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## Investigations on dynamics of juvenile hormone mimics on the economic parameters of silkworm, *Bombyx mori* L.

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#### Abstract

Juvenile hormone mimics are known to prolong larval life in insects and thus in sericulture, it has been shown to boost good cocoon and silk yield. In the present experiment, the double hybrid (FC<sub>1</sub>XFC<sub>2</sub>) silkworm was utilized and analysed the performance of economic traits. The influence of juvenile hormone mimics i.e., phytojuvenoids from Pinus (*Pinus roxburghii*), Custard apple (*Annona squamosa*) and Tapioca (*Manihot esculenta*), a commercially available juvenile hormone mimic Serimore and Pyriproxyfen has been recorded as quantitative characters of silkworm when administered topically with two different doses at two distinct larval instars. The quantum of expression of economic trait like cocoon weight, shell weight, shell ratio, filament length and denier were found to be increasing with the increase in the number of larval treatments which is double administration at the rate of 20% and 30% concentration for Pinus and Tapioca treated larvae. In case of custard apple treated larvae, double treatment at 20% concentration showed best results. Single treatment at 5 $\mu$ l and 10 $\mu$ l concentration of pyriproxyfen gave similar outcome with respect to all economic parameters. Among all, Pinus was found to be on par with Serimore for all the economic characters.

**Keywords:** Phytojuvenoid, Pinus, custard apple, tapioca, cocoon weight, shell weight, shell ratio, filament length, denier, *Bombyx mori* L. juvenile hormone mimics

#### Introduction

Sericulture is the art and science of silkworm rearing. To build its cocoon, the silkworm eats exclusively mulberry leaves, hence the silk. The two principal circulatory hormones in insects, juvenile and moulting hormone (JH and MH), regulate the majority of growth and developmental activities (Novak, 1975) [13]. Exogenous administration of analogues or mimics of these hormones has the potential to disturb normal insect growth by altering metabolic processes. The process of the event of moulting and metamorphosis are two characteristic features of growth and development in insects and is regulated by circulating hormones like a prothoracic tropic hormone (PTTH), juvenile hormone (JH), and ecdysterone. The pattern of insect development can be altered to a certain extent by exogenous administration of mimics or analogs of these circulating hormones (Sakurai, *et al.* 1989) [14].

The silkworm, *Bombyx mori* L. exhibited a stimulatory effect on the administration of exogenous JH analogs in minute quantities which leads to enhancement in commercial traits such as cocoon weight, shell weight, and silk filament length (Akai *et al.* 1985; Mamatha *et al.* 2008) [3, 8]. Some of the JH analogues /mimics have been observed to have some hormonal effects on the growth of silkworms, but the response depends on the dosage, duration, and quantity of application (Chowdhary *et al.* 1990) [4]. Ingestion of food during the time of JH analogue application contributes to the formation of silk protein. The subsequent delay in moulting is evidence for the inhibitory effect of JH on ecdysone synthesis in *B. mori* L. (Trivedy *et al.* 1997) [16]. A recent study claims that JH inhibits protein synthesis in early treated larvae, leading to later on stage protein synthesis that produces bigger silk glands, resulting in a stronger cocoon shell (Garel, 1983) [6]. In view of the biological significance of Juvenile hormone mimics on the yield of cocoons this study was undertaken in an attempt to study the effect of selected JH mimics, Pinus, Tapioca, Custard apple, Pyriproxyfen and Serimore on the economic parameters of cocoon.

#### Materials and Methods

##### Silkworm rearing

The chawki worms of multi-voltine breed, Double Hybrid (FC<sub>1</sub>XFC<sub>2</sub>) were collected from

chawki center. The silkworms were maintained in plastic trays (23 x 20 x 5 cm) under ideal rearing conditions in the silkworm rearing house, Department of Sericulture, Forest College and Research Institute, Tamil Nadu. The temperature and relative humidity were maintained at 24-26 °C and 75-80%, respectively till the spinning of cocoon. They were fed with V<sub>1</sub> variety of mulberry leaves. Plastic collapsible moutage (netrika) is used for ripened worms for spinning of cocoons.

