The Bioscan 7 (3) : 401-407, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

INFLUENCE OF PHYTOECDYSTEROID ON PUPAL PERFORMANCE OF MULTIVOLTINE MULBERRY SILKWORM (*BOMBYX MORI* LINN)

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KEY WORDS
Bombyx mori
Silk production
Phytoecdysteroid

Received on :
27.04.2012

Accepted on :
11.08.2012

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ABSTRACT
The phytoecdysteroid activates the physiological and developmental processes in the living beings, therefore, the oral application of phytoecdysteroid on silkworm larvae has been studied to observe any possibility for the heavy production of silk. 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 phytoecdysteroid concentration ($p_1 > 0.05$) of *B. mori* larvae significantly influenced the pupal length, pupal weight and survival of silkworm pupae where as pupal duration is in significant for both phytoecdysteroid concentration and number of larval treatment. The weight of pupa increased from 0.73g (control) to the maximum of 0.95g in case of 60%, double treatment of larvae. The pupal length (cm) increases with the increasing phytoecdysteroid concentration and number of treatment up to 60%, double treatment of larvae. The maximum pupal length was recorded to be 2.22g in case of 60%, double treatment of larvae while it was minimum (1.73g) in 70%, triple treatment of larvae. The survival of pupae increased with the increasing phytoecdysteroid treatment from single to double treatment in 40, 50 and 60% phytoecdysteroid concentration and it was maximum (0.91.28%) in 60%, double treatment of larvae.

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 has been known that application of hormones to *Bombyx mori* could be used to improve the quality of silk (Akai *et al.,* 1985; Mamatha *et al.,* 2006; Ahmad *et al.,* 2007). The process of moulting and metamorphosis, characteristic to larval growth and development in insects, are controlled by circulating hormones like PTTH, JH and ecdysterone (Wigglesworth, 1985). The set pattern of the insect development can be altered by manipulating compounds leading either to precocious metamorphosis or supernumerary larval moults (Sakurai, 1983).

Ecdysteriod have diverse function such as morphogenesis, regulation of gene activities, metabolism of nucleic acid, protein synthesis, development, reproduction and diapause in insects (Chow and Lu, 1980). Various ecdysteriods with moulting hormone activity in insects were isolated from plant materials (Novak, 1975; Chow and Lu, 1980; Slama *et al.,* 1993). Phytoecdysteriod simulates silkworm larvae for tolerance to toxin and viral infection (Chernysh *et al.,* 1983), enhances RNA synthesis in the silk gland (Dai *et al.,* 1985) and accelerates growth and development in silkworm. Thus, it reduces the larval duration (Chow and Lu, 1980).

The feeding of exogenous phytoecdysteriod by silkworm larvae, at certain stages of development caused synchronous development of the larvae .The response of the silkworm larvae to the treatment of ecdysteriod largely depends on the time of application and also the dose of the compound (Chou and Lu, 1980).

The perusal of literature like some references reveals that most of the work done on the influence of phytoecdysteroid on *Bombyx mori* has been restricted to the limited and scattered source of information but in the parameters like development and growth, silk producing potential and biochemical constituents, many gap still exit in our knowledge. Keeping this in view, it has been proposed to undertake a comprehensive study on the effect of phytoecdysteroid on the performance of pupae in multivoltine mulberry silkworm *Bombyx mori*.

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 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 h/day dim light condition. After four h. of mating, the paired moths were decoupled manually. The female moths were allowed for egg laying. After 24h 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* and *Cassia tora* act as bioactive phytoecdysteroid compound on *Bombyx mori* larvae (Lafont *et al*., 2004). In the present study *Achyranthes aspera* and *Cassia tora* were 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 hormone with respect to the treatment of IIIrd, IVth and Vth instar larvae. For extraction of phytoecdysteroid, the leaves of *Achyranthes aspera* and *Cassia tora* were collected, washed thoroughly with distilled water and dried in incubator at 37ºC. The dried leaves were powdered separately with the help of mechanical device. Further, 50g powder, thus obtained was subjected to extraction separately through soxlet apparatus with 250mL distilled water for 40h. After 40h of extraction a little amount of concentrated solution was obtained which was dried and 6.75g powdered material was obtained. The dried powder was dissolved in distilled water as 5g in 25mL 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 10mL on 100 g mulberry leaf which were air dried and given as food to larvae, The larvae treated with *Cassia* extract showed very poor growth and high mortality, whereas, the survival of larvae and their growth performance was satisfactory at 50% concentration of *Achyranthes* extract. Therefore, 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 10mL on 100g 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 *i.e.* 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 pupal duration length, weight and survival.

