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IMPACT OF PHYTOECDYSTEROID TREATMENT ON THE LARVAL PERFORMANCE OF MULTIVOLTINE MULBERRY SILKWORM *Bombyx mori* LINN

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ABSTRACT

Phytoecdysteroid are being used commercially to shorten the larval duration for synchronize spinning of cocoons and labour saving. In this experiment *Achyranthes aspera* plant extract containing phytoecdysteroid activity was taken. The experiments were conducted with the phytoecdysteroid concentration of 40, 50, 60 and 70 % and number of treatment viz; single, double, triple. Variation in the number of treatment (P_1>0.05) of *B. mori* larvae significantly influenced the larval duration, larval length, larval weight and survival of larvae. The larval duration decreased from 22.90 days (control) to the minimum of 21.52 days in case of 60%, double treatment of larvae. The larval length increases with the increasing phytoecdysteroid concentration and number of treatment up to 60%, double treatment of larvae. The maximum larval length was recorded to be 6.98 cm in case of 60%, double treatment of larvae while it was minimum (4.90 cm) in 70%, triple treatment of larvae. The weight of larvae increased with the increasing phytoecdysteroid treatment from single to double treatment in 40, 50 and 60% phytoecdysteroid concentration and it was maximum (0.14.36 gm) in 60%, double treatment of larvae. The maximum survival of larvae was noticed to be 95.25±1.55 per cent in case of double treatment of larvae by 60% phytoecdysteroid concentration.

Key words: *Bombyx mori,* phytoecdysteroid, larval performance

INTRODUCTION

Nistari is a resistant variety of multivoltine mulberry silkworm (*Bombyx mori* Linn.) which contributes up to a great extent in the commercial production of cocoon in India. The protein contents are the most important factors that influence the production of silk on commercial scale. Efforts are being made to evolve new technologies that are effective, labour saving and eco-friendly in order to increase, in the production of silk. Attempts have been made to study the effect of ecological factors (Upadhyay *et al.*, 2004), relative humidity (Upadhyay & Mishra, 2002), refrigeration of eggs (Pandey & Upadhyay, 2000), refrigeration of cocoons (Upadhyay *et al.*, 2009), magnetization of eggs (Upadhyay & Tripathi, 2006) and magnetization of cocoons (Upadhyay & Prasad, 2010a; Upadhyay & Prasad, 2010b) on the performance of silkworm. Nowadays biotechnology has become leading field of scientific researches which are directly concerned with the life quality of human beings.

It is hypothesized that if the *B. mori larvae* fed with different phytoecdysteroid concentration, there may be some beneficial effects on the life pattern of silkworm and the productivity of silk. An attempt has been made to investigate the Biotechnological effect of phytoecdysteroid treatment on the larval performance of multivoltine mulberry silkworm *Bombyx mori* Linn. This study may be helpful in devising the biotechnological application of phytoecdysteroid for the heavy production of silkworm silk.

MATERIALS AND METHODS

The seed cocoons of multivoltine mulberry silkworm (*Bombyx mori* Nistari) were obtained from the silkworm grainage Behraich, Directorate of Sericulture Uttar Pradesh, and were maintained in the plywood trays (23x20x5cm) under the ideal rearing conditions (Krishnaswami *et al.*, 1973) in the silkworm laboratory. The temperature and relative humidity were maintained in the BOD incubator at 26 ± 1ºC and 80 ± 5% RH respectively until the emergence of moths from the seed cocoons. The

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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 Krishnaswami *et al.* (1973).

Moth have a tendency to pair immediately after the emergence, therefore sufficient pairs, each containing one male and one female from newly emerged moth were allowed to mate at 26 ± 1ºC and 80 ± 5%RH in 12 ± 1 hour/day dim light condition. After four hours of mating, the paired moths were decoupled manually. The female moths were allowed for egg laying. After 24 hours of eggs laying the female moths were individually examined for their disease free ness and after formaline treatment 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

Some plants like *Achyranthes aspera* act as bioactive phytoecdysteroid compound on *Bombyx mori* larvae (Lafont *et al.,* 2004, Maribashetty *et al.,* 1997). In the present study *Achyranthes aspera* was taken for experiment due to their good availability. To observe the influence of bioactive phytoecdysteroid hormone on the performance of *Bombyx mori,* the experiments were performed with different concentrations of phytoecdysteroid 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 separately with the help of mechanical device. Furthermore, 50gm powder, thus obtained was subjected to extraction separately through soxlet apparatus with 250 ml distilled water for 40 hours. After 40 hours of extraction a little amount of concentrated solution was obtained which was dried and 6.75 gm powdered material was obtained. The dried powder was dissolved in distilled water as 5gm in 25 ml water and used this solution for further experiment as 100% concentration of phytoecdysteroid.

