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Juvenile Hormone Mimics as Biochemical Enhancers for Silk Production in *Bombyx mori* L.: Evidence from Plant Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the Effect of juvenile hormone mimics on the biochemical parameters of silkworm.
Study Design: Factorial Complete Random Design.
Place and Duration of Study: Department of Sericulture, Forest College and Research Institute, Mettupalayam during 2021-2022.
Methodology: Topical application of 20% and 30% acetone extracts of Juvenile hormone mimics at different interval of larval duration *viz.* IV and V instar of silkworm as single treatment (on first day of

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V instar) and double treatment (twice – first day of IV and V instar). Silkworm breed used Double Hybrid (FC1XFC2).

Results: Double administration at 20% and 30% concentration for Pinus and Tapioca treated larvae—increases, it is observed that the amount of expression of biochemical traits increases. Significantly higher silk gland protein content of (119.85 mg/g) was recorded in double dose @ 30% Pinus with highest fat body protein of 51.00 mg/g and least was recorded in untreated control. Serimore recorded highest aspartate aminotransferase activity (264.24 µg/ml) followed by Pinus (269.93 µg/ml) whereas 563.22 µg/ml and 558.31 µg/ml alanine aminotransferase activity recorded when treated with pinus and serimore respectively.

Conclusion: Among the phutojuvenoids, 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.

Keywords: Phytojuvenoid; Pinus; custard apple; tapioca; haemolyph protein; silk gland.

1. INTRODUCTION

The science and skill of raising silkworms is called sericulture. The silkworm only eats mulberry leaves in order to construct its cocoon, which is the reason that the silk exists. Most aspects of growth and development are controlled by the two main circulatory hormones in insects, juvenile and moulting hormone (JH and MH) [1]. By changing metabolic pathways, exogenous treatment of analogues or mimics of these hormones may disrupt normal insect growth [2,3]. Two essential aspects of insect growth and development are the process of moulting and metamorphosis, which are controlled by circulating hormones such as ecdysterone, juvenile hormone (JH), and prothoracicotropic hormone (PTTH). Exogenous treatment of mimics or analogs of these circulating hormones can modify the pattern of insect growth to some degree [4,5].

When exogenous JH analogs are administered in minute amounts to silkworms, such as *Bombyx mori* L., they show a stimulatory impact that improves commercial features like cocoon weight, shell weight, and silk filament length [6-9]. It has been noted that many JH analogs and mimics have some hormonal effects on silkworm growth; however, the response varies according to application quantity, duration, and dose [10,11]. Food consumption throughout the JH analog application period aids in the production of silk protein. The ensuing postponement in moulting serves as proof that JH inhibits the production of ecdysone in *B. mori* L. [12,13]. According to a recent study, JH prevents protein synthesis in early treated larvae, which triggers later stages of protein synthesis that develop larger silk glands and a more robust cocoon shell [14-17]. This study was conducted to investigate the effects of specific juvenile hormone mimics, including Pinus, Tapioca, Custard apple, Pyripoxyfen, and Serimore, on the economic parameters of silkworm, given the biological significance of these mimics on cocoon yield.

2. MATERIALS AND METHODS

2.1 Silkworm Rearing

The Double Hybrid (FC1XFC2) chawki worms were obtained from the 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 during 2021-2022. The temperature and relative humidity were maintained at 24-26 ºC and 75- 80%, respectively till the spinning of cocoon. They were fed with V1 variety of mulberry leaves. Plastic collapsible mountage (netrika) was used for ripened worms for spinning of cocoons.

Extraction of phytojuvenoids from plants:

S.no.	Common name	Scientific name	Plant part used
1.	Pinus	*Pinus roxburghii*	Needles
2.	Tapioca	*Manihot esculenta*	Leaf and branches
3.	Custard apple	*Annona squamosa*	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 [18].

