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

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

B Sannappa
Department of Sericulture Science, Manasagangotri, University of Mysore, Mysuru, Karnataka, India

Jashwanth S
Department of Sericulture Science, Manasagangotri, University of Mysore, Mysuru, Karnataka, India

Apeksha L
Department of Sericulture Science, Manasagangotri, University of Mysore, Mysuru, Karnataka, India

Linganandа
Department of Sericulture Science, Manasagangotri, University of Mysore, Mysuru, Karnataka, India

Spoorthi BM
Department of Sericulture Science, Manasagangotri, University of Mysore, Mysuru, Karnataka, India

Corresponding Author:
B Sannappa
Department of Sericulture Science, Manasagangotri, University of Mysore, Mysuru, Karnataka, India

Investigating the effects of ayurvedic-enriched fortified mulberry leaves on the biochemical composition of *Bombyx mori* L.

Kishan Kumar R, Tanvi Rahman, B Sannappa, Jashwanth S, Apeksha L, Linganandа and Spoorthi BM

Abstract
Sericulture is a crucial agro-industry in India, driving rural employment and silk production. The mulberry silkworm (*Bombyx mori*) depends solely on mulberry leaves, emphasizing the importance of leaf quality for silk yield. This study investigated the biochemical effects of fortifying mulberry leaves with Shatavari (*Asparagus racemosus*) and Vidarikand (*Pueraria tuberosa*) at concentrations of 0.5%, 1.0%, 1.5%, and 2.0%. Results showed significant increases in protein and carbohydrate content ($p \leq 0.01$). The highest protein levels in the fat body were observed with Shatavari @ 2.0% (5.510 mg/g) and Vidarikand @ 2.0% (5.283 mg/g), while haemolymph protein peaked at Shatavari @ 2.0% (19.55 mg/ml). Similarly, carbohydrate content was highest with Vidarikand @ 2.0% in haemolymph (22.01 mg/ml) and Shatavari @ 2.0% in the fat body (3.317 mg/g). These improvements are attributed to the bioactive compounds in Shatavari and Vidarikand, which enhance metabolic efficiency and nutrient synthesis. This study highlights the potential of Ayurvedic fortification as an efficient and eco-friendly approach to improving silkworm productivity, offering a sustainable solution for enhancing silk yield while supporting rural livelihoods.

Keywords: Sericulture, *Bombyx mori*, mulberry leaves

Introduction
Sericulture, an agro-based industry dedicated to rearing silkworms for raw silk production, is a vital contributor to India's economy and rural development. It provides employment to approximately 9.5 million people, primarily in rural and semi-urban areas, with a significant portion of the workforce comprising women, thereby empowering them economically and socially. India holds the distinction of being the world's largest consumer of silk and the second-largest producer globally. During the 2023-24 period, India's raw silk production reached 38,913 metric tons (MT), with the mulberry silk sector accounting for 77% (29,892 MT) of this total, as reported by the Central Silk Board (CSB). This industry not only supports rural households but also preserves traditional crafts, despite facing challenges such as competition from synthetic fibers and high production costs. Government initiatives like "Silk Samagra-2" aim to enhance productivity and quality while uplifting rural livelihoods and strengthening India's position in the global silk market.

The silkworm, *Bombyx mori L.* is a monophagous insect and consumes only the leaves of mulberry during larval stage in order to restore energy for the completion of its life cycle. In order to achieve higher productivity of mulberry silk, we should have high yielding mulberry varieties and silkworm breeds, besides providing quality nutrition to silkworm. The quality of mulberry leaf is utmost importance to harvest superior quality of cocoons with higher productivity [Pant, 1978] [8]. Mulberry leaves forms the basic food material for the silkworm, *B. mori* and nutrition is the most important growth regulating factor in the silkworm. Synthetic chemicals, fertilizers, pesticides, herbicides, growth promoter sand other inputs though enhance productivity of mulberry but adversely affect the ecosystem and increase prices of agriculture inputs [Patil *et al*., 2005] [9].