In the beginning, for the general survey experiment, four concentrations of phytoecdysteroid viz; 25, 50, 75 and 100% were prepared by adding required amount of water and sprayed separately by sprayer as 10 ml on 100 g mulberry leaf which were air dried and given as food to larvae. The growth performance was satisfactory at 50% concentration of *Achyranthes* extract. Therefore, for further experiment the suitable narrow range of *Achyranthes* phytoecdysteroid concentrations.

Thus, four phytoecdysteroid concentrations were applied topically by spraying as 10 ml on 100 gm mulberry leaves and the larvae were fed on the treated leaves. Three sets 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. 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 leaf for 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 leaves as food with 40% concentration of *Achyranthes* leaf extract. 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 Vth instar larvae i.e. just before two days of spinning.

Triple treatment: For triple treatment, the third instar larvae just before IIIrd moulting, were separated from BOD incubator. In the first treatment, 100 larvae of IIIrd instar were treated by providing 40% 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 ie. at the final stage of IVth instar larvae and transferred in BOD incubator for further rearing. The 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.

The parameters selected for observation were determined from the respective stages of *Bombyx mori*, obtained during the course of experimental rearing with phytoecdysteroid hormone. The parameters taken under study were the duration, length, weight, survival per cent of larvae.

Larval Performance

For determining the larval duration, the time required from the hatching of larvae to 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 the observation. Three replicates of each experiment were made.

For determining the larval length, the length of 30 larvae (three batches of 10 larvae in 10 batch) were recorded for each replicate. Three replicates of each experiment were made. The larval length of fifth instar larvae was taken as its normal length on the day when fifth instar larvae stop feeding.

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

For recording the survival of larvae, 90 larvae (three batches of 30, first instar, larvae in each batch) were taken under observation. The number of larvae, which obtained the pupal stage, was counted for the calculation of the survival of larvae as following:

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

RESULTS

Larval Duration

The data presented in Table 1a and Figure 1 show that change in the phytoecdysteroid concentration and the number of larval treatment influenced the duration of larvae. With the increasing number of larval treatment from one to two times, the rate of larval development increases thus the duration of larvae decreased in case of 40, 50 and 60% concentration of phytoecdsteroid treatment but the triple treatment of larvae caused an increase in the larval duration in all the above concentrations. 70% phytoecdysteroid treatment caused notable increase in the larval duration with the increase in the number of larval treatment from single to triple. The trend of decrease in the larval duration with the increasing number of larval treatment has been recorded to be almost similar in case of 40, 50 and 60% phytoecdysteroid treatment. The minimum larval duration was noticed to be

Table 1a. Effect of phytoecdysteroid treatment on the larval duration (days) of *Bombyx mori*

Stage of treatment (Larval instar)	Control X_1	Phytoecdysteroid concentration (%) 40 X_2	50 X_3	60 X_4	70 X_5	F_1-ratio n_1=4
Single (V)	22.90±1.20	22.65±1.05	22.40±1.25	22.10±1.30	23.10±1.15	
Double (IV-V)	22.90±1.20	22.45±1.02	22.05±1.05	21.52±0.221	23.55±1.35	**2.9301***
Triple (III-V)	22.90±1.20	23.25±1.06	23.50±1.08	23.85±0.87	24.45±1.45	

F_2– ratio= 7.8852** **n_2=2**
$^{**}P_2 < 0.05$ ***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 (days) of *Bombyx mori* in control, 40, 50, 60 and 70% phytoecdysteroid concentration respectively.