2.2 Topical Application of Serimore and Pyriproxyfen

Synthetic growth promoter Serimore is most frequently used to significantly increase silkworm larval growth and both quantitative and qualitative features. It is obtained from Sericare, a division of Health Care Private Limited. During the fifth instar, 0.2 ml of Serimore was applied topically V instar at 48 hours to silkworm larvae (5 ml of dissolved Serimore diluted in 2.5 liters of potable water and sprayed on the healthy silkworm). Serimore served as a check. Pyriproxyfen 10% EC is a chemical insect growth regulator that is applied topically as a single treatment or a double treatment at concentrations of 5 µl and 10 µ.

2.3 Design of Experiment for Single Treatment of Larvae

Just after the fourth moult, at the beginning of the fifth instar, a single dose was given to the larvae. Initially, 100 larvae in their fifth instar were sprayed with 10 ml of a 20% concentrated Pinus needle extract solution using a sprayer. The custard apple leaf, custard apple branch, custard apple plant (leaves and branches together), tapioca leaf, tapioca branch, and tapioca plant (leaves and branches together) extracts were all prepared using the same technique [19].

2.4 Design of Experiment for Double Treatment of Larvae

From the initial stage of the fourth instar larvae, the larvae were given double treatment. In the first treatment, 10 mL of a 20% concentrated Pinus needle extract solution were sprayed over 100 IV instar larvae. The ideal conditions for growth and development were provided for the rearing of the treated larvae. Additionally, at the beginning of the fifth instar, the same larvae received a second treatment that was identical. Thus, larvae in their fourth and fifth instars received JH mimics in a double treatment.

The custard apple leaf, custard apple branch, custard apple plant (leaves and branches together), tapioca leaf, tapioca branch, and tapioca plant (leaves and branches together) extracts were all prepared using the same technique. Comparable trials were conducted with a 30% concentration of phytojuvenoids derived from extracts of Pinus needles, custard apples, custard apple branches, custard apple plants (leaves and branches combined), tapioca leaves, tapioca branches, and tapioca plants (leaves and branches combined). After being on the bed for thirty minutes, the silkworm larvae were given fresh mulberry leaves to eat. To compare the outcomes, a parallel control group that received no treatment was kept. Three replications of each treatment were done using fifty healthy silkworm larvae for each replication.

2.5 Data Collection

2.5.1 Haemolymph protein

Three fifth instar larvae were randomly selected from each replication. The thoracic proleg was cut and the haemolymph was collected in 1.50 ml Eppendorf tubes. Few crystals of phenyl-thiourea (PTU) were added in each tube to prevent the melanization.

The samples were then centrifuged for 10 minutes at 10,000 rpm. The supernatant was removed and kept at -20°C for analysis. The recovered haemolymph was diluted with 0.5ml of distilled water. Alkaline copper reagent (5 ml) was added to the 0.5 ml aliquot of this solution and kept for 10 minutes. 0.5 ml of Folin Ciocalteu's reagent was added, carefully mixed

and kept for 20 minutes for colour development. The readings were taken using UV spectrophotometer at 650 nm. The reference employed was Bovine Serum Albumin (BSA). The mean was denoted by mg/ml [20].

2.5.2 Silk gland protein

The individual silk gland was macerated in phosphate buffer (pH 7.0) to extract the protein. The above macerated mixture subjected for 5-minute centrifugation at 5000 rpm. For the determination of the total proteins in the haemolymph, the supernatant was collected, and protein concentration was determined [20].

2.5.3 Fat body protein

The tissue protein was precipitated by adding 1 ml of 30per cent trichloroacetic acid (TCA) and the mixture was centrifuged for 30 minutes at 3000 rpm. Repeating two more times of this, the precipitate was mixed with 1 ml of 0.1 N NaOH. Alkaline copper reagent (5 ml) was added to the 0.50 ml aliquot. After 10 minutes, 0.50 ml of Folin Ciocalteu's reagent was added, properly mixed, and left for 20 minutes to develop the colour. On the UV spectrophotometer, readings were taken at a wavelength of 650 nm [20].