Like all living organisms, silkworms require energy and nutrients to survive and reproduce, making nutrition a critical factor in sericulture productivity. The essential nutrients-proteins, carbohydrates, fats, vitamins, and minerals-found in their food are digested and absorbed to varying degrees based on breed, variety, and environmental conditions [Lindroth, 1993 & Chakravorty *et al*., 2005] [7, 3]. Research has highlighted silk nutrition as a major area of focus within sericulture [Legay 1958] [6], with studies emphasizing that proper dietary management can directly enhance both the qualitative and quantitative aspects of silk yield. Effective care of silkworms through optimized dietary practices is therefore essential for maximizing sericulture output and stabilizing the economy of farmers involved in this industry. By ensuring that silkworms receive the appropriate nutrition, it is possible to significantly improve silk production and support the livelihoods of those dependent on sericulture.

Proteins, carbohydrates, and lipids are the primary biomolecules essential for the growth, development, and cocoon production of silkworms. The protein content in the diet significantly impacts not only the growth and silk yield but also the digestibility and protein levels in the haemolymph (Seol, 1982) [12]. Carbohydrates and other biomolecules largely depend on the quality of mulberry leaves provided to the silkworms and are utilized as energy sources for lipid and amino acid synthesis. The silkworm *Bombyx mori* requires ten essential amino acids for its growth and development: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine [Ito *et al.*, 1978] [5].

Recent studies have demonstrated that supplementing silkworm diets with fortifying agents can enhance biomolecule levels and improve economic parameters, leading to better silk production outcomes. Efforts have been made to enrich mulberry leaves with various nutrients, including proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics, salts, and other chemicals, to optimize the economic performance of silkworms (Kabila *et al*., 1994). However, there is limited information available regarding the changes in biomolecules within silkworms when supplemented with medicinal plant extracts. Additionally, our understanding of drug metabolism pathways in insects, including silkworms, is still quite limited. To address this gap, the current experiment aims to fortify mulberry leaves with Ayurvedic medicines to assess their effectiveness in improving silkworm productivity through the consumption of these treated leaves.

Materials and Methods

The experiment was conducted in a mulberry garden maintained by the Department of Sericulture Science at Manasagangotri Campus, Mysuru. Tender and medium mulberry leaves of the S-36 variety were used for young-age silkworm rearing followed V1 variety was used to rear late age silkworm. To ensure a pathogen-free environment, the rearing house and appliances were thoroughly cleaned using water, followed by disinfection with a 2% bleaching powder solution and 0.5% Serifighter solution at a rate of 1.5 liters per square meter (Dandin and Giridhar, 2010) [4]. Disease-free layings (eggs) of the popular bivoltine double hybrid silkworm strain *FC1 × FC2* were procured from the cold storage facility of the National Silkworm Seed Organization (NSSO), Mysuru. The eggs were subjected to black boxing for about 48 hours at pin head stage (Benchamin and Nagaraj, 1987) [2] later Brushing and rearing was conducted in model rearing house DoS, Sericulture science.

Fortification Agents Used: Shatavari Churna and Vidarikand Churna.

Shatavari Churna: Asparagus racemosus, or Shatavari, is a medicinal climber known for its rich composition of lignins, flavonoids, glycosides, alkaloids, and essential vitamins like A, B6, D, E, and K. Its roots and bioactive compounds offer numerous health benefits, including boosting immunity, reducing cholesterol, treating kidney stones, and acting as a diuretic. Widely used in traditional medicine, Shatavari is effective in addressing ailments such as colds, diarrhea, and even chronic conditions like AIDS.

Vidarikand Churna: Pueraria tuberosa, commonly known as Indian Kudzu or Vidarikand, is a climber with tuberous roots rich in lignins, flavonoids, glycosides, alkaloids, and essential vitamins like A, B6, D, E, and K. It is widely used in traditional medicine to treat urinary and reproductive system disorders, boost immunity, protect the liver, enhance fertility, and address skin diseases. Additionally, it offers relief from alcohol-related issues and supports overall health.