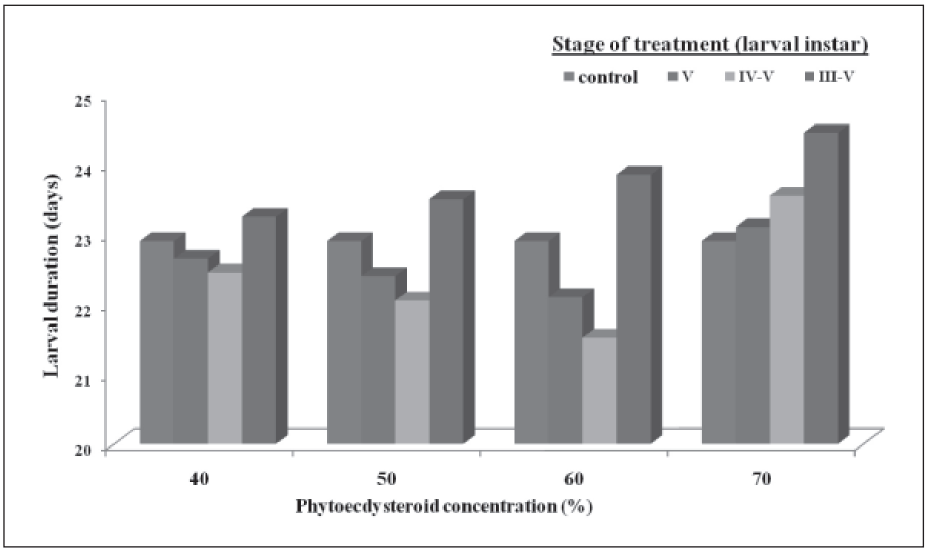

Fig. 1. Effect of phytoecdysteroid treatment on the larval duration (days) of *Bombyx mori.*

21.52±0.221 days in case of double treatment of larvae by 60% phytoecdysteroid concentration and maximum larval duration of 24.45±1.45 days in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment significantly (P_2<0.05) influenced the larval duration while phytoecdysteroid concentration did not cause significant effect on the duration of larvae. The post–hoc test (Table 1b) indicates significant group difference in the larval duration, in the double treatment of larvae in between 50% and 70%, 60 and 70% and in triple treatment of larvae significant group difference was noticed in between control and 70% concentration of phytoecdysteroid treatment. In single treatment of larvae no significant group difference was noticed in any of the group combinations.

Larval length

The data presented in Table 2a and Figure 2 shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the larval length. With the increasing

Table 1b. Post-hoc test showing effect of phytoecdysteroid treatment on the larval duration (days) of *Bombyx mori*

Mean difference in between groups	Phytoecdysteroid concentration (%)		
	Single	Double	Triple
$X_1 \sim X_2$	0.25	0.45	0.35
$X_1 \sim X_3$	0.50	0.85	0.60
$X_1 \sim X_4$	0.80	1.38	0.95
$X_1 \sim X_5$	0.20	0.65	*1.55
$X_2 \sim X_3$	0.25	0.40	0.25
$X_2 \sim X_4$	0.55	0.93	0.60
$X_2 \sim X_5$	0.45	1.10	1.20
$X_3 \sim X_4$	0.30	0.53	0.35
$X_3 \sim X_5$	0.70	*1.50	0.95
$X_4 \sim X_5$	1.00	*2.03	0.60

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

$$= 5.05 \frac{\sqrt{0.226}}{3}$$

$$= 1.39$$

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 the mean values of the larval duration (days) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

Table 2a. Effect of phytoecdysteroid treatment on the larval length (cm) of *Bombyx mori*

Stage of treatment (Larval instar)	Control X_1	Phytoecdysteroid concentration (%) 40 X_2	50 X_3	60 X_4	70 X_5	F_1-ratio n_1=4
Single (V)	5.47±0.30	5.68±0.52	5.88±0.42	6.00±0.32	5.25±0.35	
Double (IV-V)	5.47±0.30	6.02±0.38	6.55±0.52	6.98±0.60	5.09±0.38	**2.2467***
Triple (III-V)	5.47±0.30	5.25±0.40	5.15±0.60	5.02±0.55	4.90±0.43	

F_2– ratio=5.4521** n_2=2
$^{**}P_2$< 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 length (cm) of *Bombyx mori* in control, 40, 50, 60 and 70% phytoecdysteroid concentration respectively.