Pupal performance: For determining the effect of bioactive phytoecdysteroid on the pupal duration, pupal length, pupal weight and survival of pupae, the ripe worms (fifth instar larvae when stop feeding), reared at 26 ± 1ºC, 80 ± 5% RH and 12 ± 1h light a day, were put on mountages for spinning. Thus, the formation of cocoon takes place and larvae changed in the pupal stage. The pupae were remain maintained in the same BOD incubator at similar conditions. After few days of pupal period, the moths emerged from the pupae.

For determining the pupal duration : The time required from the third day of spinning (formation of pupae) to the emergence of moth was considered. For this purpose, 75cocoon along with their pupae (three batches of 25cocoon in each batch) were taken for observation. Three replicates of each experiment were made.

For determining the pupal length: The lengths of 30 pupae (three batches of 10 pupae in each batch) were recorded for each replicate. Three replicates of each experiment were made. The cocoon shells were dissected to obtain pupae and pupal length was taken on the 3rd day of spinning. Three replicates of each experiment were made.

For estimating the pupal weight: The weights of 30 normal pupae (three bathes of 10 pupae in each batch) were recorded for each replicate. The pupal weight was taken on 7th day of the formation of pupae. Three replicates of each experiment were made.

For recording the survival of pupae: 75 pupae (three batches of 25 normal pupae in each batch) were observed. The number of pupae emerged as moth was counted for calculating the survival of pupae as following:

$$\text{Percent survival of pupae} = \frac{\text{No. of moths emerged}}{\text{No. of pupae taken for observation}} \times 100$$

RESULTS

Pupal Duration: The data presented in Table 1a shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the duration of pupae. With the increasing number of larval treatment from one to two times, the pupal duration decreased in case of 40, 50 and 60%

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

Stage of treatment (Larval instar)	Phytoecdysteroid concentration (%) Control X_1	40 X_2	50 X_3	60 X_4	70 X_5	F_1-ratio $n_1 = 4$
Single (V)	10.04 ±1.02	9.95 ±0.82	9.79 ±0.65	9.56 ±0.62	10.46 ±1.08	
Double (IV-V)	10.04 ±1.02	9.84 ±0.96	8.60 ±0.75	8.24 ±0.95	11.25 ±1.05	2.9914*
Triple (III-V)	10.04 ±1.02	10.21 ±0.92	10.37 ±0.93	11.46 ±0.76	12.75 ±1.05	

F_2– ratio = 4.3892* $n_2 = 2$; *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 pupal duration (days) of *Bombyx mori* in control, 40, 50,60; 70% phytoecdysteroid concentration respectively

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

Mean difference in between groups	Phytoecdysteroid concentration (%) Single	Double	Triple
$X_1 \sim X_2$	0.09	0.20	0.17
$X_1 \sim X_3$	0.25	1.44	0.33
$X_1 \sim X_4$	0.48	1.80	1.42
$X_1 \sim X_5$	0.42	1.21	*2.71
$X_2 \sim X_3$	0.16	1.24	0.16
$X_2 \sim X_4$	0.39	1.60	1.25
$X_2 \sim X_5$	0.51	1.41	*2.54
$X_3 \sim X_4$	0.23	0.36	1.09
$X_3 \sim X_5$	0.67	*2.65	*2.38
$X_4 \sim X_5$	0.90	*3.01	1.29

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

= $5.05\ \frac{\sqrt{0.575}}{3}$ = 2.21

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 pupal duration (days) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively

concentration of phytoecdsteroid treatment but the triple treatment of larvae caused an increase in all the above concentrations. 70% phytoecdysteroid treatment caused notable increase in the pupal duration with the increase in the number of larval treatment from single to triple. The trend of decrease 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 pupal duration was noticed to be 8.24 ±0.95 days in case of double treatment of larvae by 60% phytoecdysteroid concentration and maximum pupal duration of 12.75±1.05 days was

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

Stage of treatment (Larval instar)	Phytoecdysteroid concentration (%) Control X_1	40 X_2	50 X_3	60 X_4	70 X_5	F_1-ratio $n_1 = 4$
Single (V)	2.02 ±0.034	2.04 ±0.035	2.07 ±0.034	2.10 ±0.079	1.96 ±0.047	
Double (IV-V)	2.02 ±0.034	2.06 ±0.061	2.10 ±0.056	2.22 ±0.068	1.90 ±0.014	2.4148*
Triple (III-V)	2.02 ±0.034	1.92 ±0.013	1.88 ±0.014	1.81 ±0.027	1.73 ±0.012	

