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The Phytojuvenoid caused beneficial effect on the length of filament of multivoltine mulberry silkworm (*Bombyx Mori* Linn.)

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Abstract
The topical application of phytojuvenoid on *Bombyx mori* larvae has been proved to be of biotechnological significance in the sericulture industry. The filament length increased with the increasing number of larval treatment from single to triple in 10, 20 and 30% phytojuvenoid concentration and the length was highest (893.13m) in 30% phytojuvenoid concentration- triple treatment of larvae. Variation in the larval treatment of larvae and phytojuvenoid concentration did not cause significant influence in the filament length of *Bombyx mori*. 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 generate more employment opportunities.

Keywords: Phytojuvenoid, filament length, larval treatmant, *Bombyx mori*

1. Introduction
The silkworm, *Bombyx mori* L. is a typical monophagous insect and mulberry (Morus spp.) leaf is its sole food. Sericulture is an age-old land-based practice in India with high employment potential and economic benefits to agrarian families. Though India is the second largest producer of mulberry silk next only to China, the twin problems of low productivity and poor fibre quality continue to impair an increase in production. 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 growth and development of an insect is under the control of various intrinsic and extrinsic factors such as hormones and nutrition [3-6]. The circulating hormones viz., prothoracicotropic hormone (PTTH), juvenile hormone (JH) and ecdysone are directly and indirectly manifest the phenomenon of moulting and metamorphosis. The response of silkworm to very small quantities of phytojuvenoids 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 [7]. The phytoecdysteroid has been noticed to influence the development, silk producing and reproductive potential of *B. mori* [8, 9]. The juvenile hormone analogue also has been noticed to influence the commercial potential of *Bombyx mori* [10, 11]. 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* [12]. 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 [13]. The phytojuvenoid caused beneficial effect on the the life pattern of silkworm [14]. Some plants like *Pinus longifolia, Abies balsamea, Psoralea corylifolia a*nd *Azadirachta indica* act on *Bombyx mori* larvae as bioactive juvenoid compounds [15]. 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. In the present study *Pinus longifolia* was taken for experiment due to its good availability and containing juvenile compound i.e. called phytojuvenoid compound.

Material and Method

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 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 ^{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* were 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 soxlet 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.

Filament length: For determining the length of filament, cocoons were cooked properly and treated with boiling water and cold water. 24 cocoons 3 batches of 8 cocoons in each batch were taken for determining the length of filament with following formula.

$$\text{Average filament length (m)} = \frac{\text{Length of raw silk reeled x no. of cocoons maintained /end}}{\text{No. of reeling cocoons}}$$

No. of reeling cocoons = no. of cocoons taken for testing – no. of unreelable cocoons + number of converted carry over cocoons

Results

The data presented in table-1 clearly indicates that the phytojuvenoid concentration and number of larval treatment influenced the filament length. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the filament length increased gradually and reached to the maximum level of 893.13±0.367 m in case of triple treated larvae with 30% phytojuvenoid concentration. In case of the larval treatment with 40% phytojuvenoid concentration, the filament length increased in single treated larvae but further increase in the number of larval treatment caused decline in the filament length which reached to the minimum level of 830.06±2.550 m in triple treated larvae. The trend of increase in the filament length 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 filament length but number of larval treatment did not cause significant influence on the filament length. The Post–hoc test (table-2) shows significant group difference in the filament length in between control and 40%, control and 30%, 10 and 30% and 30 and 40% in single treated larvae. In the double treated larvae significant group difference in the filament length was noticed in between all the group combinations except in control and 40%, 10 and 20% and 20 and 30%. In triple treated larvae significant group difference in the filament length was recorded in between all the group combinations except in control and 40% and 10 and 20% phytojuvenoid concentration.

Table 1: Effect of phytojuvenoid treatment on the filament length (m) of *Bombyx mori.*

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)	838.30 ±2.053	847.12 ±6.133	859.32 ±1.109	870.32 ±4.012	846.80 ±0.785	
Double (IV-V)	838.30 ±2.053	858.16 ±2.454	868.36 ±2.424	881.26 ±0.590	837.74 ±2.702	16.24*
Triple (III-V)	838.30 ±2.053	864.20 ±3.670	875.86 ±0.270	893.13 ±0.367	830.06 ±2.550	

F_2-ratio = 1.19** $n_2 = 2$

$P_1 > 0.01$ ** 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 filament length (m) in control, 10, 20, 30 and 40 % of phytojuvenoid concentration respectively.

Table 2: Post - hoc test showing effect of phytojuvenoid treatment on the filament length (m) of *Bombyx mori*

Mean difference in between groups	stage of treatment		
	Single	**Double**	**Triple**
X_1~X_2	8.82	*19.86	*25.9
X_1~X_3	*21.02	*30.06	*37.56
X_1~X_4	*32.02	*42.96	*54.83
X_1~X_5	8.5	0.56	8.24
X_2~X_3	12.2	10.2	11.66
X_2~X_4	*23.2	*22.84	*28.93
X_2~X_5	0.32	*20.42	*34.14
X_3~X_4	11.00	12.9	*17.27
X_3~X_5	12.52	*30.62	*45.8
X_4~X_5	*23.52	*43.52	*63.07

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

$= 5.05\sqrt{\frac{66.226}{3}}$

$= 13.70$

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 filament length (m) *Bombyx mori* in control, 10, 20, 30 and 40 per cent phytojuvenoid concentration respectively.

Discussion

The variation in the phytojuvenoid concentration and the number of larval treatment of *Bombyx mori* influenced the filament length of cocoon. 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 filament length of cocoon increased in single treatment and further decreased with increasing the number of larval treatment. The minimum filament length of cocoon was noticed in case of larvae treated with 40% phytojuvenoid concentration - triple treated larvae, whereas the maximum filament length was recorded in case of 30% phytojuvenoid concentration – triple treated larvae. The thyroxine treated mulberry species of *Morus multicaulis* had significant effect on the length of *Bombyx mori* silk filament [17]**.** Juvenile hormone analogues have been tested in *Bombyx mori* as insect growth regulators in order to increase the silk production [7, 18-22]. The administration of plant growth hormone Indloe-3- acetic acid increased the filament length in *Bombyx mori* [23] and the phytoecdysteroid administered at different age of 5th instar *Bombyx mori* larvae influenced the filament length [24]**.** Methoprene and fenoxycarb treated *Bombyx mori* showed significantly enhanced the filament length [25]**.** The expression for filament length of *Bombyx mori* of hybrid vigour is different for hybrids at different temperature treatments [26]**.** The positive general combining abilities effects could be used in breeding programmes for improvement of filament length of *Bombyx mori* [27]. The hybrids can be influenced by the environmental factors viz. temperature and humidity and the filament length of *Bombyx mori* was effected [28]. The maternal inheritance affects regarding the temperature tolerance and may have better performance in filament length of *Bombyx mori* [29, 30]. The effect of high temperature and high humidity was noticed decrease the filament length of *Bombyx mori* [31]. The Indian double hybrid (CSR2 X CSR27) X (CSR6 X CSR26) is better than the Chinese double hybrids in respect to filament length [32]. 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 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 filament length.

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