Formulation and application of ayurvedic supplements in silkworm rearing

The study focused on the preparation and supplementation of Ayurvedic formulations using Shatavari and Vidarikand. These formulations were dissolved in distilled water and diluted to concentrations of 0.5%, 1.0%, 1.5%, and 2.0%. The resulting solutions were then sprayed onto fresh mulberry leaves, which were shade-dried before being fed to silkworm larvae daily from the beginning of the fourth instar until the end of the fifth instar during morning feeding. Each treatment consisted of 50 larvae with three replications, and both distilled water control and absolute control groups were established for comparison. The experiment was designed using a completely randomized layout.

T_1: Shatavari @ 0.5% T_2: Shatavari @ 1.0% T_3: Shatavari @ 1.5% T_4: Shatavari @ 2.0% T_5: Vidarikand@0.5% T_6: Vidarikand@1.5%, T_7: Vidarikand@1.5% T_8: Vidarikand@2.0% T_9: Distilled Water T_{10}: Absolute Control [Plate 1].

Estimation of biomolecules

Collection and preservation of tissue samples

Biochemical analysis was carried out in the fat body and haemolymph in respective treatment and control batches. The fat body and haemolymph tissues were collected by dissecting fifth instar fifth day old larvae and preserved at -20ºC until further use. One percent tissue homogenate was prepared and samples were used for the estimation of biomolecules**.**

Estimation of protein

The protein content in fat body and haemolymph were estimated by adopting the method of Lowry *et al.* [1951]. About 1ml of tissue homogenate was taken with 5 ml protein reagent then the reaction mixture was incubated at room temperature for 10 min and 0.5 ml protein reagent was added. The contents were mixed thoroughly and kept for 30min until the development of blue color. About 1ml Bovine Serum Albumin [BSA] was used as standard. A blank was prepared using 1ml of distilled water. The optical density [OD] was measured by using spectrophotometer at 660nm against blank. The results were expressed in mg/g of for fat body and mg/ml for haemolymph.

$$\text{Amount of protein} = \frac{\text{OD of the sample X mg of BSA standard}}{\text{OD of the standard X mg of tissue taken}} \text{ X } 1000$$

Estimation of carbohydrate

Total carbohydrate was determined by Anthrone method [Dubois *et al.*, 1956] using glucose as standard. To 1ml sample, 4ml Anthrone reagent was added and contents were boiled for 8 min using water bath, then cooled with running water. The absorbance was measured using Spectro photo meter at 630 nm against blank and carbohydrate content was expressed in mg/g of for fat body and mg/ml for haemolymph.

$$\text{Amount of Carbohydrate} = \frac{\text{OD of the sample X mg of Glucose standard}}{\text{OD of the standard X mg of tissue taken}} \text{ X } 1000$$

Results

The observations pertaining to the influence of feeding mulberry leaf fortified with ayurvedic formulations at different concentrations on protein and carbohydrate contents in fat body and haemolymph of silkworm are tabulated in Table 1 and illustrated in Graph 1 and 2.

Protein content in Fat body

Fortification of mulberry leaf with ayurvedic formulations namely Shatavari and Vidarikand at different concentrations on the protein content in the fat body was found highly significant [$p \le 0.01$, F-value: 61.09**]. The highest protein content in the fat body was observed in the treatment with T_9 [5.657±0.061 mg/g] followed closely by T_4: Shatavari @ 2.0% [5.510±0.395 mg/g], T_8: Vidarikand @ 2.0% [5.283±0.159 mg/g] and T_7: Vidarikand @ 1.5% [5.267±0.120 mg/g]. On the other hand, T_3: Shatavari @ 1.5% [5.233±0.024 mg/g] and T_2: Shatavari @ 1.0% [5.053±0.167 mg/g] were found next best. However, both T_{10}: Absolute control [4.090±0.046 mg/g] and T_1: Shatavari @ 0.5% [4.120±0.006 mg/g] recorded lowest protein content in the fat body.

