be almost same in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum weight of ovary was noticed to be 0.330±0.067 g in case of the double treatment by 60% phytoecdysteroid concentration and the minimum weight of 0.253±0.021 g 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 weight of ovary. The Post – hoc test (Table 1b) indicates significant group difference in the weight of ovary in between control and 60% and 50 and 70% and 60 and 70% in the double treatment with phytoecdysteroid.

Table 1: (a) Effect of phytoecdysteroid treatment on the weight of ovary (gm) of *Bombyx mori*

Number of treatment (Larval instar)	Phytoecdysteroid concentration (%) Control (X1)	40 (X2)	50 (X3)	60 (X4)	70 (X5)	F_1 ratio $n_1 = 4$
Single (V)	0.276±0.058	0.283±0.017	0.290±0.030	0.300±0.023	0.270±0.011	2.55*
Double (IV-V)	0.276±0.058	0.295±0.037	0.307±0.044	0.330±0.067	0.260±0.025	
Triple (III-V)	0.276±0.058	0.272±0.030	0.267±0.032	0.262±0.028	0.253±.021	

F_2 ratio =5.82** $n_2 = 2$

*Non-significant **P_2 <0.05

Each value represents mean±S.E. of three replicates.

X_1, X_2, X_3, X_4 and X_5 are the mean values of the weight of ovary (gm) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration, respectively

Table 1: (b)Post-hoc test showing group difference in the effect of phytoecdysteroid treatment on the weight of ovary (gm) of *Bombyx mori*

Mean difference in between groups	Number of treatment Single	Double	Triple
X1~X2	0.007	0.019	0.004
X1~X3	0.014	0.031	0.009
X1~X4	0.024	*0.054	0.014
X1~X5	0.006	0.016	0.023
X2~X3	0.007	0.012	0.005
X2~X4	0.017	0.035	0.010
X2~X5	0.013	0.035	0.019
X3~X4	0.010	0.023	0.005
X3~X5	0.020	*0.047	0.014
X4~X5	0.030	*0.070	0.009
Honestly significant difference (HSD)	$= {}^{q}\sqrt{}$ MS within / n		
	$= {}^{5.05}\sqrt{}$ .0001957/ 3		
	= 0.040		

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 weight of ovary (gm) just after laying in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

Table 2: (a)Effect of phytoecdysteroid treatment on the weight of egg (mg) just after laying of *Bombyx mori*

Number of treatment (Larval instar)	Phytoecdysteroid concentration (%) Control (X1)	40 (X2)	50 (X3)	60 (X4)	70 (X5)	F_1 ratio $n_1 = 4$
Single (V)	0.353±0.063	0.360±0.024	0.365±0.011	0.376±0.068	0.347±0.074	2.47*
Double (IV-V)	0.353±0.063	0.375±0.021	0.382±0.052	0.398±0.092	0.336±0.112	
Triple (III-V)	0.353±0.063	0.350±0.078	0.345±0.094	0.340±0.114	0.330±0.034	

F_2 ratio = 6.06** $n_2 = 2$

*Non-significant **$P_2 < 0.05$

Each value represents mean±S.E. of three replicates.

X_1, X_2, X_3, X_4 and X_5 are the mean values of the weight of egg (mg) just after laying in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

Table 2: (b) Post-hoc test showing group difference in the effect of phytoecdysteroid treatment on the weight of egg (mg) of *Bombyx mori* just after laying.

Mean difference in between groups	Number of treatment Single	Double	Triple
X1~X2	0.007	0.022	0.003
X1~X3	0.012	0.029	0.008
X1~X4	0.023	*0.045	0.013
X1~X5	0.006	0.017	0.023
X2~X3	0.005	0.007	0.005
X2~X4	0.016	0.023	0.010
X2~X5	0.013	*0.039	0.020
X3~X4	0.011	0.016	0.005
X3~X5	0.018	*0.046	0.015
X4~X5	0.029	*0.062	0.010

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

$= {}^{5.05}\sqrt{}\ 0.1442 / 3$

$= 0.037$

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 weight of egg (mg) just after laying in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively

Weight of Egg: The data presented in Table 2a shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the weight of egg of *B. mori*. With the increasing number of larval treatment from one to two times, the weight of egg increased in case of 40, 50 and 60% phytoecdysteroid treatment but triple treatment caused notable decline in the weight of egg in all the above concentrations. It was found 70% phytoecdysteroid treatment caused notable decline in the weight of egg with the increase in number of treatment from single to triple. The trend of increase in the weight of egg with increasing number of treatment has been recorded to be almost same in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum weight of egg was noticed to be 0.398±0.092 mg in case of the double treatment by 60% phytoecdysteroid concentration and the minimum 0.330±0.034 mg 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 weight of egg. The Post-hoc test (Table 2b) indicates significant group difference in the weight of egg in between control and 60%, 40 and 70%, 50 and 70% and 60 and 70% concentration of phytoecdysteroid treatment in double treatment.