2.5.4 Asparate aminotransferace (AST)

The glutamic oxaloacetic transaminase (Asparate transaminase) was measured using the colorimetric method [21] using the GOT substrate (phosphate buffer + L-aspartate + ketoglutarate). Each test tube was filled with a known amount of haemolymph and 0.50 ml AST substrate. The tubes were shaken and then placed in boiling water for 60 minutes. The tubes were then filled with 0.50 ml of DNPH (Dinitrophenylhydrazine) and left at room temperature. Finally, 5 ml of NaOH was added and left to stand at room temperature for 5 minutes. Then, at 503 nm, the colour intensity was read on the spectrophotometer.

2.5.5 Alanine aminotransferace (ALT)

For the measurement of glutamic pyruvic transaminase (Alanine transaminase), ALT substrate (phosphate buffer + L-alanine + ketoglutarate) was utilized. By following the guidelines outlined in the estimation of glutamic oxaloacetic transaminase [21], the haemolymph was collected and ALT was quantified.

3. RESULTS AND DISCUSSION

All the JH mimics utilized in the research showed a significant positive response in larval characters. The data on the effect of the application of JH mimics on the biochemical traits of silkworm hybrid FC_1XFC_2 are presented in Fig. 1 and Tables 1, 2 and 3.

The protein content of silk gland was dramatically increased by several juvenile hormone mimic sources in silkworm. Pinus treated silkworms had the highest protein level (119.85 mg/g) in their silk glands among the various treatments The treatments *viz.,* Serimore (117.74 mg/g), tapioca leaf (117.67 mg/g) and tapioca plant (117.26 mg/g) were found to be statistically on par with each other. In the treatments namely, Custard apple plant (116.73 mg/g), Custard apple branch (116.67 mg/g) and Tapioca branch (116.07 mg/g) treatments on silkworm, the silk gland protein was found to be statistically not different from each another. Silk gland protein level was recorded lowest in control batch (115.40 mg/g) (Tables 1,3). Among the different treatment and doses tested, the greatest silk gland protein content was found in Pinus with double dose @ 30% (119.85 mg/g) treated silkworms followed by Pinus with double dose @ 20% (119.23 mg/g) and Pinus with single dose @ 30% (119.51 mg/g). The treatments such as Pinus a single dose @ 20% (118.48 mg/g), Tapioca leaf double dose @ 30% (117.67 mg/g) and Tapioca plant double doses (117.26 mg/g) were found to be on par with each other. The untreated batch of silkworms synthesized the least quantity of silk gland protein (115.40 mg/g).

The amount of fat body protein in silkworms administered with several juvenile hormone mimic sources varied greatly from one another. Among the various treatments, Pinus recorded significantly highest fat body protein of 51.00 mg/g followed by Serimore (49.31 mg/g). The fat body protein in the treatments like Tapioca leaf (49.33 mg/g), Tapioca plant (49.07 mg/g) and Pyripoxyfen (48.96 mg/g) were found to be statistically on par with each other. The least fat body protein was recorded in control (46.04 mg/g) (Tables 1,3). The interaction between treatments and doses showed significantly highest fat body protein recorded in Pinus double dose @ 30% (51.00 mg/g) followed by Pinus double dose @ 20% (50.15 mg/g) and Pinus single dose @ 30% (50.06 mg/g). The treatments namely single dose @ 30% Custard apple leaf (48.62 mg/g), double dose @ 30% Custard apple branch (48.64 mg/g), Single dose @ 20% Custard apple plant

(48.50 mg/g) and single dose @ 20% Tapioca branch (48.65 mg/g) did not differ statistically from each other in case of fat body protein content. Significantly least fat body protein was recorded in control (46.04 mg/g).

