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Influence of 20-Hydroxyecdysone on the Larval Performance of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn)

S. Prasad and V.B. Upadhyay

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

Abstract: The application of 20-hydroxyecdysone on *Bombyx mori* larvae has been proved to be a significance in the sericulture industry. Variation in the 20-hydroxyecdysone concentration significantly ($P<0.05$) influenced the larval performance of *B. mori* in terms of larval duration, larval weight and survival of larvae. The larval weight and survival of larvae increased with the increasing number of larval treatment of 20, 40 and 60% ecdysone concentration. The maximum level of larval weight (1.951±0.09 g) and survival of larvae (92.98±1.08%) was noticed in case of triple treatment by 60% ecdysone concentration. The minimum larval duration (23.72±0.62 days) was recorded in case of triple treatment by 60% ecdysone concentration showing good development of larvae. 20- hydroxyecdysone hormone interactions if applied tactfully may be useful for boosting up the sericulture industry as well as the economy of silkworm rearing.

Key words: 20-Hydroxyecdysone · Larval Performance · Larval Treatment · *Bombyx mori*

INTRODUCTION

Bombyx mori nistari is a resistant variety of multivoltine mulberry silkworm contributing to a great extent in the commercial production of cocoon in India. Larval weight, larval duration and survival of larvae are the most important factors which influence the production of cocoon on commercial scale. Attempts have been made to study the effects of ecological factors [1], relative humidity [2], refrigeration of eggs [3] and cocoons [4, 5] and magnetization of eggs [6-8] and cocoons [9, 10] and larval performance [11] on the performance of silkworms. The plant produced insect moulting hormone termed phytoecdysteroids (PES), functions as strategic defenses for plants against insects by acting as either feeding deterrents or through developmental disruption in insects. The response of silkworm to the small quantities of these phytoecdysteroids or its analogues may hasten the larval maturation events and also influenced the spinning process of the several silkworm larvae may prove to be very much useful for the management of rearing programme and economics of silkworm industry.

Ecdysteroids play key role in moulting and metamorphosis in insects. The ecdysone has been noticed to influence the reproductive potential of *Bombyx mori* [12-16]. It is hypothesized that *B. mori* larvae treated with 20-hydroxyecdysone may cause certain beneficial effects on the life pattern and the larval performance. Keeping this in view, an attempt has been made to investigate the effect of 20-hydroxyecdysone on the larval weight, larval duration and survival of larvae in multivoltine mulberry silkworm (*B. mori*). This investigative study may be helpful in devising the suitable means to increase the larval ability of *B. mori* and therefore, the increased production of good cocoons.

MATERIALS AND METHODS

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. Directorate of sericulture, Behraich Uttar Pradesh and were maintained in the plywood trays (23 x 20 x 5cm) under the ideal rearing conditions [17] in the silkworm laboratory, Department of Zoology, DDU 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

Corresponding Author: Surendra Prasad, Department of Zoology,
DDU Gorakhpur University Gorakhpur-273009 (UP). India. E-mail: dr.spd_gkp@rediffmail.com.

cocoons. The moths emerged generally in the morning at around 4 A.M. The tray in which seed cocoons were kept was suddenly illuminated by light in the morning at 4 O'clock on 9th and 10th day of spinning. The newly emerged moths were quickly picked up and kept sex-wise in separate trays to avoid copulation. The whole grainage operation was performed as per description given by [17].

Moths have a tendency to pair immediately after emergence and, therefore, the female moths required to copulate with the male moths, were allowed their mates for copulation. Sufficient pairs, each containing one male and one female from newly emerged moths were allowed to mate at 26±1°C and 80±5% RH in 12 hour / day dim light condition. After four hours of mating, the paired moths were decoupled manually. The female moths were allowed to lay egg. After 24 h of egg laying, the female moths were individually examined for their disease freeness and after formaline treatment the eggs were transferred to the incubator for hatching. After hatching, the larvae were reared on the mulberry leaves given as food in the trays. Further, the 3rd instar larvae were taken for experiment.

