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# The phytojuvenoid caused beneficial effect on the commercial parameters of multivoltine mulberry silkworm (*Bombyx mori* Linn.)

**Roli Srivastava and VB Upadhyay**

**Abstract**
The present study was aimed at investigate the economic parameter of the silkworm cocoon. The topical application of phytojuvenoid on *Bombyx mori* larvae has been proved to be of biotechnological significance in the sericulture industry. Maximum denier of cocoon was recorded to be 1.98 d. in case of 30% phytojuvenoid concentration – triple treated larvae while it was minimum 1.23 d. in 40% phytojuvenoid concentration at triple treated larvae. The outcome of this work is expected to have applied significance and the knowledge derived from the present study will be helpful in the rearing of silkworm on industrial scale and generate more employment opportunities.

**Keywords:** Phytojuvenoid, denier, initial and final stage of larvae, *Bombyx mori*

## 1. Introduction

The silkworm is an of economic insect used for silk production and the sericulture or silkworm rearing depends on mulberry leaves as the natural food of the silkworm *Bombyx mori* L., the consumption of the mulberry leaves has a direct effect on the normal growth of the larvae and the quality of the cocoon. The efforts are being made to evolve new technologies that are effective, labour saving and eco-friendly. In order to increase, the production of silk, efforts have been made to study effect of ecological factor [1], temperature [2]) etc on the performance of silkworm. The magnetization of eggs influences silk producing potential [3] and larval performance [4]. In insects, the process of growth and development is regulated by circulating hormones viz., prothoracicotropic hormone (PTTH), juvenile hormone (JH) and ecdysone, which directly and indirectly manifest the phenomenon of moulting and metamorphosis [5]. The response of silkworm to very small quantities of phytojuvenoid or its analogues may extend the larval maturation events and influence the spinning process. However, the response to such treatment varies depending on the dosage of compounds showing duration and number of applications [6]. The phytoecdysteroid has been noticed to influence the development, silk producing and reproductive potential of *B. mori* [7, 8]. The juvenile hormone analogue also has been noticed to influence the commercial potential of *Bombyx mori* [9, 10]. The more food ingested during this period gets converted and it turn contributes to silk protein. Delay in moulting is probably due to the inhibitory action of JH on ecdysone synthesis in *B. mori* [11]. JH is claimed to inhibit protein synthesis in early treated larvae with later on region protein synthesis resulting in bigger silk gland and the result is improvement of cocoon shell weight [12]. The phytojuvenoid caused beneficial effect on the the life pattern of silkworm [13, 14]. Some plants like *Pinus longifolia, Abies bolsomea, Psorelea corylifolia* and *Azadirachta indica* act on *Bombyx mori* larvae as bioactive juvenoid compounds [15]. In the present study *Pinus longifolia* was taken for experiment due to its good availability and containing juvenile compound. Keeping this in view, an attempt has been made in the present to study the topical effect of bioactive phytojuvenoid on the improvement in the commercial parameters in this monophagous insect (*Bombyx mori*).

## 2. 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 [16] in the silkworm laboratory, Department of Zoology D.D.U. Gorakhpur University, Gorakhpur. The temperature and relative humidity were maintained at 26 ± $1^0$C and 80 ± 5% RH respectively till the emergence of moths from the seed 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 $9^{th}$ and $10^{th}$ day of spinning. The moths emerged, were allowed their mates for copulation. After four hours of mating, the paired moths were detached manually by holding the female moths between the thumb and middle finger gently and pushing the male away by the fore finger and the female moths were allowed to egg laying. The disease free layings (D.F.L's) were treated with 2% formalin for 15 minutes to increase the adhesiveness of eggs on the paper sheet and surface disinfection. Thereafter, the egg sheets with eggs laid on were thoroughly washed with running water to remove formalin and the eggs were dried in shade. The dried eggs were transferred to the incubator for hatching. After two consecutive days of hatching, the silkworm larvae were collected with the help of feather of birds and reared to maintain a stock culture in the silkworm laboratory at 26 ± $1^0$C and 80 ± 5% RH and 12 ± 1 hours light a day. Four feedings of the small pieces of fresh and clean leaves of *Morus alba* were given to the larvae and care was taken that food always remained in excess in the rearing trays. These larvae were taken for the purpose of experiments.