Aspartate aminotransferase activity was significantly different in silkworms sprayed with various of phytojuvenoids. Serimore recorded highest aspartate aminotransferase activity (264.24 µg/ml) followed by Pinus (269.93 µg/ml) and Tapioca leaf (249.93 µg/ml). Custard apple plant and Tapioca branch registered aspartate aminotransferase activity of 229.63 µg/ml and 229.20 µg/ml, respectively which were found to be on par with each other. The least enzyme activity was observed in control (224.97 µg/ml). The interaction study between treatments and doses revealed that Pinus with double dose @ 30% recorded highest enzyme activity (269.93 µg/ml) followed by Serimore (264.24 µg/ml). In the treatments such as Custard apple leaf with double dose @ 20% , Custard apple branch with double dose @ 20% , Tapioca branch with single dose @ 20% and Pyripoxyfen with double dose 10 µl recorded the enzyme activity of aspartate aminotransferase 229.25 µg/ml, 229.15 µg/ml, 229.20 µg/ml and 228.93 µg/ml, respectively which did not differ statistically with each other. Significantly least activity of enzyme was observed in control (224.97 µg/ml).

The activity of alanine aminotransferase enzyme in silkworms was strongly altered by the application several juvenile hormone mimics (Table 2). Pinus and Serimore recorded alanine aminotransferase 563.22 µg/ml and 558.31 µg/ml, respectively which was found to be higher compared to other treatments. The next better treatments were Tapioca leaf (545.97 µg/ml), Tapioca plant (542.28 µg/ml) and Custard apple plant (535.64 µg/ml). The minimal enzyme activity was observed in control (490.92 µg/ml).

With respect to treatments and doses, Pinus with double dose @ 30% recorded significantly highest alanine aminotransferase activity (563.22 µg/ml) followed by Serimore (558.31 µg/ml). The next better treatments were Custard apple plant with double dose @ 20% (535.64 µg/ml) and Tapioca plant with single dose @ 20% (535.66 µg/ml) recorded enzyme activity which was on par each other. The least was observed in control (490.92 µg/ml) followed by Custard apple with single dose @ 20% (497.78 µg/ml).

The present study results regarding haemolymph protein content showed significant variations between treatments and concentrations (Fig. 1). Regarding treatments, Pinus (9.83 mg/ml) had the greatest haemolymph protein content followed by serimore (9.73 mg/ml), tapioca leaf (9.16 mg/ml) and tapioca plant (9.19 mg/ml). The larvae treated with custard apple plant (8.90 mg/ml) and custard apple branch (8.94 mg/ml) was found to have haemolymph protein content significantly on par with tapioca branch treated silkworm (8.85 mg/ml). The least amount of haemolymph protein (7.86 mg/ml) was found in the Custard apple leaf. In the interaction between treatments and doses, Pinus with double dose @ 30% (9.83 mg/ml) recorded the highest amount of haemolymph protein. When the silkworms were treated with Custard apple branch (8.89 mg/ml) and Custard apple plant (8.87 mg/ml), the haemolymph protein content was found to be statistically not different from Pyripoxyfen double dose @ 10µl (8.87 mg/ml). The Custard apple leaf had the lowest protein content treated single dose @ 20% (7.86 mg/ml) followed by control (7.89 mg/ml).

Silkworm has protein distributed all over the body which includes haemolymph, silk gland and fat body protein. The protein content in silkworm was significantly higher when administered with juvenile hormone mimics compared to control. Results showed that Pinus and Tapioca extract treatments, total protein increased with the increase in concentration and number of dose applied on silkworm whereas a sudden decline in protein content was observed when the larvae were applied with custard apple at double dose of higher concentration.

In some insects, haemolymph protein content gradually increased with the larval development. During the initial days of 5th instar, there is negligible quantity of ecdysone in the silkworm hamolymph but juvenile hormone is detectable. While in the later days of 5th instar, both ecdysone and juvenile hormone is detectable which results in increase in haemolumph protein [4]. When silkworms were treated with 1ng of fenoxycarb, male larvae showed increase in haemolymph protein after eighth day whereas in female larvae it started increasing after seventh day and reached peak on tenth day in both sexes [22].

