Effect of the Phytojuvenoid Treatment on the Silk Production of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.)

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Abstract: The present study has been aimed at investigating 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. The variation in the phytojuvenoid concentration and the number of larval treatment influenced the reelability of cocoon in *B. mori*. The reelability of cocoon increased from 68.80% (control) to the maximum of 79.36% in 30% phytojuvenoid concentration – triple treated larvae. The outcome of this work is expected to have applied significance and the knowledge derived from the study will be helpful in the rearing of silkworm on industrial scale and improve quality as well as quantity of silk.

**Key words:** Phytojuvenoid • Reelability • Larvae • *Bombyx mori*

INTRODUCTION

Silk has been intermingled with the life and culture of the Indians. India has a rich and complex history in silk production and its silk trade dates back to 15th century. The natural silk synthesized by the silkworm and spun in the form of a silk cocoon is originally synthesized in the silk gland. After Degumming, the leftover is fibroin made up of two brins. Silk fibre can be used for many purposes including textile, medical and industrial applications. The silk fibre is thin, long, light and soft. It is well known for its water absorbency, dyeing affinity, thermo tolerances, insulation properties and luster. Nistari is a resistant variety of multivoltine mulberry silkworm (*Bombyx mori*) which contributes up to a great extent in the commercial production of cocoon. The synchronized maturation of larvae and simultaneous spinning of cocoon is very important in the sericulture industry. Comparative biometric studies have attempted to identify which of the silk gland parameters is the target; four parameters show high correlation with silk productivity of different strain of *B. mori*. They are number of silk gland cells, silk gland weight, the DNA and the RNA content [1]. In order to increase, the production of silk, efforts have been made to study effect of magnetization of eggs influences silk producing potential [2] and incubation period of eggs [3] and larval performance [4]. The phytoecdysteroid and the juvenile hormone (JH) analogue have been noticed to influence the development, growth, silk producing and reproductive potential of *B. mori* [5-9]. However, the response to such treatment varies depending on the dosage of compounds showing duration and number of applications [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]. Thus, it is hypothesized that the application of phytojuvenoid on *B. mori* larvae may enhance the duration of larval instar resulting in the utilization of more mulberry leaves which may improve the quality as well as quantity of silk on the commercial scale. 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 to study the topical effect of bioactive phytojuvenoid on the improvement in the commercial parameters in this monophagous insect (*Bombyx mori*), which is the aim of the present investigation.

MATERIALS AND METHODS

The seed cocoons (pupa enclosed in silken case) of multivoltine mulberry silkworm (*Bombyx morinistari*), 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 [12] in the silkworm laboratory, Department of Zoology D.D.U.
Gorakhpur University, Gorakhpur. The temperature and relative humidity were maintained at 26 ± 1°C and 80 ± 5% RH respectively till the emergence of moths from the seed cocoons. The 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 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% formaline for 15 minute 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 formaline 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°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 were collected, washed thoroughly with distilled water and dried in incubator at 37°C. The dried materials were powdered separately with the help of mechanical device. Further, 50 gm powder was subjected to extraction separately through soxlet apparatus with 250 ml distilled water and dried in incubator at 37°C for one hour and after this, temperature was decreased by 15°C after every one hour to reach up to 115°C for one hour. The concentrated solution 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.

Reelability of Cocoon: For reeling, cocoon was dried in a hot chamber in different batches. The drying chamber was heated up to 120°C and then the cocoon was kept into drying chamber. The chamber was maintained at 115°C for one hour and after this, temperature was decreased by 15°C after every one hour to reach up to 55°C, so that the temperature profile of −115°C−100°C−85°C−70°C−55°C was followed. The cocoons were allowed to cool for half an hour and removed from drying chamber. The total duration of cocoon drying was five and half hours. After this, the cocoons were dipped into hot water to loosen tightly woven filaments. The loosen cocoons were brushed to locate the end of fiber. It is threaded through a porcelain eyelet and the fiber was reeled on the wheel.

\[
\text{Reliability} \% = \frac{\text{No. of reeling cocoons}}{\text{No. of feeding ends}} \times 100
\]

Where,
No. of reeling cocoons= no. of cocoons taken for testing
- no. of converted carry over cocoons
No. of feeding ends = no. of casting + no. of carry over cocoons – no. of converted carry over cocoons
Formula used to calculate the converted carry over cocoon and converted unreelable cocoons for multiend test reeling are as follows –

For multi and test reeling machine – Length (unreelable) = 1.00 new + 0.5 thick + 0.24 middle + 0.06 thin 
Weight (unreelable) = 1.00 new + 0.43 thick + 0.14 middle + 0.03 thin Length (carry over) = 0.58 thick + 0.24 middle + 0.0.06 thin 
Weight (carry over) = 0.43 thick + 0.14 middle + 0.03 thin 
For determining the reelability percentage, 300 cocoons (a lot) were taken.