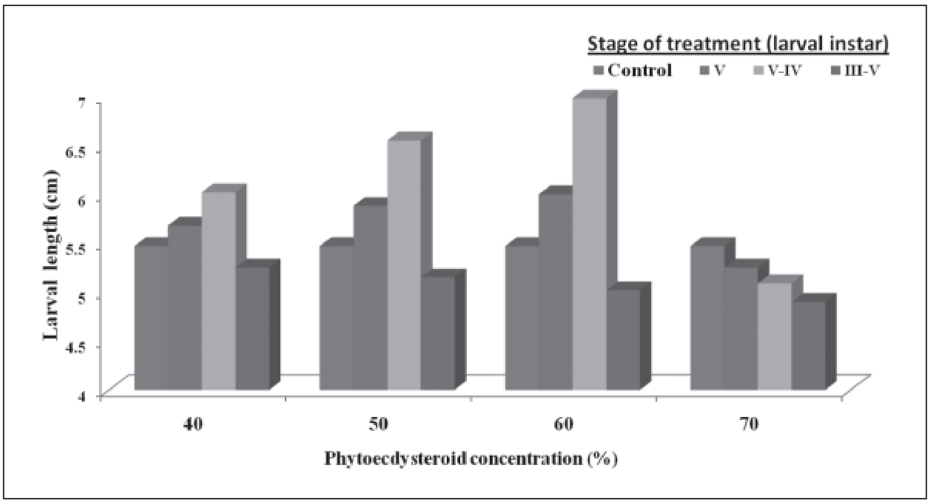

Fig. 2. Effect of phytoecdysteroid treatment on the larval length (cm) of *Bombyx mori*.

number of larval treatment from one to two times, the larval length increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the larval length in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the larval length with increase in the number of larval treatment from single to triple. The trend of increase in the larval length with the increasing number of larval treatment has been recorded to be almost similar in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum larval length was noticed to be 6.98±0.60 cm in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum larval length of 4.90±0.43 cm was recorded in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment significantly (P_2<0.05) influenced the larval length. The post–hoc test (Table 2b) indicates significant group difference in the larval length, in the 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 of the group combinations.

Larval weight

The data presented in Table 3 and Figure 3 shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the larval weight. With the increasing number of larval treatment from one to two times, the larval weight increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the larval weight in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the larval weight with increase in the number of larval treatment from single to triple. The trend of increase in the larval weight with the increasing number of larval treatment has been recorded to be almost similar in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum larval weight was noticed to be 14.36±1.02 gm in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum larval weight of 11.76±0.98 gm was recorded in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment significantly (P_2<0.05) influenced the larval weight. The post–hoc test (Table 3b) indicates significant group difference in the larval weight, in the single treatment of larvae in between 60% and 70%. In double treatment of larvae, significant group difference was noticed in between

Table 2b. Post-hoc test showing effect of phytoecdysteroid treatment on the larval length (cm) of *Bombyx mori.*

Mean difference in between groups	Phytoecdysteroid concentration (%)		
	Single	Double	Triple
X_1 ~ X_2	0.21	0.55	0.22
X_1 ~ X_3	0.41	1.08	0.32
X_1 ~ X_4	0.53	*1.51	0.45
X_1 ~ X_5	0.22	0.38	0.57
X_2 ~ X_3	0.20	0.53	0.10
X_2 ~ X_4	0.32	0.93	0.23
X_2 ~ X_5	0.43	1.10	0.35
X_3 ~ X_4	0.12	0.43	0.13
X_3 ~ X_5	0.63	*1.46	0.25
X_4 ~ X_5	0.75	*1.89	0.12

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

$= 5.05 \frac{\sqrt{0.172}}{3}$

$= 1.21$

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 the mean values of the length of larvae (cm) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

Table 3a. Effect of phytoecdysteroid treatment on the larval weight (gm) of *Bombyx mori*

Stage of treatment (Larval instar)	Control X_1	Phytoecdysteroid concentration (%)				F_1-ratio n_1=4
		40 X_2	50 X_3	60 X_4	70 X_5	
Single (V)	12.77±0.92	12.98±0.85	13.12±0.91	13.55±0.86	12.46±0.75	
Double (IV-V)	12.77±0.92	13.25±0.80	13.56±0.79	14.36±1.02	12.25±0.80	**2.6315***
Triple (III-V)	12.77±0.92	12.58±0.82	12.28±0.75	12.05±0.79	11.76±0.98	

F_2–ratio = 5.7626** **n_2** = 2
****P_2< 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 (gm) of *Bombyx mori* in control, 40, 50, 60 and 70% phytoecdysteroid concentration respectively.