Experimental Design: To observe the influence of bioactive phytoecdysteroid (20-hydroxyecdysone) hormone on the performance of *B. mori*, the experiments were performed with different concentrations of 20-hydroxyecdysone hormone with respect to the treatment of 3rd, 4th and 5th instar larvae. 5 mg 20-hydroxyecdysone purchased from the Sigma Company, was dissolved in 250 ml distilled water and used this solution as 100% concentration of 20-hydroxyecdysone. Four concentrations of 20-hydroxyecdysone viz; 20, 40, 60 and 80% were prepared by adding required amount of water and sprayed separately by sprayer as 10 ml on 100 gm mulberry leaves / 100 larvae. The larvae were fed on the treated leaves. Three sets of experiment were designed viz, single double and triple treatment of larvae. All the experiments were conducted in the BOD (Biological Oxygen Demand) incubator.

Single Treatment: Single treatment of larvae was performed with the 5th 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 20% concentration of 20-hydroxyecdysone, was given as food. Further, the treated larvae were given normal mulberry leaf for food.

Double Treatment: Double treatment of larvae was started from the final stage of 4th instar larvae. In the first treatment, 100 larvae of 4th instar were treated just before two days of 4th moulting, by providing treated mulberry leaf as food with 20% concentration of 20-hydroxyecdysone. The treated larvae then transferred in BOD incubator for further rearing and development. Further, second treatment for the same larvae was given at the final stage of 5th instar larvae i.e just before two days of spinning.

Triple Treatment: For triple treatment, the 3rd instar larvae just before 3rd moulting, were separated from BOD incubator. In the first treatment, 100 larvae of 3rd instar were treated by providing treated mulberry leaf and kept in BOD incubator for rearing. The second treatment of same larvae was done just before two days of 4th moulting i.e at the final stage of 4th instar larvae and transferred in BOD incubator for further rearing. Third treatment was given to 5th instar larvae, two days before the start of spinning. Thus, in the triple treatment 3rd, 4th and 5th instar larvae were treated.

Similar experiments were performed by 40, 60 and 80% concentration of 20-hydroxyecdysone. A control set was always maintained with each set of experiment.

Larval Weight: For the determination of larval weight the weight of 30 larvae (three batches of 10 larvae in each batch) were recorded. Three replicates of each experiment were made. The larval weight was taken on the day when fifth instar larvae stop feeding.

Larval Duration: The time required from the hatching of larvae to the third day of spinning by the fifth instar larvae was considered. For this purpose, 90 larvae (three batches of 30 larvae in each batch) were taken for observation. Three replicates of each experiment were made.

Survival of Larvae: For determining the survival of larvae, 90 larvae (three batches of 30,1st instar, larvae in each batch) were taken under the observation. The number of larvae which attained the pupal stage was counted for the calculation of the survival of larvae as following:

$$\text{Per cent survival of larvae} = \frac{\text{No. of larvae pupated}}{\text{No. of } 1^{st} \text{ instar larvae taken for observation}} \times 100$$

RESULTS

Larval Weight: The data given in Table-1a indicates that variation in the phytoecdysteroid (20-hydroxyecdysone) concentration and the number of larval treatment influenced the larval weight of 5th instar *Bombyx mori* larvae. With the increase in number of treatment by 20-hydroxyecdysone from one to three times, the larval weight increased in case of 20, 40 and 60% ecdysone treatment while in case of the treatment with 80% ecdysone concentration, the larval weight increased in single treatment of larvae but further increase in the number of treatment caused decline in the larval weight. The increase in the larval weight with the increase in number of treatment has been noticed to be almost of similar trend with 20, 40 and 60% ecdysone concentration. The maximum larval weight was noticed to be 1.951±0.09 gm (18.96% increase as compared to control) in case of triple treatment by 60% ecdysone concentration. The minimum larval weight was recorded to be 1.620±0.02 gm in case of triple treatment by 80% ecdysone concentration.

Two way ANOVA indicates that variation in the ecdysone concentration significantly ($P<0.05$) influenced the larval weight of *B. mori* larvae (Table-1a). The Post-hoc test (Table-1b) indicates significant group difference in the larval weight in between control and 60% ecdysone concentration in case of double treatment. In the triple treatment significant group difference in the larval weight was noticed in between control and 40%, control and 60%, 40 and 80% and 60 and 80% of ecdysone treatment. In case of single treatment there was no significance group difference.