F_2– ratio = 8.1849** $n_2 = 2$; *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 pupal duration (days) of *Bombyx mori* in control, 40, 50,60; 70% phytoecdysteroid concentration respectively

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

Mean difference in between groups	Phytoecdysteroid concentration (%) Single	Double	Triple
$X_1 \sim X_2$	0.02	0.04	0.10
$X_1 \sim X_3$	0.05	0.08	0.14
$X_1 \sim X_4$	0.08	0.20	0.21
$X_1 \sim X_5$	0.06	0.12	*0.29
$X_2 \sim X_3$	0.03	0.04	0.04
$X_2 \sim X_4$	0.06	0.16	0.11
$X_2 \sim X_5$	0.08	0.16	0.12
$X_3 \sim X_4$	0.03	0.12	0.07
$X_3 \sim X_5$	0.11	0.20	0.15
$X_4 \sim X_5$	0.14	*0.32	0.08

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

= $5.05\ \frac{\sqrt{0.006}}{3}$ = 0.226

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 pupal duration (days) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively

recorded in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment and phytoecdysteroid concentration did not cause significant effect on the duration of pupae. The post–hoc test (Table 1b) indicates significant group difference, in the double treatment

Table 3(a): Effect of phytoecdysteroid treatment on the pupal weight (g) of *Bombyx mori*

Stage of treatment (Larval instar)	Phytoecdysteroid concentration (%) Control X_1	40 X_2	50 X_3	60 X_4	70 X_5	F_1-ratio $n_1 = 4$
Single (V)	0.73 ±0.017	0.78 ±0.019	0.84 ±0.020	0.90 ±0.022	0.70 ±0.018	
Double (IV-V)	0.73 ±0.017	0.82 ±0.023	0.88 ±0.025	0.95 ±0.021	0.62 ±0.026	2.7065*
Triple (III-V)	0.73 ±0.017	0.70 ±0.019	0.66 ±0.021	0.61 ±0.023	0.58 ±0.033	

F_2– ratio = 6.5014** $n_2 = 2$; *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 pupal duration (days) of *Bombyx mori* in control, 40, 50,60; 70% phytoecdysteroid concentration respectively

Table 3(b): Post-hoc test showing effect of phytoecdysteroid treatment on the pupal weight (g) of *Bombyx mori*

Mean difference in between groups	Phytoecdysteroid concentration (%) Single	Double	Triple
$X_1 \sim X_2$	0.05	0.09	0.03
$X_1 \sim X_3$	0.11	0.15	0.07
$X_1 \sim X_4$	0.17	*0.22	0.12
$X_1 \sim X_5$	0.03	0.08	0.15
$X_2 \sim X_3$	0.06	0.06	0.04
$X_2 \sim X_4$	0.12	0.13	0.09
$X_2 \sim X_5$	0.08	0.17	0.12
$X_3 \sim X_4$	0.06	0.07	0.05
$X_3 \sim X_5$	0.14	*0.23	0.08
$X_4 \sim X_5$	0.20	*0.30	0.03

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

= $5.05\ \frac{\sqrt{0.005}}{3}$ = 0.206

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 pupal duration (days) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively

Table 4(a): Effect of phytoecdysteroid treatment on the survival per cent of pupae of *Bombyx mori*

Stage of treatment (Larval instar)	Phytoecdysteroid concentration (%) Control X_1	40 X_2	50 X_3	60 X_4	70 X_5	F_1-ratio $n_1 = 4$
Single (V)	86.67 ±1.25	87.12 ±1.70	88.46 ±1.63	89.86 ±1.68	84.05 ±1.12	
Double (IV-V)	86.67 ±1.25	88.33 ±1.36	90.20 ±1.50	91.28 ±1.92	81.67 ±1.05	3.4078*
Triple (III-V)	86.67 ±1.25	85.25 ±1.40	83.15 ±1.86	81.20 ±1.89	79.56 ±1.02	

F_2– ratio = 6.0437** $n_2 = 2$; *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 pupal duration (days) of *Bombyx mori* in control, 40, 50,60; 70% phytoecdysteroid concentration respectively

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

Mean difference in between groups	Phytoecdysteroid concentration (%) Single	Double	Triple
$X_1 \sim X_2$	0.45	1.66	1.42
$X_1 \sim X_3$	1.79	3.56	3.52
$X_1 \sim X_4$	3.19	4.61	5.47
$X_1 \sim X_5$	2.62	5.00	*7.11
$X_2 \sim X_3$	1.34	1.87	2.10
$X_2 \sim X_4$	2.74	2.95	4.05
$X_2 \sim X_5$	3.07	*6.66	*7.11
$X_3 \sim X_4$	1.04	1.08	1.95
$X_3 \sim X_5$	4.41	*8.53	3.59
$X_4 \sim X_5$	5.81	*9.61	1.64

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

$= 5.05\ \frac{\sqrt{5.049}}{3} = 6.55$

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 pupal duration (days) in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively

of larvae in between 50 and 70% and 60 and 70% phytoecdysteroid concentration. In triple treatment of larvae significant group difference 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.