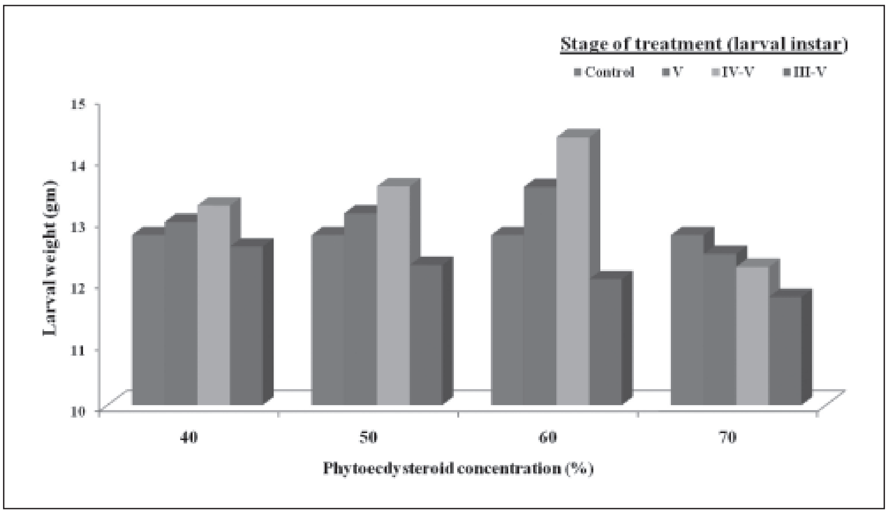

Fig. 3. Effect of phytoecdysteroid treatment on the larval weight (gm) of *Bombyx mori*.

Table 3b. Post-hoc test showing effect of phytoecdysteroid treatment on the larval weight (gm) of *Bombyx mori*

Mean difference in between groups	Phytoecdysteroid concentration (%)		
	Single	Double	Triple
$X_1 \sim X_2$	0.21	0.48	0.19
$X_1 \sim X_3$	0.35	0.79	0.49
$X_1 \sim X_4$	0.78	*1.59	0.72
$X_1 \sim X_5$	0.31	0.52	1.01
$X_2 \sim X_3$	0.14	0.31	0.30
$X_2 \sim X_4$	0.57	1.11	0.53
$X_2 \sim X_5$	0.52	1.00	0.82
$X_3 \sim X_4$	0.43	0.80	0.23
$X_3 \sim X_5$	0.66	1.31	0.52
$X_4 \sim X_5$	*1.09	*2.11	0.29

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

$= 5.05 \frac{\sqrt{0.209}}{3}$

$= 1.33$

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 the mean values of the weight of larvae (gm) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

control and 60% and 60 and 70% concentration of phytoecdysteroid treatment. In triple treatment of larvae no significant group difference was noticed in any of the group combinations.

Survival of larvae

The data presented in Table 4a and Figure 4 shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the survival of larvae. With the increasing number of larval treatment from one to two times, the survival of larvae increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the survival of larvae in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the survival of larvae with increase in the number of larval treatment from single to triple. The trend of increase in the survival of larvae with the increasing number of larval treatment has been recorded to be almost similar in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum survival of larvae was noticed to be 95.25±1.55 per

Table 4a. Effect of phytoecdysteroid treatment on the survival per cent of larvae of *Bombyx mori*

Stage of treatment (Larval instar)	Control X_1	Phytoecdysteroid concentration (%) 40 X_2	50 X_3	60 X_4	70 X_5	F_1-ratio n_1=4
Single (V)	90.33±1.66	90.99±1.07	92.12±1.21	93.45±1.82	89.46±1.35	
Double (IV-V)	90.33±1.66	91.89±1.97	93.10±1.38	95.25±1.55	84.55±1.85	**3.7900***
Triple (III-V)	90.33±1.66	89.95±1.58	88.10±1.60	85.28±1.75	78.85±2.01	

F_2–ratio = 5.08** n_2=2
****P_2< 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 per cent of *Bombyx mori* in control, 40, 50, 60 and 70% phytoecdysteroid concentration respectively.

