ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor (RJIF): 8.4
IJAR 2024; 10(5): 272-276
www.allresearchjournal.com
Received: 13-03-2024
Accepted: 16-04-2024

Tanvi Rahman
Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India

TS Jagadeesh Kumar
Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India

Kishan Kumar R
Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India

R Mahesh
Central Seri Cultural Research and Training Institute, Mysore, Karnataka, India

Varsha GS Gowda
Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India

Nandeesh
Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India

Corresponding Author:
Tanvi Rahman
Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India

Impact of serimore, a plant based growth promoter on haemolymph constituents and economic traits of silkworm double hybrid

Tanvi Rahman, TS Jagadeesh Kumar, Kishan Kumar R, R Mahesh, Varsha GS Gowda and Nandeesh

DOI: https://doi.org/10.22271/allresearch.2024.v10.i5d.11767

Abstract
Ecdysone and Juvenile hormones play a vital role in growth and development of silkworm (*Bombyx mori* L) by stimulatory metamorphosis and regulating ecdysis. Silkworm hormonal activities can be regulated with exogenous application of growth regulator. Serimore is a plant extract based silkworm growth regulator developed by CSRTI, Mysore. The study was undertaken to find out the impact of serimore application on haemolymph protein content, protease activity and economic traits in silkworm double hybrid FC_1 x FC_2. 0.2% serimore was topically administered as a single dose over the 5th instar larvae at 48 h (T_1) and 72 h (T_2) after ecdysis. Control batch (T_0) was maintained without serimore application. Protein content in haemolymph was significantly enhanced under T_1 in comparison to T_2 and T_0. In 48 h of treatment (T_1), there was significant improvement in the cocoon weight, shell weight, filament length over to that of T_2 and T_0. Thus, results clearly revealed that application of 0.2% serimore at 48 h of 5th instar silkworm double hybrid could help to enhance the silk cocoon productivity in sericulture.

Keywords: Serimore, growth regulators, juvenile hormone mimic, plant extract

Introduction
Silk industry is primarily based on cultivation of host plants and rearing of silkworm. Silk, the Queen of textile is reeled out of the cocoons produced by the silkworm. 70% of the silk industry is occupied by the mulberry sericulture. In the present day scenario, India stands at second largest silk producing country in the world following China. To breach the gap between supply and demand within the country, India should focus on quantity as well as quality of the silk production. Silkworm growth regulators (SGRs) such as juvenile and moulting hormones or their analogs (juvenoids and ecdysoids) when used judiciously have been found to be useful in sericulture industry. In insects, Juvenile hormone(JH) are a group of acyclic sequiterpenoids that helps in regulation of insect physiology especially metamorphosis and reproduction. Some plant species have been shown to contain certain compounds that can either mimic JH activity or act as antagonists by inhibiting JH biosysnthesis. These phytochemicals interfere with the endocrine system of insects especially the functioning of the JH in insects. Plants produce these phytochemicals as a protection against insect herbivory. In silkworm, these juvenile hormone mimics are often used to prolong the larval duration which shows proliferic result on the improvement of economic traits. Synthetic juvenile hormone mediates enhancement and manifestation of commercial traits. The physiological balance of juvenile hormone in insects depends on its biosynthesis and degradation pathway. Several Indian studies were carried on the subject of juvenile hormone mimic R394 (ethyl 9-cyclohexyl-3, 7-dimethy l-2, 4-non adienoate) applied topically on the abdominal tergum of silkworm (*Bombyx mori* L.), after the fourth ecdysis Results revealed that cocoon yield was improved by inducing Juvenile Hormone Analogue, SB-515 in the bivoltine silkworm (*Bombyx mori* L.) hybrid, CSR_2 x CSR_4 and its reciprocal combination. Similarly, Nagendraradhya and Kumar, (2013) [16] stated the use of synthetic juvenile hormone mediating the enhancement and manifestation of commercial traits of CSR_2 x MG_{408} and CSR_4 x MU_{852} productive bivoltine silkworm hybrids.

In another study, also reported that use of phyto-juvenile hormone mimics enhance the cocoon yield in silkworm, *Bombyx mori*. Likewise, many study reports indicated the use of JH analogues or the mimics in silkworm rearing regulates silk gland function and supports silk production. In silkworm, the synthesis of the silk fibres depend upon the accumulation of proteins in haemolymph. Thus, silk fiber synthesis is completely dependent upon the protein content of the larval body. However, protease activity plays a major role in synthesis of protein. Protease is an enzyme that catalyses the hydrolysis of peptide bonds present in proteins. Further, this enzyme liberates the amino acids needed for silkworm survival, growth and development. They are required for the regulation of various metabolic and cellular processes. Improvement of protein and protease enzyme in haemolymph could help to increase the larval development in silkworm. These improvements in silkworm can be regulated through application JHs topically. Hence, the present study was under taken to find out the impact of serimore application on the haemolymph protein content and protease activity as well the economic traits of silkworm double hybrid.