Similarly, silk gland protein content evidently differs between juvenile hormone mimic treated larvae and control batch. Maximum silk gland protein was recorded at double dose 30 *per cent* of Pinus extract (119.85 mg/g) followed by serimore (117.74 mg/g) and pyripoxyfen with single dose at 5 µl (116.90 mg/g).

Table 1. Effect of different Juvenile hormone mimics on Silk Gland protein (mg/g) and fat body protein (mg/g) of silkworm, *B. mori* L.

Treatments	Concentration / biochemical traits									
	Single Treatment@20%		Single Treatment@30%		Double Treatment@20%		Double Treatment@30%		Mean	
	SG protein	FB protein	SG protein	FB protein	SG protein	FB protein	SG protein	FB protein	SG protein	FB protein
Pinus	118.48	49.68	119.51	50.06	119.23	50.15	119.85	51.00	119.27	50.22
Custard apple leaf	113.58	48.45	113.50	48.62	113.97	48.19	113.79	48.33	113.71	48.40
Custard apple branch	115.77	48.65	115.65	48.67	116.71	48.59	115.67	48.64	115.95	48.63
Custard apple plant	115.55	48.50	116.21	48.76	116.73	48.66	116.09	48.72	116.14	48.66
Tapioca leaf	116.70	48.42	117.41	49.06	117.01	49.24	117.67	49.33	117.20	49.01
Tapioca branch	115.71	48.65	115.87	48.72	116.00	48.70	116.07	48.61	115.91	48.67
Tapioca plant	116.21	48.88	116.74	49.05	117.18	49.07	117.26	49.20	116.84	49.05
Serimore	117.74	49.31	117.74	49.31	117.74	49.31	117.74	49.31	117.74	49.31
Control	115.40	46.04	114.40	46.04	114.40	46.04	114.40	46.04	114.40	46.04
Mean	116.20	48.55	116.42	48.71	116.56	48.67	116.49	48.79		
S.Ed and CD for SG protein	S.Ed CD (P=0.05) T= 0.158 T= 0.321** C= 0.100 C= 0.203** TC= 0.317 TC= 0.642**				S.Ed and CD for FB protein		S.Ed CD (P=0.05) T= 0.113 T= 0.230** C= 0.072 C= 0.145* TC= 0.227 TC= 0.460*			

*silk gland protein (mg/g), fat body protein (mg/g), T - Treatment, D - Dose, ** Highly significant, * significant*

Table 2. Effect of different Juvenile hormone mimics on Aspartate aminotransferase (µg/ml) and Alanine aminotransferase (µg/ml) of silkworm, *B. mori* L.

Treatments	Concentration / biochemical traits									
	Single Treatment@20%		Single Treatment@30%		Double Treatment@20%		Double Treatment@30%		Mean	
	AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
Pinus	248.38	550.88	254.23	556.47	260.47	561.59	269.93	563.22	258.18	558.04
Custard apple leaf	226.12	497.78	227.33	498.89	229.25	505.12	227.91	500.84	227.65	500.66
Custard apple branch	227.00	530.00	228.13	530.47	229.15	531.08	228.66	530.16	228.23	530.42
Custard apple plant	229.63	528.91	230.00	530.25	231.46	535.64	230.34	532.84	230.36	532.16
Tapioca leaf	243.68	540.51	247.06	540.97	247.83	542.77	249.93	545.97	247.12	542.55
Tapioca branch	229.20	520.79	230.11	521.80	230.62	521.31	231.04	522.09	230.24	521.49
Tapioca plant	240.62	535.66	243.32	539.79	245.32	540.87	248.70	542.28	244.49	539.65
Serimore	264.24	558.31	264.24	558.31	264.24	558.31	264.24	558.31	264.24	558.31
Control	224.97	490.92	224.97	490.92	224.97	490.92	224.97	490.92	224.97	491.92
Mean	236.80	529.45	238.29	530.83	239.25	529.99	240.43	529.99		
S.Ed and CD for AST	S.Ed CD (0.05)				S.Ed and CD for ALT		S.Ed CD (0.05)			
	T= 0.012 T= 0.249**						T= 0.253 T= 0.512**			
	C= 0.078 C= 0.157**						C= 0.160 C= 0.324**			
	TC= 0.247 TC= 0.499**						TC= 0.507 TC= 1.025**			