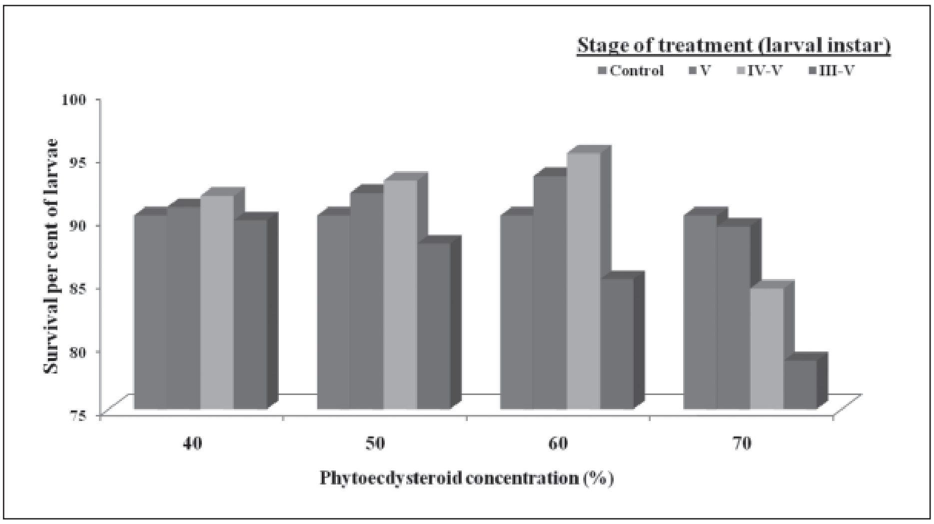

Fig. 4. Effect of phytoecdysteroid treatment on the survival per cent of larvae of *Bombyx mori*.

cent in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum survival of larvae was 78.58±2.75 per cent in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment significantly ($P_2<0.05$) influenced the survival of larvae. The post–hoc test (Table 4b) indicates significant group difference in the survival of larvae, in the double treatment of larvae in between 50 and 70% and 60 and 70%. In the triple treatment of larvae significant group difference in the survival of larvae was noticed in between control and 70%, 40 and 70% and 50 and 70% concentration of phytoecdysteroid treatment. In single treatment of larvae no significant group difference was noticed in any of the group combinations.

Table 4b. Post-hoc test showing effect of phytoecdysteroid treatment on the survival per cent of larvae of *Bombyx mori*

Mean difference in between groups	Phytoecdysteroid concentration (%)		
	Single	Double	Triple
$X_1 \sim X_2$	0.66	1.56	0.38
$X_1 \sim X_3$	1.79	2.77	2.23
$X_1 \sim X_4$	3.12	4.92	5.05
$X_1 \sim X_5$	0.87	5.78	*11.48
$X_2 \sim X_3$	1.13	1.21	1.85
$X_2 \sim X_4$	2.46	3.36	4.67
$X_2 \sim X_5$	1.53	7.34	*11.10
$X_3 \sim X_4$	1.33	2.15	2.82
$X_3 \sim X_5$	2.66	*8.55	*9.25
$X_4 \sim X_5$	3.99	*10.77	6.43

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

$= 5.05\,\frac{\sqrt{7.088}}{3}$

$= 7.762$

MS = Mean square value of ANOVA table
q = Studentized range static
n = No. of replicates
* = Shows significant group difference
$\mathbf{X_1}$, $\mathbf{X_2}$, $\mathbf{X_3}$, $\mathbf{X_4}$ and $\mathbf{X_5}$ are the mean values of the survival per cent of larvae of larvae in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