Protein content in Haemolymph

The enrichment of mulberry leaves with different concentrations of Shatavari and Vidarikand resulted in a highly significant increase in protein content in the haemolymph [$p \le 0.01$, F-value: 30.13**]. The highest protein content in the haemolymph was recorded in T_4: Shatavari @ 2.0% [19.55±0.127 mg/ml], followed by T_8: Vidarikand @ 2.0% [19.47±0.069 mg/ml], T_3: Shatavari @ 1.5% [19.40±0.129 mg/ml] and T_2: Shatavari @ 1.0% [19.04±0.30 mg/ml]. Treatments, T_7: Vidarikand @ 1.5% [19.15±0.035 mg/ml] and T_6: Vidarikand @ 1.0% [18.80±0.323 mg/ml] also showed notable protein content. Conversely, T_9: Distilled water [17.37±0.199 mg/ml] and T_{10}: Absolute control [17.26±0.206 mg/ml] exhibited the lowest protein content in the haemolymph.

Carbohydrate content in Fat body

Enhancement of mulberry leaves with varying concentrations of Shatavari and Vidarikand significantly affected the carbohydrate content in the fat body [$p \le 0.01$, F-value: 53.63**]. The highest carbohydrate content in the fat body was observed in T_9 [3.330±0.215 mg/g] followed closely by T_4: Shatavari @ 2.0% [3.317±0.133 mg/g], T_8: Vidarikand @ 2.0% [2.853±0.064 mg/g] and T_7: Vidarikand @ 1.5% [2.577±0.097 mg/g]. On the other hand, T_3: Shatavari @ 1.5% [2.463±0.026 mg/g] and T_2: Shatavari @ 1.0% [2.230±0.01 mg/g] were found next best; however, both T_{10}: Absolute control [2.100±0.017 mg/g] and T_1: Shatavari @ 0.5% [2.143±0.012 mg/g] recorded lowest carbohydrate content in the fat body.

Carbohydrate content in **Haemolymph**

Fortification of mulberry leaves with Shatavari and Vidarikand at different concentrations significantly affected the carbohydrate content in the haemolymph [$p \le 0.01$, F-value: 88.82**]. The highest carbohydrate content in the haemolymph was observed in the treatment with T_8: Vidarikand @ 2.0% [22.01±0.020 mg/ml], followed by T_4: Shatavari @ 2.0% [21.80±0.092 mg/ml], T_7: Vidarikand @ 1.5% [21.74±0.155 mg/ml] and T_3: Shatavari @ 1.5% [21.59±0.030 mg/ml]. Treatments T_2: Shatavari @ 1.0% [21.56±0.100 mg/ml] and T_1: Shatavari @ 0.5% [21.52±0.045 mg/ml] also showed notable carbohydrate content; conversely, T_9: Distilled water [20.27±0.147 mg/ml] and T_{10}: Absolute control [20.03±0.027 mg/ml] exhibited the lowest carbohydrate content in the haemolymph.

Discussion

Protein content in the fat body and haemolymph

Fortifying mulberry leaves with Shatavari and Vidarikand significantly boosts protein content in silkworms. In the fat body, the highest protein content was observed with Shatavari @ 2.0% [5.510 mg/g] followed by Vidarikand @ 2.0% [5.283 mg/g], Vidarikand @ 1.5% [5.267 mg/g]. Lower concentrations of Shatavari [1.5% and 1.0%] and the absolute control showed the least protein content. In the haemolymph, Shatavari @ 2.0% [19.55 mg/ml] was most effective, followed by Vidarikand @ 2.0% [19.47 mg/ml]. Other high-protein levels were seen with Shatavari @ 1.5% [19.40 mg/ml], 1.0% [19.04 mg/ml], and Vidarikand @ 1.5% [19.15 mg/ml], 1.0% [18.80 mg/ml]. The lowest protein content was found in distilled water and absolute control treatments. The enhanced protein content is attributed to the bioactive compounds in Shatavari and Vidarikand, which stimulate protein synthesis and improve metabolic efficiency. The current study was supported with the studies of Rima Shahin and Gad Gadelhak [2013] [10], where larvae of the mulberry silkworm, *Bombyx mori* L. were fed on mulberry leaves treated with [0.01, 0.05, 0.10, 0.50 and 1.00%] lettuce seed oil. The silk gland total protein was estimated from the 2nd day to the 10th days [spinning day] of the 5th larval instar. The highest value of total silk gland protein was obtained after feeding larvae on 0.10% lettuce seed oil.