Materials And Methods

The study was carried out in the Department of Sericulture, University of Mysore, Manasagangotri, Karantaka during 2022. Disease-free layings of popular and high yielding bivoltine silkworm double hybrid (FC_1 x FC_2) was used for this study.

Silkworm rearing

Silkworm rearing was conducted following the standard recommended rearing practices at a temperature of 25±1°C and 75±5% relative humidity under 12:12 (light:dark) photoperiod. Feeding of freshly harvested Victory-1 (V1) mulberry variety leaves were given thrice a day. Frequent and timely cleaning of rearing bed after every moult to prevent disease occurring. Care was taken of lime dusting and bed disinfection with Vijetha in order to maintain bed humidity and disease prevention. After fourth moult out, 150 larvae were selected and reared in ventilated plastic rearing trays measuring 90x60 cm. A total of 9 trays were used and reared the silkworm by segregating three trays for each treatment.

Treatments

Serimore, a bakuchoil extract was procured from Sericare, division of health care private limited, KHB Industrial area, Yelahanka New town, Bengaluru-06. Serimore at a concentration of 0.2% emulsion was prepared by dissolving 5ml serimore in 2.5 litres potable water. Two treatments were imposed in the 5th instar larvae as a single dose application of serimore, one at 48 h of 5th instar larvae as T_1, and another at 72 h of 5th instar larvae as T_2. Control was maintained with no serimore application as T_0. The serimore emulsion was then topically administrated as a single dose to silkworms in pre cleaned trays at 48h and 72h of fifth instar at the rate of 0.02 ml per 100 larvae (0.2 micro litre (µl) serimore administered per silkworm larvae). After half an hour of imposing treatments, the larvae were fed with freshly harvested clean mulberry leaves (V1). After maturation, larvae were allowed for mounting and spinning of cocoons. Later, cocoons were harvested on 6th day after mounting and assessed for economic traits.

Tissue preparation for enzyme assay

Haemolymph collections were made on 3rd, 4th, 5th, 6th, 7th and 8th days of the fifth instar silkworm larvae both in serimore treated and control batches. To prepare the tissue homogenate for enzyme estimations, haemolymph samples were collected from 6 to 7 larvae by random selection. Haemolymph was collected by ampulating the abdominal legs of the silkworm larvae and placing in the pre chilled centrifuge tube with a few crystals of phenyl thiourea @ 1 mg per sample. Use of phenyl thiourea in order to avoid the prophenol oxidase activity followed by melanization of the haemolymph samples.0.5 ml haemolymph was extracted. The samples were centrifuged for 10 minutes at 4000 rpm at -4 ºC. The supernatant was transferred to pre chilled tubes and stored at -20 ºC till the commencement of experimentation. The protein content in haemolymph was estimated by Lowry's method (1951). The protease activity in the haemolymph was estimated by following the method.

Data collection

Daily larval weight was recorded from the ten randomly selected 5th instar larvae and averaged**.** Economic characters like cocoon weight, shell weight, filament length (average of 10 males and 10 females per replication), shell ratio, denier were recorded. All the data were subjected to statistically analysis by following the t test and the significance among the treatments were denoted as *.

Results and Discussion

Protein content

In insects, the haemolymph proteins play an important role for their enzyme action as well as the transport functions of various metabolites. Serimore administration with 0.2% concentration significantly impacted the protein content in haemolymph of silkworm (Table 2). In general, haemolymph protein was increased consistently from 3rd day to 7th day and thereafter declined steeply prior to the spinning in both treated and untreated larvae. The decline curve was due to the progressive development of the larval period and conversion of haemolymph protein to silk protein in the developing silk glands. Among both the treatment, the administration of 0.2% serimore at 48 h of 5th instar recorded significantly higher haemolymph protein in comparison to serimore application at 72 h of 5th instar larvae and control batches. In both the treatments, haemolymph protein was enhanced by 49.8% at 48 h and 41.1% at 72 h in comparison to control. According to Hurliman and Chen, 1974 genetic and hormonal factors controlled the synthesis and utilization of haemolymph protein. But high rates application of juvenile analogs inhibited the intracellular fibroin transport. Kajiura and Yamashita (1989) [7] suggested that the enhanced silk production was a concerted effect of the conversion of additional quantity of leaf consumed during the extended larval period and a direct stimulatory effect of the exogenous applied compound on protein synthesis in silk gland. Akai and Kiguchi, (1980) also reported that methoprene application increased the silk production by 24%.