Larval Duration: The data given in Table-2a is indicative of the fact that variation in the ecdysone concentration and the number of larval treatment influenced the larval duration of *B. mori* larvae. With the increase in number of larval treatment by ecdysone from one to three times, the larval duration decreased in case of 20, 40 and 60% ecdysone treatment while in case of the treatment with 80% ecdysone concentration, the larval duration decreased in single treatment of larvae but further increase in the number of treatment caused increase in the larval duration. The decrease in the larval duration with the increase in number of treatment has been noticed to be almost of similar trend with 20, 40 and 60% ecdysone treatment. The minimum larval duration was noticed to be 23.72±0.62 days. in case of triple treatment by 60% ecdysone concentrations. The maximum larval duration 26.10±1.12 days was recorded in case of triple treatment by 80% ecdysone concentration.

Two way ANOVA indicates that variation in the ecdysone concentration significantly ($P<0.05$) influenced the larval duration of silkworm larvae (Table-2a). The post hoc test (Table-2b) indicates significant group difference in the larval duration in between control and 60% and 60 and 80% ecdysone concentration in case of triple treatment of larvae. In single and double treatment, there was no group difference.

Survival of Larvae: The data given in Table-3a clearly indicates that variation in the ecdysone concentration and the number of larval treatment influenced the survival per cent of *B. mori* larvae. With the increase in number of treatment by ecdysone from one to three times, the survival per cent of larvae increased in case of 20, 40 and

Table 1: Effect of 20-hydroxyecdysone on the larval weight (gm) of *Bombyx mori* larvae

Stage of treatment (larval instar)	20-hydroxyecdysone concentration (%) Control (X_1)	20 (X_2)	40 (X_3)	60 (X_4)	80 (X_5)	F_1-ratio n_1= 4
Single (5th)	1.640±0.01 (100)	1.662±0.008 (101.34)	1.721±0.02 (104.94)	1.782±0.04 (108.65)	1.797 ±0.05 (109.57)	
Double (4th -5th)	1.640±0.01 (100)	1.692±0.02 (103.17)	1.830±0.01 (111.58)	1.892±0.08 (115.36)	1.782 ±0.04 (108.65)	3.965*
Triple (3rd -5th)	1.640±0.01 (100)	1.800±0.05 (109.75)	1.900±0.03 (115.85)	1.951±0.09 (118.96)	1.620±0.02 (98.78)	

F_2 –ratio = 0.825** $n_2 = 2$ *P < 0.05

**Non Significant

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 larval weight in control, 20, 40, 60 and 80% ecdysone concentration respectively.

Figures in parentheses indicate per cent value when control was taken as 100%

Table 1b: Post-hoc test showing effect of 20-hydroxyecdysone on the larval weight (gm) of *Bombyx mori* larvae.

	Stage of treatment		
Mean difference in between groups	Single	Double	Triple
$X_1 \sim X_2$	0.022	0.052	0.160
$X_1 \sim X_3$	0.081	0.190	*0.260
$X_1 \sim X_4$	0.142	*0.252	*0.311
$X_1 \sim X_5$	0.157	0.142	0.020
$X_2 \sim X_3$	0.059	0.138	0.100
$X_2 \sim X_4$	0.120	0.200	0.151
$X_2 \sim X_5$	0.135	0.090	0.180
$X_3 \sim X_4$	0.061	0.062	0.051
$X_3 \sim X_5$	0.076	0.048	*0.280
$X_4 \sim X_5$	0.015	0.110	* 0.331

$$\text{Honesty significant difference (HSD)} = q\sqrt{\frac{\text{MS within}}{n}}$$

$$= 5.05\sqrt{\frac{0.006}{3}}$$

$$= 0.226$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of larval weight in control, 20, 40, 60 and 80% ecdysone concentration respectively

Table 2a: Effect of 20-hydroxyecdysone on the larval duration (days) *Bombyx mori* larvae

Stage of treatment (larval instar)	20-hydroxyecdysone concentration (%) Control (X_1)	20 (X_2)	40 (X_3)	60 (X_4)	80 (X_5)	F_1 = -ratio n_1= 4
Single (5th)	25.82±1.12 (100)	25.52±0.05 (98.84)	25.18±0.63 (97.52)	24.75±0.57 (95.86)	24.30±0.62 (94.11)	
Double (4th -5th)	25.82±1.12 (100)	25.30±0.02 (97.98)	24.98±0.56 (96.75)	24.24±0.46 (93.88)	24.78±1.20 (95.97)	3.187*
Triple (3rd -5th)	25.82±1.12 (100)	25.12±0.58 (97.28)	24.56 ±0.36 (95.12)	23.72±0.62 (91.87)	26.10±1.12 (101.08)	

F_2 –ratio = 0.0322** n_2 = 2

*P < 0.05

**Non Significant

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 larval duration in control, 20, 40, 60 and 80% ecdysone concentration respectively.