Carbohydrate content in the fat body and haemolymph

Enriching mulberry leaves with Shatavari and Vidarikand significantly boosts carbohydrate content in silkworms. In the fat body, the highest carbohydrate levels were observed with T_9 [3.330 mg/g] and Shatavari @ 2.0% [3.317 mg/g], followed by Vidarikand @ 2.0% [2.853 mg/g] and 1.5% [2.577 mg/g]. Lower concentrations of Shatavari also increased carbohydrates. In the haemolymph, Vidarikand @ 2.0% [22.01 mg/ml] and Shatavari @ 2.0% [21.80 mg/ml] were most effective, with Vidarikand @ 1.5% [21.74 mg/ml] and Shatavari @ 1.5% [21.59 mg/ml] also showing high levels. The lowest carbohydrate content was in distilled water and the absolute control. The increased carbohydrate content is likely due to the bioactive compounds in these ayurvedic formulations, which enhance metabolic processes related to carbohydrate synthesis and storage. Higher concentrations of these formulations appear more effective, providing greater availability of active compounds for improved carbohydrate metabolism. The current study is supported with the studies of Lindorth [1993] [7], wherein silkworms like all other living organisms require energy and nutrients to survive and reproduce. The nutrients carbohydrates, fats, vitamins and minerals in the foods are digested and absorbed at different degrees depending on breed, variety and environmental condition.

Summary

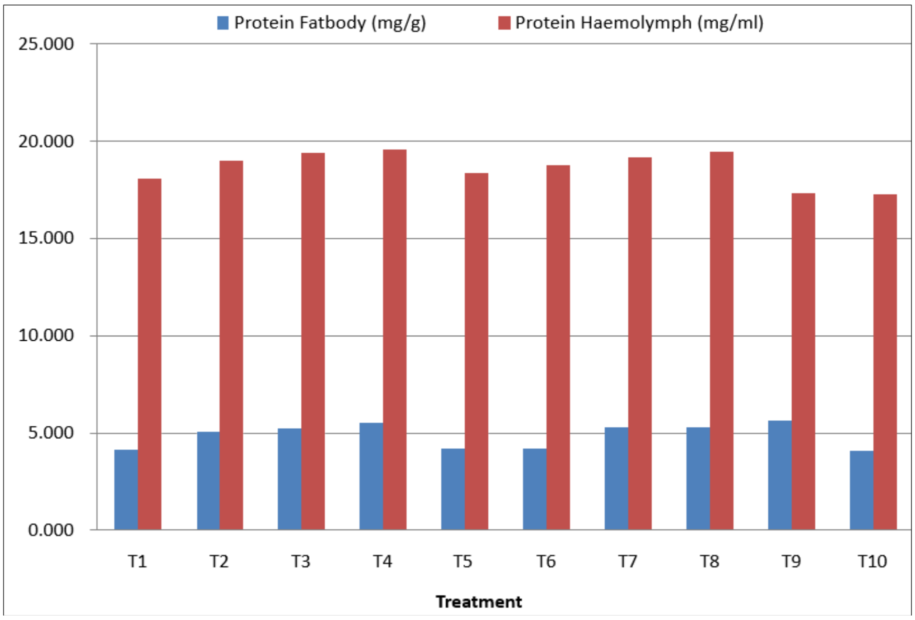
The study revealed that enriching mulberry leaves with Ayurvedic formulations, Shatavari and Vidarikand, significantly influenced the biochemical composition of silkworms. Protein content in the fat body was highest in the distilled water group, while haemolymph showed maximum protein levels with Shatavari @ 2.0%, highlighting its effectiveness at higher concentrations. For carbohydrate content, the fat body recorded higher levels in the distilled water group, whereas Vidarikand @ 2.0% resulted in the highest carbohydrate levels in haemolymph, indicating its role in enhancing carbohydrate metabolism in silkworms. The experimental results showed higher protein and carbohydrate content in the fat body of silkworms treated with distilled water. To clarify this finding, the experiment may be repeated under controlled conditions, averaging values from multiple trials.