RESULTS

Reelability (%): The data given in the table 1 shows that group difference in the reelability (%) in between control the phytojuvenoid concentration and number of larval treatment influenced the reelability (%). With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the reelability (%) increased gradually and reached to the maximum level of 79.36± 3.50% in case of triple treated larvae with 30% phytojuvenoid concentration. In case of the treatment with 40% phytojuvenoid concentration, the reelability (%) increased in single treated larvae but further increase in the number of larval treatment caused decline in Reelability (%) which reached to the minimum level of 66.76±8.60% in triple treated larvae. The trend of increase in the reelability (%) was almost of same fashion 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 < 0.01) influenced the reelability while number of larval treatment has no significant influence on it. Two-way ANOVA indicates that variation in the phytojuvanoid concentration significantly (P < 0.01) influenced the reelability of silk filament while number of larval treatment has no significant influence on the reelability (%). The Post–hoc test (table 2) indicates significant group difference in the reelability (%) in between control and 20%, control and 30%, 10 and 30%, 20 and 30% and 30 and 40% in single treated larvae. In the double treated larvae significant group difference in the reelability (%) was noticed in between all the group combinations except in control and 10%, control and 40% and 10% and 40%. In triple treated larvae significant group difference in the reelability(%) was recorded in between all group combinations except in control and 40% phytojuvenoid concentration.

Table 1: Effect of phytojuvenoid treatment on the reelability (%) of Bombyx mori silk filament.

<table>
<thead>
<tr>
<th>Phytojuvenoid concentration (%)</th>
<th>Stage of treatment (Larval instar)</th>
<th>Control</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single (V)</td>
<td>X_{i}</td>
<td>68.80±9.89</td>
<td>69.63±3.30</td>
<td>71.81±1.67</td>
<td>75.23±6.70</td>
<td>69.90±3.03</td>
</tr>
<tr>
<td>Double (IV-V)</td>
<td>X_{i}</td>
<td>68.80±9.89</td>
<td>70.52±5.70</td>
<td>73.12±10.01</td>
<td>77.08±6.69</td>
<td>68.69±1.89</td>
</tr>
<tr>
<td>Triple (III-V)</td>
<td>X_{i}</td>
<td>68.80±9.89</td>
<td>71.87±7.50</td>
<td>75.10±4.40</td>
<td>79.36±3.50</td>
<td>66.76±8.60</td>
</tr>
<tr>
<td>F-ratio = 0.99** n = 2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P &gt; 0.01 ** Non significant</td>
<td>Each value represents mean ± S.E. of three replicates.</td>
<td></td>
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</tr>
<tr>
<td>X_{1}, X_{2}, X_{3}, X_{4}, and X_{5} are the mean values of the reelability (%) of silk filament in control, 10, 20, 30 and 40 % of phytojuvenoid concentration respectively.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 2: Post - hoc test showing effect of phytojuvenoid treatment on reelability (%) of Bombyx mori silk filament.

<table>
<thead>
<tr>
<th>Mean difference in between groups</th>
<th>Stage of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
</tr>
<tr>
<td>X_{1}–X_{2}</td>
<td>0.83</td>
</tr>
<tr>
<td>X_{1}–X_{3}</td>
<td>*3.01</td>
</tr>
<tr>
<td>X_{1}–X_{4}</td>
<td>*6.43</td>
</tr>
<tr>
<td>X_{1}–X_{5}</td>
<td>1.10</td>
</tr>
<tr>
<td>X_{2}–X_{3}</td>
<td>2.18</td>
</tr>
<tr>
<td>X_{2}–X_{4}</td>
<td>*5.6</td>
</tr>
<tr>
<td>X_{2}–X_{5}</td>
<td>0.27</td>
</tr>
<tr>
<td>X_{3}–X_{4}</td>
<td>*3.42</td>
</tr>
<tr>
<td>X_{3}–X_{5}</td>
<td>1.19</td>
</tr>
<tr>
<td>X_{4}–X_{5}</td>
<td>*5.33</td>
</tr>
</tbody>
</table>

Honesty Significant difference (HSD) = qv MS within

= 5.05v 2.166 = 2.48

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 reelability (%) of Bombyx mori silk filament incontrol, 10, 20, 30 and 40 per cent phytojuvenoid concentration respectively.


DICUSSIONS

Variation in the phytojuvenoid concentration and the number of larval treatment influenced the reelability of silk filament. The minimum reelability of silk filament was noticed with 40% concentration at triple treated larvae, whereas, it reached to the maximum level in 30% phytojuvenoid concentration in triple treated larvae (Table 1). The administration of plant growth hormone Indole-3-acetic acid increased the reelability of silk filament [13]. The phytoecdysteroid administered at different age of 5th instar larvae of Bombyx mori, influenced thereelability of silk filament [14]. Methoprene and fenoxycarb treated Bombyx mori showed significantly enhanced reelability of silk filament [15]. Increase in reelability per cent is the important commercial characters in the improvement of silk quality and yield [16, 17]. The hybrids can be influenced by the environmental factors viz. temperature and humidity and the denier of Bombyx mori was effected [18]. The maternal inheritance effects regarding the temperature tolerance have better performance in denier of Bombyx mori [19, 20]. The effect of high temperature and high humidity was decreased on denier of Bombyx mori [21]. The Indian double hybrid (CSR2 X CSR27) X (CSR6 X CSR26) is better than the Chinese double hybrids in respect to reelability [22].

In the present investigation the post cocoon characters positively increased with the increasing phytojuvenoid concentration up to 30%. 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 various post cocoon characters 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 thereliability of cocoon.

REFERENCES