Figures in parentheses indicate per cent value when control was taken as 100%

60% ecdysone treatment while in case of the treatment with 80% ecdysone concentration, the survival per cent of larvae slightly increased in single treatment of larvae but further increase in the number of treatment caused decline in the survival per cent of larvae. The increase in the survival per cent of larvae with the increase in number of treatment has been noticed to be almost of similar trend with 20, 40 and 60% ecdysone concentration. The maximum survival per cent of larvae was noticed to be 92.98±1.08% (18.20% increase as compared to control) in case of triple treatment by 60% ecdysone concentration. The minimum survival per cent of larvae (77.56±1.14%) was recorded in case of triple treatment by 80% ecdysone concentration.

Two way ANOVA indicates that variation in the ecdysone concentration significantly (P<0.05) influenced the survival per cent of larvae (Table-3a). The Post-hoc test (Table-3b) indicates significant group difference in the survival per cent of larvae in between control and 60% and 60 and 80% concentration of ecdysone treatment in case of triple treatment of larvae. No group difference was found in case of single and double treatment.

Table 2b: Post-hoc Test showing effect of 20-hydroxyecdysone on the larval duration (days) of *B. mori* larvae.

	Stage of treatment		
Mean difference in between groups	Single	Double	Triple
$X_1 \sim X_2$	0.30	0.52	0.70
$X_1 \sim X_3$	0.64	0.84	1.26
$X_1 \sim X_4$	1.07	1.58	*2.10
$X_1 \sim X_5$	1.52	1.04	0.28
$X_2 \sim X_3$	0.34	0.32	0.56
$X_2 \sim X_4$	0.77	1.06	1.40
$X_2 \sim X_5$	1.22	0.52	0.98
$X_3 \sim X_4$	0.43	0.74	0.84
$X_3 \sim X_5$	0.88	0.20	1.54
$X_4 \sim X_5$	0.45	0.54	*2.38

$$\text{Honesty significant difference (HSD)} = q\sqrt{\frac{\text{MS within}}{n}}$$
$$= 5.05\sqrt{\frac{0.316}{3}}$$
$$= 1.636$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of larval duration in control, 20, 40, 60 and 80% ecdysone concentration respectively.

Table 3a: Effect of 20-hydroxyecdysone on the survival per cent of *Bombyx mori* larvae

Stage of treatment (larval instar)	20-hydroxyecdysone concentration (%) Control (X_1)	20 (X_2)	40 (X_3)	60 (X_4)	80 (X_5)	F_1-ratio n_1= 4
Single (5^{th})	78.66±0.65	80.85±0.73	82.76±1.26	84.76±0.94	85.02±0.65	
	(100)	(102.78)	(105.21)	(107.75)	(108.08)	
Double (4^{th}-5^{th})	78.66±0.65	82.25±1.12	83.35±1.27	86.48±1.25	83.04±0.92	3.778*
	(100)	(104.56)	(105.96)	(109.94)	(105.57)	
Triple (3^{rd} -5^{th})	78.66±0.65	84.96±1.15	85.75±1.30	92.98±1.08	77.56±1.14	
	(100)	(108.00)	(109.01)	(118.20)	(98.60)	

F_2 –ratio = 0.367** $n_2 = 2$

*$P < 0.05$

**Non Significant

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 survival of larvae in control, 20, 40, 60 and 80% ecdysone concentration respectively

Figures in parentheses indicate per cent value when control was taken as 100%

Table 3b: Post-hoc Test showing effect of 20-hydroxyecdysone on the survival per cent of *B. mori* larvae

	Stage of treatment		
Mean difference in between groups	Single	Double	Triple
$X_1 \sim X_2$	2.19	3.59	6.30
$X_1 \sim X_3$	4.10	4.69	7.09
$X_1 \sim X_4$	6.10	7.82	*14.32
$X_1 \sim X_5$	6.36	4.38	1.10
$X_2 \sim X_3$	1.91	1.10	0.79
$X_2 \sim X_4$	3.91	4.25	8.02
$X_2 \sim X_5$	4.17	0.79	7.40
$X_3 \sim X_4$	2.00	3.13	7.23
$X_3 \sim X_5$	2.26	0.31	8.19
$X_4 \sim X_5$	0.26	3.44	*15.42

$$\text{Honesty significant difference (HSD)} = q\sqrt{\frac{\text{MS within}}{n}}$$

$$= 5.05\sqrt{\frac{9.298}{3}}$$

$$= 8.89$$

MS = Mean square value of ANOVA Table
q = Studentized range static
n = No. of replicates
* = Shows significant group difference
X_1, X_2, X_3, X_4 and X_5 are mean values of survival of larvae in control, 20, 40, 60 and 80% ecdysone concentration respectively