Protease activity

Protease is one of the important key enzyme that helps in the degradation of the structural proteins into soluble proteins which in turn utilized for the formation of the silk fibre. In Table 3, results revealed that significant improvement was found in protease activity in the haemolymph with Serimore application. At 48h of treatment, 0.2% serimore administration enhanced the protease activity by 75.8% on 3rd day, by 46.9% on 4th day, by 31.6% on 5th day, by 52.4% on 6th day, by 17.5% on 7th day and by 2.1% on 8th day in comparison to control. Similarly, at 72 h, the protease activity was increased by 29.3% on 4th day, by 46.8% on 5th day, by 80.9% on 6th day, by 35.3% on 7th day and by 8.8% on 8th day over to the untreated larvae. There was marginal increase noticed in the protease activity of treatment at 72 h (80.9%) than 48 h. However, a steady and stable increase in daily protease activity was observed in all the batches up to seventh day except the eighth day of 5th instar. The steep decline in the protease activity during 8th day was may be due to the completed conversion of haemolymph protein to silk protein and the larvae preparing for spinning cocoons. Control treatment (without serimore spplication) was recorded significantly lesser protease activity compared to that of Serimore application at 48 h and 72 h. Waldbauer (1968) [2], reported significantly high activity of midgut protease during fifth instar larval development of silkworms as result of a higher rate of enzyme synthesis corresponding to enhanced food intake. This might facilitate a greater utilization of proteins for larval growth and silk production as well. Moreover, protease activity was reported to be high in bivoltines compared to multivoltines which might result in better conversion of exogenous proteins that ultimately lead to production of more silk (Tanaka 1964) [23].

The administration of serimore (Juvenile hormone mimic) on larval body resulted in significant changes in larval weights, cocoon weight and shell weight of silkworm double hybrid.

Results revealed that 0.2% Serimore application significantly influenced the larval weight. The larval weight improvement was 26.2% at 72 h and 14.7% at 48h compared to control. (Table 1).

Table 1: Average larval weight (g) of FC_1 X FC_2 silkworm treated with 0.2% of serimore after 48h and 72h during fifth instar silkworm

Treatments	Average larval weight(g) during fifth instar							
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
T_0 (Control)	1.346±0.022	1.750±0.138	3.083±0.107	4.173±0.295	4.193±0.208	4.400±0.188	5.360±0.330	4.790±0.324
T_1 (48 h)			3.093±0.068	4.106±0.290	4.72±0.168	5.056±0.118	5.623±0.092	5.606±0.068
Percent change			0.324	1.605	12.56	14.77	4.906	17.035
T_2 (72 h)				4.503±1.325	4.840±1.484	5.553±1.690	5.496±1.755	5.653±1.784
Percent change				7.907	15.430	26.200	2.530	18.010

Table 2: Haemolymph protein content (u moles/mg protein/min/ml) of FC_1 X FC_2 silkworm treated with 0.2% of serimore after 48h and 72h during fifth instar silkworm

Treatments	Haemolymph protein content during fifth instar							
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
T_0 (Control)	2.406±0.043	2.746±0.049	3.179±0.359	3.659±0.086	3.766±0.211	4.178±0.022	3.202±0.089	3.166±0.034
T_1 (48 h)			3.06±0.049	3.046±0.101	4.036±0.601	4.277±0.369	4.797±0.413	3.396±0.095
Percent change			3.743	16.75	7.169	2.369	49.812	8.985
T_2 (72 h)				3.00±0.072	3.116±0.201	3.887±0.180	4.521±0.510	3.283±0.495
Percent change				3.212	17.259	6.965	41.193	5.359

Table 3: Haemolymph protease activity (u moles/mg protein/min/ml) of FC_1 X FC_2silkworm treated with 0.2% of serimore after 48h and 72h during fifth instar silkworm.

Treatments	Haemolymph protease activity during Fifth instar							
	1stday	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
T_0 (Control)	0.453±0.043	0.876±0.067	0.870±0.033	1.113±0.187	1.736±0.137	2.286±0.060	4.156±0.048	2.456±0.051
T_1 (48h)			1.530±0.294	1.636±0.152	2.286±0.049	3.483±0.453	4.886±0.247	2.403±0.314
Percent change			75.860	46.990	31.682	52.490	17.565	2.157
T_2 (72 h)				1.440±0.112	2.550±0.441	4.136±0.259	5.626±0.498	2.673±0.040
Percent change				29.380	46.88	80.927	35.370	8.835

Table 4: Evaluation of economic parameters of FC_1 X FC_2 silkworm treated with 0.2% of serimore after 48h and 72h during fifth instar silkworm.