DISCUSSION

The change in the phytoecdysteroid concentration and the number of larval treatment influenced the duration of larvae. With the increasing number of larval treatment from one to two times, the duration of larvae decreased in case of 40, 50 and 60% concentration of phytoecdsteroid treatment but the triple treatment of larvae caused an increase in the larval duration in all the above concentrations. 70% phytoecdysteroid treatment caused notable increase in the larval duration with the increase in the number of larval treatment from single to triple. The variation in the larval duration of silkworm has been reported by a number of workers viz., Das & Vijayrahavan (1990), Bhargava & Datta (1996) and Khan *et al.* (1997). It is well known that an ideal race is one which has a shorter duration thus causing low consumption of leaf (Mathur & Subba Rao, 1987, Maribashetty *et al.,* 2002). Phytoecdysteroid is recognized as one of the most important components in the silkworm rearing (Nair *et al.,* 2005). The rich-nutrients of mulberry leaves enhanced its nutritional status causing reduction in the larval duration (Soo Hoo & Frenkal, 1966). The present investigation shows positive co-relation with larval duration. Phytoecdysteroid 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 phytoecdysteroid concentration from 40 to 60%, double treatment of larvae, while at 70%, triple treatment of larvae, larval duration increased because of stress response at higher concentration.

Variation in the phytoecdysteroid concentration and the number of larval treatment influenced the larval length. With the increasing number of larval treatment from one to two times, the larval length increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the larval length in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the larval length with increase in the number of larval treatment from single to triple. The maximum larval length was noticed to be 6.98±0.60 cm in case of double treatment of larvae by 60% phytoecdysteroid concentration. Developmental stages are significantly related to the body length and head capsule width (De Loach, 1976; Goyer & Stark, 1983; Oke *et al.,* 2009). The application of hormones to *Bombyx mori* could be used to improve the development and growth of silkworm (Akai *et al.,* 1985; Ahmad *et al.,* 2007; Mamatha *et al.,* 2006, 2008). The application of thyroxine to *B. mori* larvae increased the ecdysteroid titer 33.34% higher than control and the higher titer of ecdysteroid promote larval growth (Ahmad *et al.,* 2007). Maximum larval length was observed after treatment with the test compound R394 in *Bombyx mori* larvae (Gangwar*,* 2009). The good availability of vitamin and mineral mixture (filibon) increased the larval length in silkworm (Muniandy *et al.,* 1995). Thus, it may be envisaged that phytoecdysteroid, by way of enhancing *B. mori* larval tissue ATPase and cytochrome-c-oxidase activity, may contribute to generation of more ATP necessary for the larva for its developmental activity.

The change in the phytoecdysteroid concentration and the number of larval treatment influenced the larval weight. With the increasing number of larval treatment from one to two times, the larval weight increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment while 70% phytoecdysteroid treatment caused notable decline in the larval weight with increase in the number of larval treatment from single to triple. Supplementation with vitamin B increased the resistance against poor environmental conditions and increased body weight in silkworm (Das & Medda, 1998). The larval weight of *Bombyx mori* significantly increased by the hormonal treatment (Khan *et al.,* 1997). Thus, phytoecdysteroid 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 phytoecdysteroid treatment may cause stress response leading to the decrease in the larval weight of *Bombyx mori.*

The change in the phytoecdysteroid concentration and the number of larval treatment influenced the survival of larvae. In larval treatment from one to two times, the survival of larvae increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment while 70% phytoecdysteroid treatment caused notable decline in the survival of larvae with increase in the number of larval treatment from single to triple. The maximum survival of larvae was noticed to be 95.25±1.55 % in case of double treatment of larvae by 60% phytoecdysteroid concentration. The ecological factors (Upadhyay & Gaur, 2002) have been noticed to be important for regulating the survival of larvae. Survival per cent of silkworm larvae was significantly affected by exogenous application of phytoecdysteroid (Shivakumar *et al.,* 1995; Nair *et al.,* 2008). Maximum larval survival of 95% was observed after larval treatment with the test compound R394 in *Bombyx mori* larvae (Gangwar*,* 2009).

Thus, it is concluded that the higher survivability of larvae may be due to the resistance developed in the larvae at low phytoecdysteroid treatment up to 60% concentration, double treatment of larvae while the higher phytoecdysteroid concentration, triple treatment of larvae may cause toxic response resulting in the high mortality of larvae.

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