Pupal length: The data presented in Table 2a shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the pupal length. With the increasing number of larval treatment from one to two times, the pupal length increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the pupal length in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the pupal length with increase in the number of larval treatment from single to triple. The trend of increase in the pupal 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 pupal length was noticed to be 2.22 ± 0.068cm in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum pupal length of 1.73 ± 0.012cm 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 pupal length. The post–hoc test (Table 2b) indicates significant group difference in the pupal length, in the double treatment of larvae in between 60 and 70%. In triple treatment of larvae significant group difference in the pupal length 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.

Pupal weight: The data presented in Table 3a shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the pupal weight. With the increasing number of larval treatment from one to two times, the pupal weight increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the pupal weight in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the the pupal weight with increase in the number of larval treatment from single to triple. The trend of increase in the pupal 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 pupal weight was noticed to be 0.95 ± 0.021g in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum pupal weight 0.58 ± 0.033 g 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 pupal weight. The post–hoc test (Table 3b) indicates significant group difference in the pupal weight, 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.

Survival of pupae: The data presented in Table 4a shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the survival of pupae. With the increasing number of larval treatment from one to two times, the survival of pupae 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 pupae in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the survival of pupae with increase in the number of larval treatment from single to triple. The trend of increase in the survival of pupae 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 pupae was noticed to be 91.28 ± 1.92 per cent in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum of 79.56 ± 1.02 per cent 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 survival of pupae. The post–hoc test (Table 4b) indicates significant group difference in the survival of pupae, in the double treatment of larvae in between 50 and 70%, 50 and 70% and 60 and 70% phytoecdysteroid concentration. In triple treatment of larvae

significant group difference in pupal survival was noticed in between control and 70% and 40 and 70% concentration of phytoecdysteroid treatment. In single treatment of larvae no significant group difference was noticed in any of the group combinations.

DISCUSSION

The change in the phytoecdysteroid concentration and the number of larval treatment influenced the duration of pupae. With the increasing number of larval treatment from one to two times, the pupal duration decreased in case of 40, 50 and 60% concentration of phytoecdsteroid treatment but the triple treatment of larvae caused an increase in all the above concentrations. 70% phytoecdysteroid treatment caused notable increase in the pupal duration with the increase in the number of larval treatment from single to triple. The minimum pupal duration was noticed in case of double treatment of larvae by 60% phytoecdysteroid concentration. The pupal duration of *Bombyx mori* has been noticed to be influenced by the change in varieties of mulberry, given as food to the larvae (Bheemanna *et al.,* 1989), secretion of specific hormone (Khan *et al.,* 1997) and ecological factors (Gaur and Upadhyay, 2002). It is a well known fact that the insect larval moulting is determined by the interaction of JH secreted by corpora allata and MH secreted by prothoracic gland activated by the brain hormone PTTH. The larvae, pupae and adult transformations are determined mainly by the functions of MH (Bharathi and Yungen, 2000). The major objective of using phytoecdysteroid in sericulture is to hasten the larval maturation events, thus reduces larval as well pupal duration (Nair *et al.,* 2002; Trivedy *et al.,* 2003). The difference in the larval and mounting duration is because of a physiological role played by the exogenous ecdysteroid on the insect development system. The feeding larvae always contain a baseline level of ecdysone but reaches to pupation inducing peak before pupation (Sehnal, 1989). In the present investigation pupal duration decreased with increasing phytoecdysteroid concentration up to 60% and double treatment of larvae, while higher concentration and number of treatment caused adverse effect. The possible explanation for this is that the feeding larvae always contain a baseline level of ecdysone which reaches to pupation inducing peak before pupation. By giving an extra dose of plant based ecdysteroid at the critical time, the pupation inducing peak of ecdysteroid content in silkworm is advanced and thereby change the larval behavior as such.