#### Extraction of phytojuvenoids from plants

S.no.	Common name	Scientific name	Plant part used
1.	Pinus	<i>Pinus roxburghii</i>	Needles
2.	Tapioca	<i>Manihot esculenta</i>	Leaf and branches
3.	Custard apple	<i>Annona squamosa</i>	Leaf and branches

For extraction of phytojuvenoids, the needle of Pinus, leaves and branches of tapioca and custard apple were collected, cut into small pieces and shade dried. The dried materials were powdered separately with the help of mechanical device. Separate extraction of phytojuvenoid was carried out from leaves, branches and together as leaves and branch. 25 gm powder of concerned part was subjected to extraction separately through soxhlet apparatus with 250 ml acetone for 6-8 hours. After extraction, a little amount of concentrated solution of plant extract was obtained. The concentrated solution was dried and 100 gm material was obtained in paste/semi-liquid form. The paste thus obtained, was dissolved in 25 ml acetone and used this solution for further experiment, as 100% concentration of phytojuvenoid as stock. For further experiment the suitable narrow ranges of Pinus, tapioca and custard apple phytojuvenoid concentrations *viz.* 20 and 30% were made using distilled water as working solution. Thus, this two different phytojuvenoid concentrations were applied topically by spraying as 10 ml on to 100 larvae separately. Three sets of experiments were designed *viz.*, single, double and triple treatment of larvae.

#### Topical application of serimore and pyriproxyfen

Serimore is a synthetic growth promoter most commonly used for the substantial improvement in larval growth and quantitative and qualitative traits of the silkworm procured from Sericare, division of health care private limited. Serimore was administered topically during the fifth instar at 48h of interval at a concentration of 0.2 microliter/silkworm larvae (5ml Serimore dissolved in 2.5 liters potable water and sprayed on the healthy silkworm). Serimore was used as check. Pyriproxyfen 10% EC, a chemical Insect growth regulator used at a concentration of 5µl and 10µl concentration sprayed topically as single and double treatment.

#### Design of experiment

##### Single treatment of larvae

Single treatment of larvae was performed at the initial stage of fifth instar larvae just after fourth moult. One hundred larvae of fifth instar at the initial stage were treated with 10ml of 20% concentrated solution of Pinus needle extract by sprayer. Same method was followed for custard apple leaf extract, custard apple branch extract, custard apple plant (leaves and branch together) extract, tapioca leaf extract, tapioca branch extract and tapioca plant (leaves and branch together) extract. Double treatment of larvae.

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 10 ml of 20% concentrated solution of Pinus needle extract by spraying. The treated larvae were reared under optimum condition for growth and development. Further, similar second treatment for the same larvae was given at the initial stage of fifth instar. Thus, in double treatment, fourth and fifth instar larvae were treated.

Same method was followed for custard apple leaf extract, custard apple branch extract, custard apple plant (leaves and branch together) extract, tapioca leaf extract, tapioca branch extract and tapioca plant (leaves and branch together) extract. Similar experiments were performed for 30% concentration of phytojuvenoid obtained from Pinus needle extract, custard apple leaf extract, custard apple branch extract, custard apple plant (leaves and branch together) extract, tapioca leaf extract, tapioca branch extract and tapioca plant (leaves and branch together) extract.

The silkworm larvae were left on the bed for 30 min and then fed with fresh mulberry leaves. An untreated control was maintained in parallel to compare the results. Each treatment was replicated into 3 times with 50 healthy silkworm larvae per replication.

#### Data Collection

**Economic traits of cocoons:** The cocoons were matured after 6 days, after the 5<sup>th</sup> instar was completed. Some important economic traits of sericulture, such as the cocoon weight, shell weight, shell ratio, filament length and denier were recorded from ten randomly selected cocoons and the data were calculated by the following formulae. Data were analyzed using AGRESS as means ± SD. P values < 0.05 were regarded as statistically significant.