DISCUSSION

Variation in the phytoecdysteroid concentration and number of larval treatment influenced the weight of ovary of *Bombyx mori* moth. The weight of ovary increased with the increasing number of larval treatment from one to two times in case of 40, 50 and 60% phytoecdysteroid concentration. The highest weight of ovary was recorded to be maximum in case of double treatment of larvae by 60% phytoecdysteroid concentration, while the weight of

ovary treated with 70% phytoecdysteroid concentration, declined sharply. The rate of increase in the weight of ovary in single treatment of larvae in 40, 50 and 60% concentration were almost similar to the double treatment. In the triple treatment, a considerable decline was noticed with all the concentrations. In female insects, the steroid hormone 20- hydroxyecdysone (20 E) plays a vital role in activating vitellogenesis, a process required for egg development [12]. The PEs are not hypersensitive androgenic, oestrogenic or antioestrogenic and do not induce virilisation [18]. Insect reproductive activity is controlled by juvenile hormone [19] and ecdysone [13]. 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 [20]. Effect of 20-hydroxyecdysone on egg production of silkworm resulted large eggs in addition to normal eggs [14]. 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, *B.mori* [21]. In *B. mori*, the prothoracic gland hormone is responsible for the development of ovaries during the pupal period [22].

Variation in the phytoecdysteroid concentration and number of larval treatment caused considerable influence on the weight of egg. The weight of egg of *B. 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 weight of egg was recorded to be maximum in case of double treatment of larvae with 60% phytoecdysteroid concentration, while the weight of egg treated with 70% phytoecdysteroid concentration, declined sharply. Antijuvenile hormone is generally known to prevent egg maturation when applied to feeding adults [23]. Insect reproductive activity is controlled by juvenile hormone [19] and ecdysone hormone [13]. Corpus allatum (CA) the source of JH regulated egg formation and the presence of an active carpus allatum is necessary for the successful yolk deposition and egg maturation [19]. Effect of 20- hydroxyecdysone on egg production of silkworm resulted into more large egg in addition to normal eggs [14]. In female insects, the steroid hormone 20- hydroxyecdysone (20E) plays a major role in activating vitellogenesis, a process required for egg development [12]. In *Hyalophora cercopia*, isolated pupal abdomens implanted with prothoracic gland laid eggs after their emergence [24]. In *Malacosoma pluviale*, application of β-ecdysone to the isolated pupal abdomen stimulated an increase in the number of oocytes [25]. All the silkworm lines produce poor quality eggs when larvae were reared under low (50%) and high (90%) RHs [26]. In *Samia cynthia*, prothoracic gland hormone stimulated the ovarian development and the critical period of hormone secretion for the formation of mature eggs was 80 h after larval pupal ecdysis at 24°C [27] . The vitellogenic female protein necessary for the growth of oocytes is already abundant in the haemolymph of *B. mori* pupae before the maximum secretion of ecdysone from the prothoracic glands [28].

Thus, it is concluded that the weight of ovary and weight of egg, obtained from the larvae treated with low phytoecdysteroid concentration may be due to ultra structural changes in the cell contents and enzyme activity during larval and pupal stages caused positive effect on the weight of ovary and egg resulting increase in the weight of ovary and weight of egg of *B. mori.* The higher concentration of phytoecdysteroid caused adverse effect on the cellular level, therefore, weight of ovary and weight of egg declined.

REFERENCES

1. Upadhyay, V.B., K.P. Gaur and S.K. Gupta, 2004. Effect of ecological factors on the silk producing potential of the mulberry silkworm (*Bombyx mori* Linn.) Malays. Appl. Biol., 33: 13-18.
2. Upadhyay, V.B. and A.B. Mishra, 2002. Influence of relative humidity on the nutritive potential of mulberry silkworm *Bombyx mori.* Linn. Larvae. J. Adv. Zool., 23: 54-58.
3. Pandey, A.K. and V.B. Upadhyay, 2000. Impact of refrigeration of eggs and pre-refrigeration period on the weight of shell of *Bombyx mori* Linn. J. Adv. Zool., 21: 98-101.
4. Upadhyay, V.B., R. Singh and S. Prasad, 2006. Refrigeration of pupae influenced the hatchability and fecundity of *Bombyx mori* Linn. Malaysian J. Appl. Biol., 35: 13-19.
5. Upadhyay, V.B., R. Singh and S. Prasad, 2009. Refrigeration of cocoons influences the pupal characteristics of multivoltine mulberry silkworm ((*Bombyx mori* Linn.) J. Appl. Biosci., 35: 166-168.
6. Tripathi, S.K. and V.B. Upadhyay, 2005. Magnetization of eggs influences the incubation period of multivoltine mulberry silkworm *Bombyx mori* Linn Eggs. J. Adv. Zool., 26: 24-28.
7. Upadhyay, V.B. and S.K. Tripathi, 2005. Magnetization of the eggs on the reproductive potential of multivoltine mulberry silkworm (*Bombyx mori* Linn). Indian J. Seric., 44: 212-214.