Variation in the phytoecdysteroid concentration and the number of larval treatment influenced the pupal length. With the increasing number of larval treatment from one to two times. The pupal length increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the pupal length in all the above concentrations. The maximum pupal length was noticed in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum pupal length was recorded in case of triple treatment of larvae by 70% phytoecdysteroid concentration. Large pupal size or weight has been associated with greater longevity (Bloem *et al.,* 1994). The puparial length increased as puparial weight increased (Alfredo *et al.,* 2006). The silkworm Chinese strain and Japanes strain have more pupal length than normal (Alimurong *et al.,* 1986). Positive relationship between pupal length, width or size and weight of female pupae of *Bombyx mori* were noticed by (Rithinam *et al.,* 1991). On the basis of present observation and above information it may be concluded that the application of phytoecdysteroid to *B. mori* larvae activate cellular activity in silk gland enhancing rapid growth and development in silkworm at certain extent while higher phytoecdysteroid concentration and number of treatment cause adverse effect on length of pupae due to ecdysteriod which have diverse function such as morphogenesis, regulation of gene activities, metabolism of nucleic acid, protein synthesis, development, reproduction and diapause in insects.

The change in the phytoecdysteroid concentration and the number of larval treatment influenced the pupal weight. With the increasing number of larval treatment from one to two times, the pupal weight increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the pupal weight in all the above concentrations. 70% phytoecdysteroid treatment caused notable decline in the pupal weight with increase in the number of larval treatment from single to triple. The pupal weight of *Bombyx mori* has been noticed to be influenced by the variation in the level of secreted hormones (Khan *et al.,* 1997) and genotype variations (Rajashekher Gouda *et al.,* 1997)**.** The difference between the weight of cocoon and shell is the weight of the pupa (Gaviria *et al.,* 2006)**.** 20 % increase in cocoon and pupal weight with big cocoon formation was noticed when the larvae were treated with methylenedioxy phenyl derivatives (Chang, 1980). Oral supplementation of ascorbic acid to *Bombyx mori* larvae resulted in significant increase in the cocoon characteristics such as cocoon weight, pupal weight, shell weight, shell ratio and filament length when compared to control (Thilsath *et al.,* 2008). Organic manures are having strong hold not only on the growth and development of silkworm, but also have a direct effect on the cocoon, pupal and silk weight (Shashidhar *et al.,* 2009). An enhanced yield of 20-35 % in cocoon and pupal weight were noticed by Murakoshi *et al.* (1972). Large pupal size and weight has been associated with greater longevity (Bloem *et al.,* 1994). Puparial length increased as puparial weight increased (Alfredo *et al.,* 2006). Rithinam *et al.* (1991) studied the positive relationship between pupal length, width, size and weight of female pupae of *Bombyx mori.* The feeding leaves supplemented with distilled water alone slightly increased the weight of larvae and pupae (Verma and Atwal, 1968**).** The highest pupal weight of 3.10g was recorded 0.1μL /1arva at 72h. treatment, with the test compound R394 which was 37.96 % higher than the control in *Bombyx mori* larvae (Gangwar *et al.,* 2009). On the basis of present observation and above information it may be concluded that the application of phytoecdysteroid to *B. mori* larvae increased the ecdysteroid titer which activate cellular activity enhancing rapid growth and development in silkworm at certain extent while higher phytoecdysteroid concentration and number of treatment cause toxic effect.

The variation in the phytoecdysteroid concentration and the number of larval treatment influenced the survival of pupae.

With the increasing number of larval treatment from one to two times, the survival of pupae increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment while in 70% phytoecdysteroid treatment, the trend of the survival of pupae was lower with increase in the number of larval treatment from single to triple. The maximum survival of pupae was noticed to be 91.28 ± 1.92 % in case of double treatment of larvae by 60% phytoecdysteroid concentration. The survival and development of insects are at the mercy of nature and developmental activities are restricted in accordance with the prevailing ecological conditions and to a certain extent to their genetic built up (Andrewartha and Birch, 1954; Robertson, 1957; Krishnaswamy *et al.,* 1973). The physiological changes due to variation in the rearing temperature influenced the survival of pupae in *Bombyx mori* (Pandey and Upadhyay, 1999a, 1999b). The oral administration of folic acid during 5th instar silkworm significantly influenced the survival per cent of silkworm (Rahmathulla *et al.,* 2007; Nirwani and Kaliwal, 1996a; Rai *et al.,* 2002; Etebari, 2002). Rithinam *et al.* (1991) studied positive relationship between pupal length width or size and weight of female pupae of *Bombyx mori*. Thus, the increased survival per cent of pupae with increasing phytoecdysteroid treatment from one to two times in 40, 50, 60% phytoecdysteroid concentration of *Bombyx mori* larvae may be defined as stimulatory physiological and developmental changes, whereas, decline in survival per cent of pupae at 70% phytoecdysteroid concentration, triple treatment of larvae may be due to the toxic effect caused by the higher concentration of phytoecdysteroid concentration.

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