**Cocoon weight (g):** The cocoons were randomly selected from each treatment replication-wise, weighed individually and the average single cocoon weight was completed.

**Shell weight (g):** After removing the pupae and larval exuvium from cocoons the individual shell weight was recorded.

**Shell ratio (%):** The shell ratio was calculated using the formula.

$$\text{Shell ratio} = (\text{Shell weight (g)} / \text{Cocoon weight (g)}) \times 100$$

**Filament Length (m):** Ten cocoons were randomly selected from each batch was reeled to find out the single filament of the cocoon using epprouvette and was determined by adopting the formula.

$$L = R \times 1.125 \quad (R = \text{Number of revolutions recorded by an epprouvette in a meter})$$

**Denier (d):** This denotes the thickness of filament, 9000 meters of the silk filament weighing 1g is considered as 1 denier. It was calculated using the following formula.

$$\text{Denier} = (\text{Weight of the filament (g)} / \text{length of the filament (m)}) \times 9000$$

#### Results and Discussion

All the JH mimics utilized in the research showed a significant positive response in cocoon characters. The data

on the effect of the application of JH mimics on the economic traits of silkworm hybrid FC<sub>1</sub>XFC<sub>2</sub> are presented in figure 1 and tables 1, 2 and 3.

Table 1 shows that in case of pine, tapioca leaf, tapioca branch and plant, there were increase in cocoon weight and shell weight with increase in dose and the number of dosage application which was double treatment@30% concentration whereas in case of custard apple series, it was found to be slight decline in both the parameters at high dosage of double application. Pine and serimore were found on par with each

other.

The maximum cocoon weight for double treatment at 30% concentration was found to be 1.78g for pinus followed by 1.71g for serimore, 1.59g, 1.57g and 1.49g for tapioca leaf, plant and branch respectively. Further for custard apple series, maximum cocoon weight was seen in double treatment at 20% concentration that is 1.58g, 1.42g and 1.40g for custard apple plant, branch and leaf respectively. In case of control, it was found to be 1.39g cocoon weight.

**Table 1:** Effect of different Juvenile hormone mimics on cocoon weight (g) and shell weight (g) of silkworm, *B. mori* L.

Treatments	Concentration / economic traits									
	Single Treatment@20%		Single Treatment@30%		Double Treatment@20%		Double Treatment@30%		Mean	
	Cocoon weight	Shell weight	Cocoon weight	Shell weight	Cocoon weight	Shell weight	Cocoon weight	Shell weight	Cocoon weight	Shell weight
Pinus	1.54	0.37	1.63	0.38	1.69	0.39	1.78	0.39	1.66	0.38
Custard apple leaf	1.33	0.28	1.34	0.28	1.40	0.31	1.36	0.29	1.35	0.29
Custard apple branch	1.34	0.26	1.37	0.27	1.42	0.27	1.39	0.27	1.37	0.27
Custard apple plant	1.41	0.32	1.43	0.33	1.58	0.34	1.47	0.33	1.44	0.33
Tapioca leaf	1.42	0.31	1.47	0.31	1.53	0.32	1.59	0.33	1.51	0.32
Tapioca branch	1.41	0.29	1.45	0.30	1.47	0.31	1.49	0.32	1.45	0.31
Tapioca plant	1.39	0.33	1.47	0.34	1.52	0.35	1.57	0.35	1.48	0.34
Serimore	1.71	0.37	1.71	0.37	1.71	0.37	1.71	0.37	1.71	0.37
Control	1.39	0.27	1.39	0.27	1.39	0.27	1.39	0.27	1.39	0.27
Mean	1.46	0.32	1.51	0.33	1.54	0.33	1.58	0.33		
S.Ed and CD for cocoon weight	S.Ed CD (0.05) T= 0.03 T= 0.05** C= 0.02 C= 0.04** TC= 0.07 TC= 0.13**				S.Ed and CD for shell weight		S.Ed CD (0.05) T= 0.005 T= 0.013* C= 0.004 C= 0.008* TC= 0.013 TC= 0.025*			