DISCUSSION

The larval weight of *Bombyx mori* varied with variation in the varieties of some host plant [18] and declined due to the starvation of larvae [19]. The nutritional status of mulberry leaf has been noticed to be the major factor in deciding the larval weight of silkworm [20-22]. Larval weight was noticed to be highest during the winter and lowest during the summer [23]. The use of antibiotics increased the larval weight of *B. mori* [24]**.** Supplementation with vitamin B increased the resistance against poor environmental conditions and increased body weight in silkworm [25]. Increase in larval weight was noticed when silkworm races were administrated with thiamine [26] thyroxine [27] and ascorbic acid [28, 29]. The nutritional elements of mulberry leaf determine the growth and development of the larvae [30]**.** Positive results in larvae were noticed when larvae fed on the mulberry leaves treated with *Nux vomica* [31]. Organic manures have strong hold on the growth and development of silkworm [32]. The larval weight of *B. mori* increased significantly by the hormonal treatment [33]. The treatment of *B. mori* egg with HCl was also helpful for increasing the larval weight [34]. Cocoon magnetization also influences the larval weight [11]. The feeding leaves supplemented with distilled water alone slightly increased the weight of larvae and pupae [35]. Thus, 20-hydroxyecdysone treatment in low concentration cause, an increase in the larval weight due to increased rate of metabolism resulting in the consumption of more food by the silkworm larvae while higher concentration of 20-hydroxyecdysone treatment may cause stress response leading to the decrease in the larval weight of *B. mori.*

The variation in the larval duration of silkworm has been reported by a number of workers [36, 33]. It is well known that an ideal race is one which has a shorter duration thus causing low consumption of leaf [37]. Phytoecdysteriod is recognized as one of the most important components in the silkworm rearing [38]. The rearing condition was also reported to be effective in deciding the larval span and growth [39]. The topical application of methoprene (Juvenile Hormone Analog) prolonged larval period and caused an increase in the weight of silk gland and cocoon of *B. mori* [40]. The nutrition plays a major role in improving the growth and development of *B. mori* [41]. Cocoon magnetization also influences the larval duration [11]. The present investigation shows positive co-relation with larval duration. 20-hydroxyecdysone simulates silkworm larvae for tolerance to toxin and viral infection, accelerates growth and development in silkworm. Thus, it reduces the larval duration with the increasing 20-hydroxyecdysone concentration from 20 to 60%, in single, double and triple treatment of larvae, while at 80%, triple treatment of larvae, larval duration increased because of stress response at higher concentration.

The ecological factors [42] and genome of silkworm [43] have been noticed to be important for regulating the survival of larvae. The oral administration of folic acid during 5th instar silkworm significantly influenced the survival per cent of silkworm larvae [26, 28, 44, 45]. Cocoon magnetization also influences the survival of larvae [11]. Ascorbic acid is reported to enhance the larval survival rate [46, 47]. Survival per cent of silkworm larvae was significantly affected by exogenous application of phytoecdysteriod [48, 49]. Thus, it is concluded that the higher survivability of larvae may be due to the resistance developed in the larvae at low 20-hydroxyecdysone treatment up to 60% concentration at triple treatment of larvae while the higher 20-hydroxyecdysone concentration, triple treatment of larvae may cause toxic response resulting in the high mortality of larvae.