Treatments	Economic parameters							
	Cocoon weight (g)	Shell weight (g)	Pupal weight (g)	Shell ratio (%)	Filament length (m)	Filament weight (g)	Denier (u)	Renditta (kg)
T_0 (Control)	2.091±0.233	0.482±0.050	1.605±0.207	23.233±2.676	1060.43±38.670	0.441±0.036	3.746±0.248	5.530±0.195
T_1 (48h)	2.644±0.276	0.571±0.056	2.154±0.184	20.913±2.067	1268.067±40.266	0.530±0.006	3.780±0.134	5.590±0.114
Percent change	26.440**	18.464**	34.205**	9.98**	19.579**	21.088**	0.907**	1.0848*
T_2 (72 h)	2.621±0.374	0.528±0.081	2.069±0.317	20.339±2.556	1118.330±77.650	0.500±0.003	4.200±0.290	5.242±0.465
Percent change	25.346**	9.543**	28.909**	12.456**	5.460**	13.370**	12.119**	5.207**

*Represents as significance at 1%

** Represents as significance at 5%

Plate 1: Control batch of silkworm larvae and cocoons

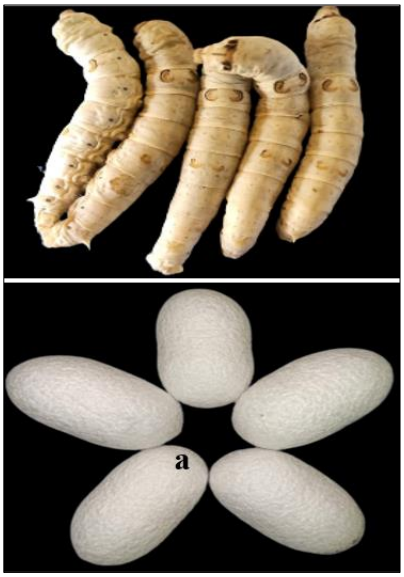

Plate 2: Silkworm larvae treated with Serimore after 48h and cocoons.

Plate 3: Silkworm larvae treated with Serimore after 72h and cocoons

Similarly, Serimore usage profusely influences the expression pattern of economic characters in silkworm cocoon. The performance being assessed at 48h (T_1) and 72h (T_2) of treatment in the 5th instar larval period. Administration of 0.2% Serimore at 48 h significantly increased the cocoon weight (26.4%), shell weight (18.4%), pupal weight (34.2%), shell ratio (9.9%), filament length (19.5%), filament weight (21.0%), denier (0.90%), and renditta (1.08%) in comparison to control. At 72 h also 0.2% Serimore application improved the cocoon weight by 25.3%, shell weight by 9.5%, pupal weight by 28.9%, shell ratio by 12.4%, filament length by 5.4%, filament weight by 13.3%, denier by 12.1%, and renditta by 5.2% over to control. In the present study, results revealed that economic traits of cocoon were higher in 0.2% Serimore spray at 48 h in comparison to 72 h and control. This result is in agreement with the findings of Nair *et al*. (2003) who reported that the use of juvenile hormone analog, Bakuchoil induced the hermetic effect in silkworm in turn enhanced cocoon yield. Nair *et al* (2000) reported application of JH compound enhanced the silk protein rate, cocoon weight and shell weight. Silkworm bivoltine breed performed better than multivoltine breed with JH spray. Repeated use of juvenoids during 3rd to 5th instar silkworm resulted in the increase in shell weight of the cocoon by 26% (Akai *et al*, 1985) [3]. It is very much clear that serimore, the bakuchoil extract implicit a positive impact on the silk production. Contrastingly, a notable decline in the cocooning percentage has been observed in Samia Cynthia ricini on application of Juvenile hormone analogs. (Magadum and Magadum, 1991) [12]. Another juvenile hormone analog, methoprene induction leads to the production of dauver larvae with characteristic growth of middle silk gland was reported by Kajiura *et al.*, 1989 [7]. This investigation gives a clear indication that though serimore influence the silk production positively, it is largely dependent on the dose and time of application as stated earlier (Akai *et al*., 1985; Trivedy *et al*., 1993) [3, 25].

Conclusion

In the present study, impact of serimore application at 48 h and 72 h of fifth instar silkworm double hybrid (*Bombyx mori* L) was studied. Results revealed that 0.2% serimore application at 48 h of fifth instar significantly enhanced the haemolymph protein content (49.8%) whereas, protease activity (75%) in comparison to the control. Significant improvement in cocoon weight, filament length, shell weight were also found under 0.2% serimore application at 48 h over to that of 72 h of serimore treatment and control. Thus, based on the present findings, results indicated that single dose topical administration of 0.2% serimore on fifth instar silkworm larvae at 48 h increased the protein content, protease activity in haemolymph and economics traits over to 72 h and control. Therefore, it can be concluded that application of 0.2% serimore topically at 48 h facilitate for better cocoon productivity fetching higher economic returns to the sericulture industry.

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