The study highlighted the significant impact of enriching mulberry leaves with Ayurvedic formulations, Shatavari and Vidarikand, on the biochemical composition of silkworms. Shatavari @ 2.0% demonstrated maximum protein levels in the haemolymph, showcasing its effectiveness at higher concentrations, while Vidarikand @ 2.0% resulted in the highest carbohydrate levels in the haemolymph, underscoring its role in enhancing carbohydrate metabolism. These findings indicate that both Ayurvedic formulations can positively influence the nutritional profiles of silkworms, contributing to improved growth and development. To further validate these results and ensure accuracy, it is recommended to repeat the experiment under controlled conditions and average values across multiple trials. This approach will deepen our understanding of the biochemical effects of these formulations, ultimately paving the way for optimized sericulture practices.

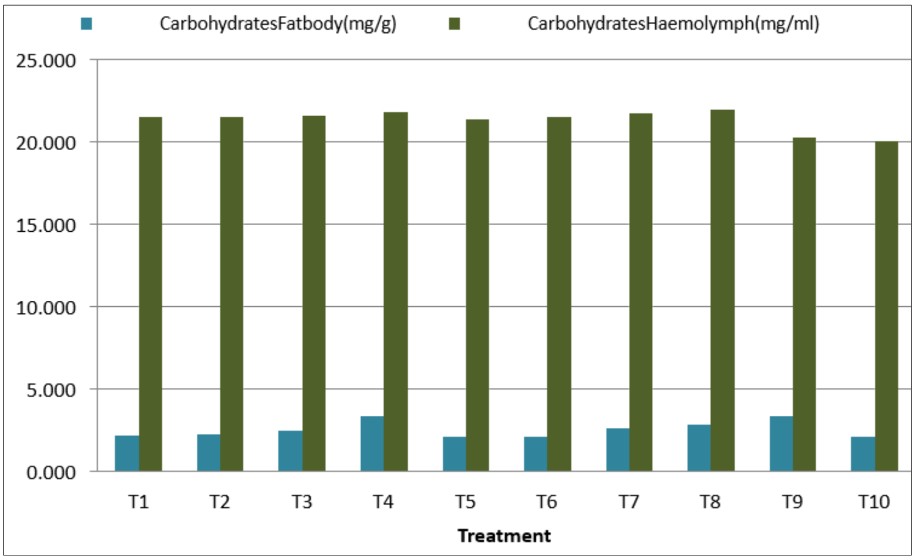
Table 1: Fortification of mulberry leaf with ayurvedic formulations at varied concentrations on biomolecules in fat body and haemolymph of silkworm