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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(1): 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(1): 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(2): 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 Jour. Appl. Biol., 35(2): 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(2): 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(1): 24-28.
7. Upadhyay, V.B. and S.K. Tripathi, 2005. Magnetization of eggs on the reproductive potential of multivoltine mulberry silkworm *(Bombyx mori* Linn.). Indian J. Seric., 44(2): 212-214.
8. Upadhyay, V.B. and S.K. Tripathi, 2006. Effect of magnetization of eggs on the silk producing potential of multivoltine mulberry silkworm (*Bombyx mori* Linn.).Sericologia, 46(3): 269-278.
9. Upadhyay, V.B. and S. Prasad, 2010a. Magnetization for the improvement of silk producing potential in multivoltine mulberry silkworm (*Bombyx mori* Linn). The Bioscan, 5(2): 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(4): 461-672.
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(5): 565-572.
12. Prasad, S. and V. B. Upadhyay, 2012. Effect of 20-hydroxyecdysone on the reproductive potential of multivoltine mulberry silkworm (*Bombyx mori* Linn).Acad. J. Entomol., 5(2): 86-93.
13. Pandeville, E., A. Maria, J.C. Jacques, C. Bourgouin and C.D. Villemant, 2008. *Anapheles gambience* males produce and transfer the vitellogenic steroid hormone 20-hydroxyecdysone to females during mating. Proc. Nati. Acad Sci. USA, 16, 105(50): 619-631.
14. 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.
15. 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.
16. 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. Biochem. Physiol., 25(2): 121-135.
17. Krishnaswamy, S., M.M. Narasimhanan and S.K. Suryanarayana, 1973. Sericulture Mannual 2 silkworm rearing F.A.O. Agric. Services Bull., 15(2): 1-131.

18. Kaul, O., K. Tikku, B.P. Saxena and O.K. Atal, 1979. Growth and silk production in *Bombyx mori* L. fed on 3 different varieties of mulberry. Indian J. Seric., 18: 1-5.
19. Alagumalai, K., R. Ramraj, M. Thiravalluvan, N. Nagendran and R. Ranjaly, 1991. Effect of antibiotics on larval, cocoon characters and fecundity of silkworm, *Bombyx mori* L. Environment and ecology, 9(3): 795-796.
20. Mishra, A.B. and V.B. Upadhyay, 1995. Influence of temperature on the silk producing potential of multivoltine *Bombyx mori* L race nistari Sericologia, 35: 217-222.
21. Qader, M.A., R. Haqque and N. Absar, 1995. Nutritive effects of bush lowent and tree mulberry leaves on economic characters of silkworm *Bombyx mori* L. Pakistan J. Zool., 27: 77-81.
22. Srivastava, A.D. and S.D. Mishra, 1995. Effect of crowding on fecundity of eri silkworm *Philosamia ricini* Hutt. Indian J. Seric., 24: 41-43.
23. Jyothermayala, D. and D. Bharathi, 1993. Effect on the growth pattern of silkworm, *B. mori* (L.) with reference to seasonal variations. Environment and Ecology, 11(1): 220-222.
24. Deehu, P.S., R. Govindan, M.C. Deraiah and T.K.N. Swamy, 1997. Effect of antibiotics on growth and cocoon parameters of silkworm, *Bombyx mori* (L). Mysore J. Agric. Sci., 31(1): 41-46.
25. Das, S. and A. Medda, 1988. Effect of cyanocobalamine on protein and nucleic acid contents of ovary of silkworm, *Bombyx mori* L. during larval, pupal and adult stages of development. Insect Sci. Appl., 9: 641-646.
26. Nirwani, R.B., I.I. Hugar and B.B. Kaliwal, 1998. Supplementation of riboflavin on economic parameters and biochemical changes of the silkworm, *Bombyx mori* L. Bull. Sericult. Res., 9: 37-41.
27. Rajashekhargouda, R., M. Goplan, S. Jayra and N. Natarajan, 1997. Feed of pure breeds and single cross hybrids of *Bombyx mori* L. Entomon, 22(3-4): 223-227.
28. Etebari, K., R. Ebadi and L. Matindoost, 2004. Effect of feeding mulberry's enriched leaves with ascorbic acid on some biological, biochemical and economical characteristics of silkworm *Bombyx mori* L. Int. J. Indust. Entomol., 8: 81-87.
29. Babu, M., M.T. Swamy, P.K. Rao and M.S. Rao, 1992. Effect of ascorbic acid enriched mulberry leaves on rearing of *Bombyx mori* L. Indian J. Seric., 31: 111-114.
30. Seidavi, A.R., A.R. Bizhannia, R. Sourati and M. Mavvajpour, 2005. The nutritional effects of different mulberry varieties on biological characters in silkworm. Asia Pac. J. Clin. Nutr., 14: 122.
31. Hiware, C.J., 2006. Effect of fortification of mulberry with homeopathic drug *Nux vomica* on *Bombyx mori*. L. Homeopathy Jul., 95(3): 148-50.
32. Shashidhar, R.K., T.K. Narayanaswamy, T. Sarithakumari, K. Sudhir and N.L. Naveena, 2009. Cocoon parameters of PM X CSR2 fed with mulberry raised through organic based nutrient management. J. Biol. Sci., 1(1): 2076-9946.
33. Khan, A., S. Rahman and N. Birendra, 1997. Effect of vertebrate sex hormone, mestranol norethindrone, on growth and development of *Bombyx mori* (Lepidoptera). Bangladesh J. Zool., 25(2): 103-109.
34. Hurkadli, H.K., 1998. Hatching of silkworm eggs. Indian textile Journal, 108(9): 72-74.
35. Verma, A.N. and A.S. Atwal, 1968. Effect of temperature and food on the development and silk production of *Bombyx mori* L. Beitr. Entomol., 18(1-2): 249-258.
36. Das, P.K. and K. Vijayaraghavan, 1990. Studies on the effect of different mulberry varieties and season on the larval development and cocoon characters of the silkworm, *Bombyx mori* (L). Indian J. Seric., 29(1): 44-53.
37. Mathur, S.K. and G. Subba Rao, 1987. The saga of Murishidabad Silk industry. Indian Silk, 26(5): 16-17.
38. Nair, K.S., Y.G. Miao and S.N. Kumar, 2005. Differential response of silkworm, *Bombyx mori* L. to phytoecdysteroid depending on the time of administration. J. Appl. Sci. and Environ. Manage., 9(3): 81-86.
39. Ueda, S., R. Kimura and K. Suzuki, 1971. Studies on growth of silkworm, *B. mori* L. III. Relative increase in body weight and silk gland weight in the fifth instar larvae. Bull. Seric. Expt. Stn., 25: 1-20.
40. iranda, J.E., S.A.D. Bortoli and R. Takahashi, 2002 Development and silk production by silkworm larvae after topical application of methoprene. Sci. Agric., 59(3): 585-588.
41. Kanafi, R.R., R. Ebadi, S.Z. Mirhosseini, R.R. Seidavi, Zolfaghari and K. Etebari, 2007. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm *Bombyx mori* L. Inv. Sur. J., 4: 86-91.
42. Upadhyay, V.B. and K.P. Gaur, 2002. Effect of ecological factors on the performance of *Bombyx mori* L. larvae. Zool. Soc., India, pp: 91-104.