**Design of Experiment**

For extraction of phytojuvenoid the needle of *Pinus longifolia* was collected, washed thoroughly with distilled water and dried in incubator at $37^0$C. The dried materials were powdered separately with the help of mechanical device. Further, 50 gm powder was subjected to extraction separately through soxhlet apparatus with 250 ml distilled water for 40 hours. After 40 hours of extraction a little amount of concentrated solution of plant extract was obtained. The concentrated solution was dried and 6.45 gm material was obtained in powdered form. The dried powder thus obtained, was dissolved in distilled water as 5 gm in 25 ml water and used this solution for further experiment, as 100% concentration of phytojuvenoid. For further experiment the suitable narrow ranges of *Pinus* phytojuvenoid concentrations viz. 10, 20, 30 and 40% were taken. Thus, four phytojuvenoid concentrations were applied topically by spraying as 1 ml on to 100 larvae separately. Three sets of experiments were designed viz., single, double and triple treatment of larvae. Single treatment of larvae was performed at the initial stage of fifth instar larvae just after fourth moulting. One hundred larvae of fifth instar at the initial stage were taken out from the BOD incubator and treated with one ml of 10% concentrated solution of *Pinus* needle extract by sprayer. Double treatment of larvae was started from the initial stage of fourth instar larvae. In the first treatment, one hundred larvae of fourth instar were treated by 1 ml of 10% concentrated solution of *Pinus* needle extract by spraying. The treated larvae were then transferred in BOD incubator for rearing and development. Further, similar second treatment for the same larvae was given at the initial stage of fifth instar larvae. Thus, in double treatment, fourth and fifth instar larvae were treated. For triple treatment, the third instar larvae in the initial stage were separated from BOD incubator. In the first treatment one hundred, third instar larvae, were treated by 1 ml of 10% concentrated solution of *Pinus* needle extract by sprayer and kept in BOD for rearing. The second treatment of same larvae was done just after third moulting i. e. at the initial stage of fourth instar larvae and transferred in BOD incubator for rearing. Third treatment was given at the initial stage of fifth instar i.e. just after fourth moulting of the same treated larvae as earlier. Thus, in the triple treatment third, fourth and fifth instar larvae were treated. Similar experiments were performed by 20, 30 and 40% concentrations of phytojuvenoid obtained from *Pinus* needle extract. A control set was always maintained with each set of experiment. All the data obtained by the experiment were analyzed statistically by two-way ANOVA and Post-hoc test.

**Denier of cocoon:** Denier is a unit for the linear mass density of fibers. It is defined as the mass in gram per 9000 meters of filament. Denier was calculated as follows-

$$\text{Denier (d)} = \frac{\text{Wt. of cocoon filament (gm)}}{\text{Length of cocoon filament (m)}} \times 9000$$

**3. Results**

**Denier of silk filament -** It is clear from the data given in table-1 that the phytojuvenoid concentration and number of larval treatment influenced the denier of silk filament. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the denier of silk filament increased gradually and reached to the highest level of 1.98± 0.057 d (denier) in case of triple treated larvae with 30% phytojuvenoid concentration. In case of larval treatment with 40% phytojuvenoid concentration, the denier of silk filament increased in single treated larvae but further increase in the number of larval treatment caused decline in the denier of silk filament which reached to the lowest level of 1.23±0.055 d in triple treated larvae. The trend of increase in the denier of silk filament was almost same in 10, 20 and 30% phytojuvenoid concentration in relation to the number of larval treatment. Two-way ANOVA indicates that variation in the phytojuvenoid concentration significantly ($P_1$ < 0.01) influenced the denier of silk filament while number of larval treatment has no significant influence on the denier of silk filament. The Post–hoc test (table-2) indicates significant group difference in the denier of silk filament in between control and 10% and control and 30% in single treated larvae. In the double treated larvae significant group difference in the denier of silk filament was noticed in between all the group combinations except in control and 40%, 10 and 20%, 10 and 30% and 20 and 30% and in triple treated larvae significant group difference in the denier of silk filament was recorded in between all group combinations except in control and 40% and 10 and 20% phytojuvenoid concentration.