Treatment	Protein		Carbohydrates	
	Fatbody [mg/g]	**Haemolymph [mg/ml]**	**Fatbody [mg/g]**	**Haemolymph [mg/ml]**
T_1= Shatavari @0.5%	4.120±0.006 [0.733]	18.11±0.340 [4.925]	2.143±0.012 [2.048]	21.52±0.045 [7.439]
T_2= Shatavari @1.0%	5.053±0.167 [23.545]	19.04±0.300 [10.31]	2.230±0.015 [6.190]	21.56±0.100 [7.789]
T_3= Shatavari@1.5%	5.233±0.024 [27.946]	19.40±0.129 [12.399]	2.463±0.026 [17.28]	21.59±0.030 [8.837]
T_4= Shatavari@2.0%	5.510±0.395 [34.719]	19.55±0.127 [13.26]	3.317±0.133 [57.95]	21.80±0.092 [8.837]
T_5 =Vidarikand@0.5%	4.200±0.057 [2.689]	18.36±0.644 [6.373]	2.130±0.012 [1.429]	21.40±0.149 [6.830]
T_6=Vidarikand @ 1.0%	4.200±0.057 [2.689]	18.80±0.323 [8.922]	2.130±0.012 [1.429]	21.52±0.132 [7.439]
T_7 =Vidarikand@1.5%	5.267±0.120 [28.77]	19.15±0.035 [10.95]	2.577±0.097 [22.71]	21.74±0.155 [8.537]
T_8=Vidarikand @ 2.0%	5.283±0.159 [29.16]	19.47±0.069 [12.80]	2.853±0.064 [35.85]	22.01±0.020 [9.885]
T_9=Distilled water control	5.657±0.061 [38.31]	17.37±0.199 [0.637]	3.330±0.215 [58.57]	20.27±0.147 [1.198]
T_{10} =Absolute control	4.090±0.046	17.26±0.206	2.100±0.017	20.03±0.027
F-value	61.09**	30.13**	53.63**	88.82**

±: Standard error values **: $p \leq 0.01$ []: Percent change values over absolute control



Graph 1: Effect of feeding mulberry leaf fortified with ayurvedic formulations at different concentrations on protein content in fat body and haemolymph of silkworm



Graph 2: Effect of feeding mulberry leaf fortified with ayurvedic formulations at different concentrations on carbohydrate content in fat body and haemolymph of silkworm

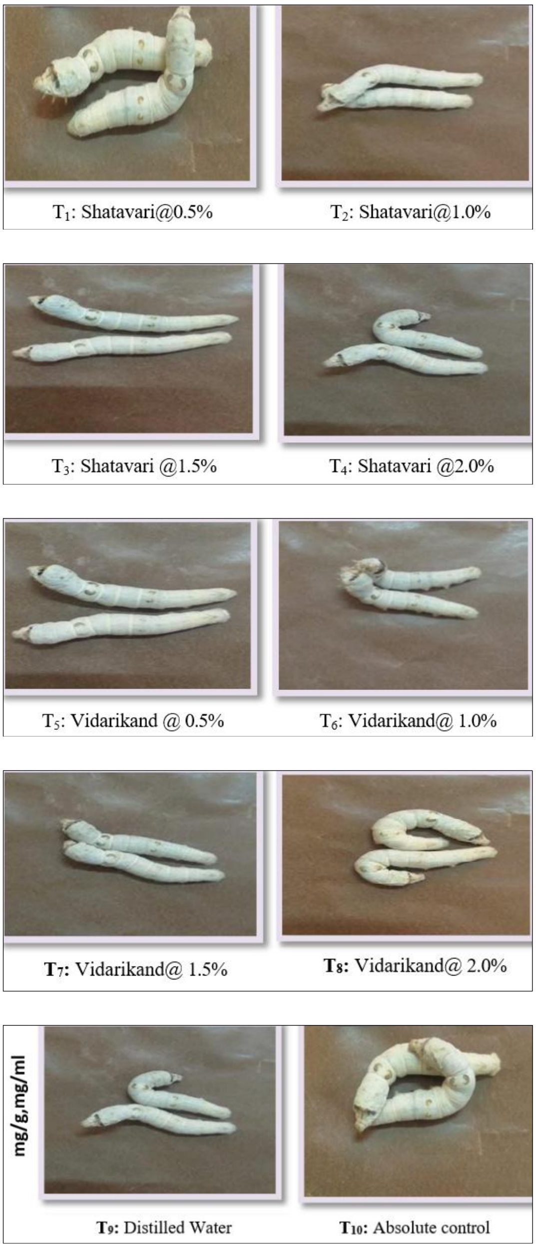


Plate 1: Matured larvae of bivoltine double hybrid silkworm [FC_1XFC_2]

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