Aspartate aminotransferase (AST) (µg/ml), Alanine aminotransferase (ALT) (µg/ml)
*T - Treatment, D - Dose, ** Highly significant, * significant*

Table 3. Effect of Pyriproxyfen on different biological parameters of silkworm, *B. mori* L

Economic Traits	Concentration				
	Single treatment@ 5 µl	Single treatment@ 10 µl	Double treatment@ 5 µl	Double treatment@ 10 µl	Mean
SG protein (mg/g)	116.90	116.23	115.63	115.40	116.04
FB protein (mg/g)	48.96	48.84	48.83	48.75	48.84
AST (µg/ml)	234.14	233.50	229.25	228.93	231.45
ALT (µg/ml)	540.75	539.42	512.32	511.92	526.10

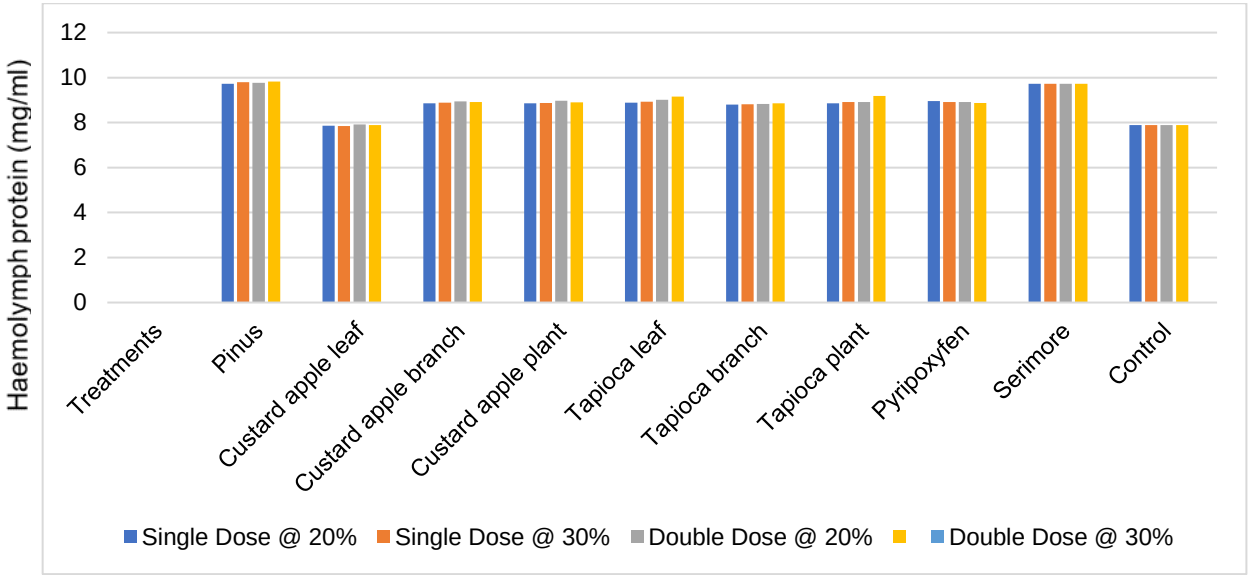

Fig. 1. Effect of different Juvenile hormone mimics on haemolymph protein of silkworm *B. mori* L.
*Significant from normal control, *P<0.05*

Least was recorded in untreated larva batch (115.40 mg/g). This result was supported where the topical application of 1ng of fenoxycarb on zero day of 5th instar, caused surge in juvenile hormone esterase two times *i.e.* first immediately after two hours of its application and next surge just before pupation [23]. During this time, highest activity of mRNA in silk gland was seen which caused increase in silk gland protein.