Similarly, for shell weight, pine and tapioca series showed increased trend with the highest dose and the times it had applied on silkworms. The shell weight was 0.39g, followed by 0.37g, 0.35g, 0.33g and 0.32g for pinus, serimore, tapioca

plant, leaf and branch. However, it was highest for double treatment at 20% concentration i.e. 0.34g, 0.31g and 0.27g for custard apple plant, leaf and branch respectively. The shell weight was 0.27g for control batch (table1).

**Table 2:** Effect of different Juvenile hormone mimics on shell ratio (%) and filament length (m) of silkworm, *B. mori* L.

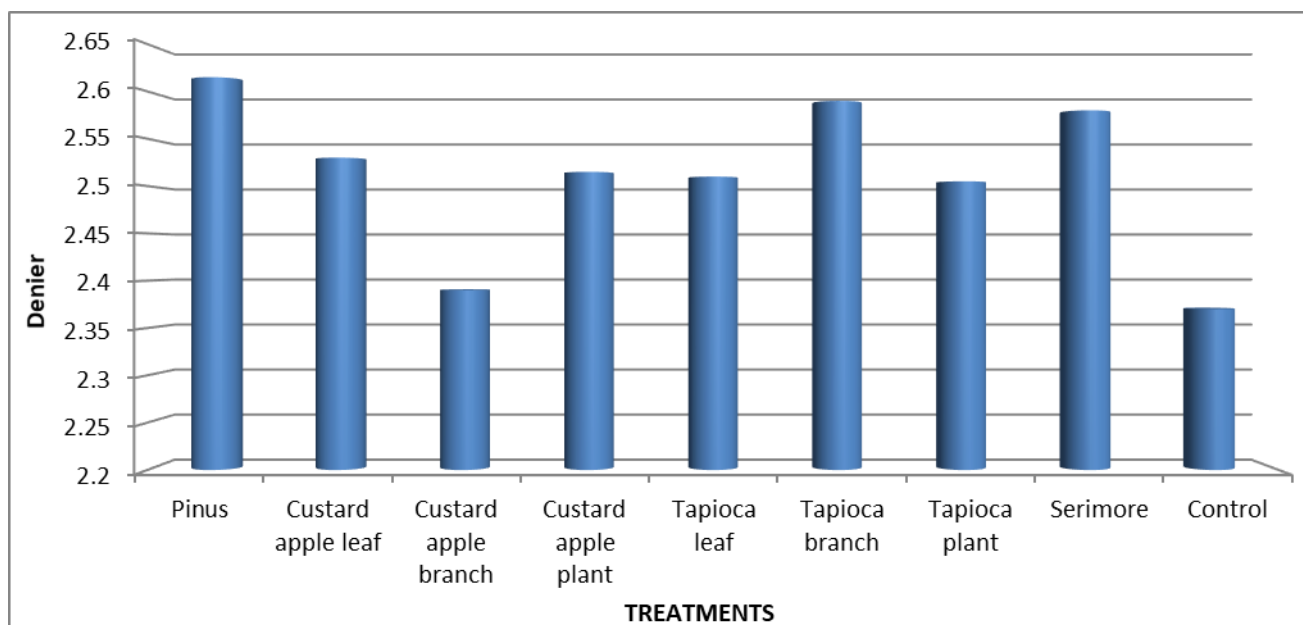
Treatments	Concentration / economic traits									
	Single Treatment@20%		Single Treatment@30%		Double Treatment@20%		Double Treatment@30%		Mean	
	Shell ratio	Filament length	Shell ratio	Filament length	Shell ratio	Filament length	Shell ratio	Filament length	Shell ratio	Filament length
Pinus	23.96	1329.31	23.19	1333.71	22.01	1334.35	21.91	1350.76	23.425	1340.04
Custard apple leaf	21.05	1223.15	20.89	1228.23	22.14	1243.91	21.32	1241.15	21.375	1232.15
Custard apple branch	19.48	1235.67	19.34	1237.19	19.22	1241.36	19.35	1238.54	19.415	1237.11
Custard apple plant	22.91	1265.13	23.01	1267.28	21.71	1268.31	22.52	1269.25	22.715	1267.19
Tapioca leaf	21.48	1276.71	21.36	1276.51	20.82	1281.49	20.92	1293.17	21.2	1284.94
Tapioca branch	20.64	1238.37	20.62	1240.25	20.88	1262.51	21.41	1265.38	21.025	1251.88
Tapioca plant	23.81	1239.35	23.12	1240.78	23.02	1241.70	22.29	1257.31	23.18	1248.33
Serimore	21.75	1331.46	21.75	1331.46	21.75	1331.46	21.75	1331.46	21.75	1331.46
Control	19.28	1210.59	19.28	1210.59	19.28	1210.59	19.28	1210.59	19.28	1210.59
Mean	21.62	1269.95	21.24	1272.15	20.65	1272.47	21.09	1280.68		
S.Ed and CD for Shell ratio	S.Ed CD (0.05) T= 0.39 T= 0.78* C= 0.29 C= 0.57* TC= 0.96 TC= 1.90*				S.Ed and CD for Filament length		S.Ed CD (0.05) T= 20.27 T= 40.11** C= 14.97 C= 29.62** TC= 49.66 TC= 98.24*			