**Table 1:** Effect of phytojuvenoid treatment on the denier of *Bombyx mori* silk filament.

| | Phytojuvenoid concentration (%) | | | | | |
|---|---|---|---|---|---|---|
| **Stage of treatment (Larval instar)** | **Control $X_1$** | **10 $X_2$** | **20 $X_3$** | **30 $X_4$** | **40 $X_5$** | **$F_1$ – ratio $n_1$ =4** |
| Single (V) | 1.38 ±0.017 | 1.52 ±0.019 | 1.58 ±0.013 | 1.63 ±0.03 | 1.46 ±0.086 | |
| Double (IV-V) | 1.38 ±0.017 | 1.61 ±0.019 | 1.67 ±0.075 | 1.74 ±0.027 | 1.32 ±0.045 | 8.91* |
| Triple (III-V) | 1.38 ±0.017 | 1.66 ±0.085 | 1.78 ±0.074 | 1.98 ±0.057 | 1.23 ±0.055 | |

$F_2$-ratio = 0.89** $n_2$ = 2

$P_1$ > 0.01 ** 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 the denier of silk filament in control, 10, 20, 30 and 40 % of phytojuvenoid concentration respectively.

**Table 2:** Post - hoc test showing effect of phytojuvenoid treatment on the denier of cocoon (d) of *Bombyx mori*

| Mean difference in between groups | stage of treatment | | |
|---|---|---|---|
| | Single | Double | Triple |
| $X_1$~$X_2$ | *0.14 | *0.23 | *0.28 |
| $X_1$~$X_3$ | 0.02 | *0.29 | *0.40 |
| $X_1$~$X_4$ | *0.25 | *0.36 | *0.60 |
| $X_1$~$X_5$ | 0.08 | 0.06 | 0.15 |
| $X_2$~$X_3$ | 0.06 | 0.06 | 0.12 |
| $X_2$~$X_4$ | 0.11 | 0.13 | *0.32 |
| $X_2$~$X_5$ | 0.06 | *0.29 | *0.43 |
| $X_3$~$X_4$ | 0.05 | 0.07 | *0.20 |
| $X_3$~$X_5$ | 0.12 | *0.35 | *0.55 |
| $X_4$~$X_5$ | 0.17 | *0.42 | *0.75 |

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

$= 5.05\sqrt{\frac{0.012}{3}}$

$= 0.19$

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 denier of cocoon (d) of *Bombyx mori* in control, 10, 20, 30 and 40 per cent phytojuvenoid concentration respectively.

## 4. Discussion

**Denier of silk filament-** The variation in the phytojuvenoid concentration and the number of larval treatment of *Bombyx mori* influenced the denier of silk filament. With the increasing number of larval treatment from single to triple, the filament length of cocoon increased in case of 10, 20 and 30% phytojuvenoid concentration, while in 40% concentration, the denier of silk filament increased in single treatment and further decreased with the increasing the number of larval treatment. The thyroxine treated mulberry species as *Morus multicaulis* had significant effect on the denier [17]**.** The administration of plant growth hormone Indloe-3- acetic acid increased the denier [18]. The phytoecdysteroids when administered at different age of 5th instar larvae of *Bombyx mori* influenced the denier [19]**.** Methoprene and fenoxycarb treated *Bombyx mori* larvae showed significant enhance in the denier [20]**.** The hybrids can be influenced by the environmental factors viz. temperature and humidity and the denier of *Bombyx mori* was affected [21]**.** The maternal inheritance affects regarding the temperature tolerance and has better performance in denier of *Bombyx mori* [22, 23]. The effect of high temperature and high humidity was decreased the denier of *Bombyx mori* [24]**.** The Indian double hybrid (CSR2 X CSR27) X (CSR6 X CSR26) is better than the Chinese double hybrids in respect to denier [25]**.**

## 5. Conclusion

In the present investigation the post cocoon character positively increased with the increasing phytojuvenoid concentration up to 30%. The maximum level of denier of silk filament was recorded in case of 30% phytojuvenoid concentration – triple treated larvae, whereas, the minimum denier was noticed in case of larvae treated with 40% phytojuvenoid concentration - triple treated larvae. The increase in the silk production might be due to direct stimulatory effect of phytojuvenoid on the protein synthesis of silk gland. The stimulatory ability of phytojuvenoid on post cocoon character contributing to silk yield may be attributed to the synthesis of protein and nucleic acid in the silkworm. The increase in fibroin content may lead to the superior quality of silk. The higher concentration of phytojuvenoid may cause stress response, resulting in the decline of the denier.

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