43. Ghosh, B., S. Chattopadhyay, P.R.T. Rao, S.K. Das, G.C. Roy, S.K. Sen and S.S. Sinha, 1993. Analysis of quantitative traits of multivoltine silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) in varied environments. Uttar Pradesh J. Zool., 13(1): 47-51.
44. Rahmathulla, V.K., D. Priyabrata, M. Ramesh and R.K. Rajan, 2007. Growth rate pattern and economic traits of silkworm, *Bombyx mori* L under the influence of folic acid administration. J. Appl. Sci. Environ. Manage., 11(4): 81-84.
45. Rai, M.M., M.K. Rathod and A.M. Khurad, 2002. Improvement in economic characters of silkworm *Bombyx mori* L. by folic acid administration. Entomon, 27: 99-104.
46. Ito, T. and M. Niminura, 1966a. Nutrition of silkworm *Bombyx mori*. Its specific requirement and its nutrition in relation to the mineral nutrition of its host plant mulberry *Morus indica.* Indian J. Exp. Biol., 4: 31-36.
47. Ito, T. and M. Niminura, 1966b. Nutrition of silkworm *Bombyx mori*. XII Nutritive effects of minerals. Bul. Sericult. Expt. Sta., 20: 373.
48. Shivakumar, G.R., K.V. Anantha Raman, K.V.R. Reddy, S.B. Magadum, R.K. Datta, S.S. Hussain, A. Banerji and S.K. Chowdhury, 1995. Effect of phytoecdysteroid on larval maturation and economic parameters of the silkworm, *Bombyx mori* L. Indian J. Seric., 34(1): 46-49.
49. Nair, K.S., B.K. Kariappa and C.K. Kamble, 2008. Impact of individual and co-administration of juvenile hormone analogue and phytoecdysteroid on the crop management and performance of silkworm *Bombyx mori* L. J. Biol. Sci., 8(2): 470-473.