8. Upadhyay, V.B. and S.K. Tripathi, 2006. Effect of the magnetization of eggs on the silk producing potential of multivoltine mulberry silkworm leaves on economic characters of silkworm (*Bombyx mori* Linn.). Sericologia, 46: 269-278.
9. Upadhyay, V.B. and S. Prasad, 2010 a. Magnetization for the improvement of silk producing potential in multivoltine mulberry silkworm (*Bombyx mori* Linn).The Bioscan, 5: 285-289.
10. Upadhyay, V.B. and S. Prasad, 2010b. Biotechnological importance of cocoon magnetization with particular reference to the reproductive potential of multivoltine mulberry silkworm (*Bombyx mori* Linn). Sericologia, 50: 461-472.
11. Prasad, S. and V.B. Upadhyay, 2011. Biotechnological importance of cocoon magnetization with particular reference to the larval performance of multivoltine mulberry silkworm (*Bombyx mori* Linn*.)*. Middle-East J. Sci. Res., 10: 565-572.
12. Pondeville, E., A. Maria, J.C. Jacques, C. Bourgouin and C.D. Villemant, 2008. *Anopheles gambience* males produce and transfer the vitellogenic steroid hormone 20-hydroxyecdysone to females during mating. Proc. Nati. Acad Sci. USA. 16, 105: 619-631.
13. Parlak, O., S. Sakurai, M. Kaya and T. Ohotaki, 1992. Content and possible role of ecdysteroids in the larval ovary of the silkworm *Bombyx mori*. Invertebr. Reprod. Dev., 21: 1-6.
14. Kawaguchi, Y., Y. Banno, K. Koga, H. Doira and H. Fuji, 1993. Polygonal patterns on egg shells of giant egg mutant and large egg induced by 20-hydroxyecdysone in *Bombyx mori*. J. Insect Physiol., 36: 437-443.
15. Okuda, K.K., W. Amornsak and O. Yamashita, 1993. Controlled ecdysteroid accumulation in eggs of silkworm *Bombyx mori* L. by an imidazole compound (KK42) and embryogenesis in these eggs. Arch. Physiol Biochem., 25: 121-135.
16. Lafont, R. and D.H.S. Horn, 2004. Ecdysone from chemistry to mode of action. Ed. J. Koolmann heor Thieme Verlag, pp: 39.
17. Krishnaswamy, S., M.N. Narasimhanna, S.K. Suryanaryan and S. Kumar Raja, 1973. Sericulture Manual 2 Silkworm Rearing F.A.O. Agric Serves Bull. Rome, 15: 1-131.
18. Lafont, R., L. Dinan, J. Harmatha and V. Volodin, 2009. Phytoecdysteroids: Diversity, Biosynthesis and distribution. In: Ecdysones:Structures and Functions.(Guy, S. ed.) Springer, pp: 3-45.
19. Doane, W.W., 1973. Role of hormone of insect development. In: Developmental Systems. Insects, S.J. Counce and C.H. Waddington (Eds), Academic Press, New York, pp: 291-297.
20. Kawaguchi, Y., Banno, Y., Doira, H. and H. Fuji, 1993b. Manifestation of characteristics in the giant egg mutant of *Bombyx mori* (Lepidoptera: Bombycidae) 4 Surface structure of egg shell in Ge egg. Jpn. J. Appl. Entomol. Zool., 37: 91-95.
21. Kawaguchi, Y., Y. Banno, K. Koga, H. Doira and H. Fuji, 1993a. Polygonal patterns on egg shells of giant egg mutant and large eggs induced by 20-hydroxyecdysone in *Bombyx mori*. J. Insect Physiol, 36: 437-443.
22. Chatani, F. and E. Ohnishi, 1976. Effect of ecdysone on the ovarian development of *Bombyx* silkworm. Dev. Growth Differ., 18: 481-484.
23. Bowers, W.S., 1982. Endocrine strategies for insect control.Entomol. Exp. Appl., 31: 3-4.
24. Williams, C.M., 1952. Physiology of Insect Diapause,IV.The Brain and Prothoracic gland asan Endocrine System in *Cercopia* Silkworm. Biol. Bull.(Woods Hole), 103: 120-138.
25. Sahota, T.S., 1969. Hormonal control of ovarian development and metamorphosis in *Malacosoma pluviale*. Can. Jan. Zool., 47: 917-920.
26. Hussain, M., A. Khan, M. Naeem and A. Mohsin, 2011. Effect of Relative Humidity on Factors of Seed Cocoon Production in some inbred Silk Worm (*Bombyx mori*). Int. J. Agric. Biol., 13: 57-60.
27. Takahashi, S. and H. Mizohata, 1975. Critical period necessary for the imaginal moulting and egg maturation of the eri-silkworm, *Samia cynthia Ricini. Zo*ol. Mag., 84: 283-295.
28. Kawaguchi, Y. and H. Doira, 1973. Gene-controlled incorporation of haemolymph protein into the ovaries of *Bombyx mori***.** J. Insect Physiol., 19: 2083-2096.