Shell ratio is inversely proportional to the cocoon weight. Thus, lower cocoon weight gives higher shell ratio in percentage. Table 3 shows that highest shell ratio was found to be 23.96% for pine followed by 23.81% for tapioca plant, 22.91% for custard apple plant, 22.14% for custard apple leaf, 21.75% for serimore, 21.48% for tapioca leaf, 20.88% for tapioca branch, 19.48% for custard apple branch and 19.28% for control (table 2).

Since, single treatment at 20% concentration on larvae gave minimum cocoon weight for most of the treatments. Hence, when comparison made within each treatment, highest shell ratio was seen at single dose of 20% concentration in case of pine, Custard apple branch, Tapioca leaf and Tapioca plant. The filament length (m) gave similar pattern in case for pine and tapioca series i.e. best results at double treatment 30% concentration. Whereas for the custard apple series, best

results at double dosage 20% concentration. Pine and serimore were found to be on par with each other. Highest filament length was recorded as 1350.76m for pinus, followed by 1331.46m for serimore. Next best filament length

was found as 1293.17m, 1265.38m, 1257.31m, 1269.25m, 1243.91m, 1241.36m and 1210.59m for tapioca leaf, branch, plant, custard apple plant, leaf branch and control respectively (table 2).



**Fig 1:** Effect of different Juvenile hormone mimics on denier of silkworm *B. mori* L.

With regard to the treatments, highest denier was observed in pine 2.63, followed by tapioca branch with 2.61. Next best results were followed as 2.58 for serimore, 2.54 for custard apple plant and leaf, 2.53 for tapioca leaf, 2.51 for tapioca plant, 2.40 for custard apple branch and lastly 2.37 for the control (figure 1).

Denier for double treatment at 20% and 30% concentration was found to be on par with each other in all the phytojuvenoid treatments. Among all concentration, single dosage at 20% concentration gave least results for denier with respect to all treatments.

**Table 3:** Effect of Pyriproxyfen on economic traits of silkworm *B. mori* L.

Economic Traits	Concentration				Mean
	Single treatment@ 5µl	Single treatment@ 10µl	Double treatment@ 5µl	Double treatment@ 10µl	
Cocoon weight(g)	1.62	1.55	1.61	1.54	1.58
Shell weight (g)	0.36	0.35	0.31	0.30	0.33
Shell ratio (%)	21.97	22.45	19.44	19.35	20.80
Filament length (m)	1294.45	1281.31	1257.12	1256.13	1272.25
Denier	2.55	2.56	2.43	2.42	2.49

(CD (0.05) for cocoon weight, shell weight, shell ratio, filament length and denier were 0.13\*\*, 0.025\*, 1.90\*, 98.24\* and 0.13\*\*respectively.)