The highest fat body protein was observed in case of treatment with double dose at 30 *per cent* of Pinus extract (51.00 mg/g) whereas least was recorded in Custard apple leaf extract with treatment as single dose at 20 *per cent* (48.45 mg/g) followed by Pyripoxyfen with double dose at10 µl (48.75 mg/g). This was supported where administration of methoprene at 2 µg concentration on first day of 5th instar caused gradual increase in the fat body protein [24].

Distinguishable differences with regard to aspartate aminotransferase enzyme were recorded in different treatments of silkworm compared to untreated batches. Maximum aspartate aminotransferase activity was seen in treatment with Pinus extract double dose at 30 *per cent* (269.93 µg/ml) followed by pyripoxyfen at single dose of 5 µl concentration which recorded the enzyme activity of 234.14 µg/ml after 120 hours of application. This result was supported, where aspartate aminotransferase activity recorded at 120 hours after application of pyripoxyfen at the rate of 1, 10, 75, 150 ppm and found that 75 ppm concentration resulted in maximum enzyme activity of 384.7 IU/L followed by 10 ppm concentration (333.0 IU/L) [25]. Significant difference was observed between the juvenile hormone treated larvae and control batch. Highest alanine aminotransferase enzyme activity was seen in Pinus with double dose at 30 *per cent* concentration (563.22 µg/ml). In case of pyripoxyfen, it was recorded highest with single dose at 5 µl (540.75 µg/ml). This is supported where the administration of 3 fg/larva fenoxycarb causes higher activity in alanine aminotransferase enzyme inside muscle and silk gland which were recorded as 4.52 µM pyruvate/mg protein/hr and 2.94 µM pyruvate/mg protein/hr after fourth day of application in muscle and silk gland respectively whereas 5.01 µM pyruvate/mg protein/hr in muscle and 4.02 µM pyruvate/mg protein/hr in silk gland after sixth day of application [26]. The highest activity of alanine aminotransferase enzyme showed in 1 ppm pyripoxyfen treated larvae [25].

One of the enzymes that limits the rate at which juvenile hormone (JH) is produced in insects is juvenile hormone acid methyltransferase (JHAMT). To create JH, it transfers the methyl group of S-adenosyl methionine to either the farnesoic acid or the carboxyl group of JH acids. In Bombyx mori, six JHAMT paralogues have been found; of them, JHAMT1 and JHAMT2 exhibit methyltransferase activity [27]. The most well-known insulin signaling pathway is just one of several determinants that affect longevity, which is a complex biological phenomenon. This route controls the transcription factor Forkhead box O (FoxO), which in turn controls various physiological processes in many animals, including growth, development, metabolism, and longevity. Sex hormones (such as testosterone and estrogen) and dehydroepiandrosterone affect longevity in eukaryotes in addition to insulin and insulin-like growth factors [28].

4. CONCLUSION

Based on the current investigation, pinus was the most effective phutojuvenoids, followed by tapioca and custard apple. The tapioca plant produced the best results out of the tapioca series, followed by the tapioca leaf and tapioca branch. When it came to the custard apple series, the custard apple leaf was the least preferred option, followed by the custard apple branch. Pine was discovered to be comparable to JHA serimore that is sold commercially.

The optimal treatments for the tapioca and pine series were determined to be repeated treatments at a 30% concentration since this lengthens the larval period, which in turn improves the amount of protein and silk produced. When treated at a high concentration of 30% twice administration, the economic features of the cocoon diminish. This is because custard apple leaves have a strong odor, which reduces leaf eating and results in poor growth and development. Results with a single dose at 5µl and 10µl concentrations for Pyriproxyfen 30%EC were comparable. Because pyriproxyfen is a chemical growth regulator for insects, double treatment at both concentrations resulted in a decrease in economic features because of the residual influence on the fat bodies and haemolymph of silkworms. Therefore, using these JH mimics at the appropriate dosage and correct instar will increase economic traits of cocoons of silkworm, *Bombyx mori* L.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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