Single treatment at 5µl and 10µl concentration of pyriproxyfen was found be on par with each other. There was decline in results i.e. cocoon weight, shell weight, shell ratio, filament length and denier for larvae administered doubly both at 5µl and 10µl concentration.

Table 3 shows different economic traits of silkworm treated with pyriproxyfen. Highest cocoon weight 1.62g followed by 1.55g, shell weight 0.36g followed by 0.35g, filament length 1294.45m followed by 1281.31m for single treatment at 5µl and 10µl concentration respectively. Further for shell ratio 22.45% followed by 21.97%, denier 2.56 followed by 2.55 in case of single treatment at 10µl and 5µl concentration respectively.

Kajiura and Yamashita (1989) [7] suggested that the conversion of an additional quantity of leaf consumed during an extended period and stimulatory effect of the compound on protein synthesis and silk gland. These changes attributed to founding at the molecular level might be the result of alterations in the ratio of circulating hormone. The economic characters of the silkworm hybrid exhibited a positive

response in juvenoid treated breeds without any difference in the development simultaneously as reported by Muroga (1975) [10]. The increased shell weight represents the converted silk content in the form of bave which is indicated as a qualitative parameter. The role of JH mimics for the stimulation and conversion of silk protein synthesis at the molecular level and elicits substantially an improvement in the filament characters including the denier.

Literature survey revealed that most of (JH) mimics applied on the silkworms were synthetic (Gangwar 2009; Nair *et al* 2011; Trivedy *et al* 1997) [5, 12, 16]. It is noteworthy that the JH hormonal mimic is a bioactive compound formulated in such a way for the third generation and employed for the improvement of all most all the commercial traits especially larval weight, shell weight, shell ratio, filament length, filament weight are phenomenal achievements of the goal in the sericulture industry. Nair *et al.* (2002) [11] studied the effect of diluted bakuchiol on the economic traits of the silkworm, *Bombyx mori* L. The results showed that when bakuchiol was administered to the fifth instar silkworm hybrid

KA x NB<sub>4</sub>D<sub>2</sub>, the highest increase in cocoon yield (5.74% compared to untreated control) was observed when treated at 48 hours. Further, the data from this work indicates a net increase of cocoon weight at 11.82%, increase of shell weight 14.54%, and an increase of shell ratio by 2.51%. Marghitas *et al.* (2007)<sup>[9]</sup> studied the expression of quantitative characters of the silkworm after topical application of methoprene and fenoxycarb. They reported that both positively influenced the duration of the fifth instar silkworm.

### Conclusion

From the present study, we can conclude that among the phytjuvenoids, pinus was best followed by tapioca and then custard apple. Among tapioca series, tapioca plant showed best result followed by tapioca leaf and tapioca branch. In case of custard apple series, custard apple plant was better followed by custard apple branch and least by custard apple leaf. Pine was found to be on par with commercially available JHA serimore.

For pine and tapioca series treatments, double treatment at 30% concentration was found best since it increases larval duration thus feeding duration increases leads to increase in protein and silk production. There is decline in economic traits of cocoon when treated at high concentration of 30% double administration. This is due to pungent smell of custard apple leaves, leads to reduction in feeding of leaves followed by poor growth and development. Pyriproxyfen 30%EC showed on par results with single treatment at 5 $\mu$ l and 10 $\mu$ l concentration. Since, pyriproxyfen is a chemical insect growth regulator, double treatment at both concentration showed reduction in economic traits due to residual effect in to the silkworm haemolymph and fat bodies. Thus, application of these JH mimics at right dose and correct instar will increase economic traits of cocoons of silkworm, *Bombyx mori* L.

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