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Biofortifying castor leaves with *Moringa oleifera* to enhance the biochemical composition and commercial features of eri silkworms (*Samia ricini*)

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Abstract
The present study explored the effect of varied levels of *Moringa oleifera* leaf powder (T_1-T_5) and distilled water (T_6) on castor leaves consumed by *Samia ricini* with T_7 as the absolute control. Important economic characteristics such as mature larval weight, cocoon weight, pupal weight, shell weight, shell ratio, and silk productivity were affected by *moringa* supplementation. Highest values in most of the parameters were obtained with the T_5 treatment (1.0% M. *oleifera*), with the larvae attaining a weight of > 22 g, cocoon weight of ~14 g, and pupal weight of ~9 g, reflecting enhanced digestion and physiological efficiency. T_3 (0.6%) showed maximum shell ratio, whereas T_5 showed better silk productivity.
Biochemical analysis indicated that T_2 (0.4%) and T_3 (0.6%) contained the highest concentration of proteins in haemolymph (~85 mg/ml and ~75 mg/ml, respectively), while the fat body and silk gland showed maximum protein accumulation in T_5. Carbohydrate content was maximum in T_5 with the fat body recording ~28 mg/g followed by elevated concentrations in the haemolymph and silk glands, indicating higher metabolic activity. Correlation analysis identified high positive correlations between fat body carbohydrate content and principal economic characters such as larval weight (r = 0.785*), cocoon weight (r = 0.939**), pupal weight (r = 0.908**), and productivity in silk (r = 0.886**). Haemolymph carbohydrate, however, demonstrated negative correlation with these characters. Protein concentrations in the tissues did not correlate significantly with the economic traits.
These results reveal that 1.0% *Moringa oleifera* diet supplementation significantly improves eri silkworm growth, silk production, and metabolic activity mainly through the improvement of carbohydrate reserves in fat body and haemolymph. *M. oleifera* thus holds great promise as a natural solution for enhancing the commercial traits of *S. ricini* in ecologically friendly sericulture operations.

Keywords: ERI silkworm, bio-chemicals, bioassay, castor leaf

Introduction
Silkworms are the group of insects belonging to Lepidoptera, and are domesticated insects mainly recognized for their use in the production of silk. The most widely cultivated species in sericulture is the mulberry silkworm, *Bombyx mori* (Shahzadi *et al.*, 2022) [14]. India is unique in that, it produces non-mulberry silkworms, including assam tasar (*Antheraea assamensis*), tropical tasar (*Antheraea mylitta*), and erosion silkworm (*Samia ricini*). These illustrations show how different species of silkworms differ in terms of their host plants, ecological adaptations, and silk quality (Tikader *et al.*, 2013) [15]. Millions of farmers and artists have made money from silkworms through spinning, weaving, cocoon rearing, and silk reeling. Silkworms are therefore essential to rural livelihoods.
Silkworms produce the natural protein fabric known as silk, which is valued for both its unique mechanical properties and visual appeal. Fibrin and sericin are the proteins, which make up the majority of silk, and gives strength, structure, flexibility, and inherent sheen. Silk's brightness, durability, and dye retention make it a premium yarn or textile (Kunz *et al.*, 2016) [7]. Sericulture is therefore a sustainable agricultural method as well as a business venture. Additionally, sericulture has significant economic effects on rural regions in China, India, and other nations that produce silk. India is the only country that produces all five of the economically exploited types of silk, making it the second-largest producer in the world (Mushtaq *et al.*, 2023) [10]. Eri silk (*Samia ricini*) known as endi or ahimsa silk, is unique among non-mulberry silks because of its environmentally friendly and ethical production

method. Eri silkworms only feed on castor (*Ricinus communis*) and sometimes tapioca leaves (Birari *et al.*, 2019) [2]. The production of Eri silk lets the silk moth emerge naturally, unlike mulberry silkworms that die in the reeling process, thus being cruelty-free and increasing popularity in sustainable design companies. The silk produced is woolly, strong, and has tremendous thermal insulation, so it works well when combined with cotton, wool, or synthetic fibres (Das *et al.*, 2021) [3]. Eri sericulture is rising in popularity in the north eastern and southern India regions, both because it is adaptable to a wide variety of agro-climatic conditions, relatively easy for limited investments, and because the hand spinning industries have taken higher regard of eri silk (Reddy & Parasuramudu, 2024) [13]. Factors externally affecting the nutritional characteristics of the leaves that eri silkworms consume, ultimately affect their productivity and the silk. If it can be established that phytochemicals like *Moringa oleifera* sprayed castor leaves will improve the nutritive value of the castor leaves to be given to the eri silkworms, this biological supplement would certainly serve as a viable option for improving both yield and quality of eri silk (Narzary *et al.*, 2024) [11].

Castor (*Ricinus communis*) is cultivated in India and is the primary host plant for eri silkworms (*Samia ricini*), which provide for nutrition and water during development and cocoon formation. Castor leaves are said to contain moderate levels of protein, amino acids, and moisture required for larval growth, but their nutritional limitations often hinder potential higher silk yield and cocoon quality (Department of Botany, University of Science and Technology, Meghalaya, Ri-Bhoi, India *et al.*, 2023) [9].

Moringa oleifera and other plant-based feed additives have been explored as options for this issue. The "Miracle Tree," also called *moringa*, is well-known for its unique nutritional qualities, including the presence of essential amino acids, protein, minerals (such as calcium, potassium, and iron), and vitamins A, C, E, and B-complex (Islam *et al.*, 2021). Additionally, it has a high concentration of phytochemicals that promote growth and a variety of antioxidants that improve metabolic and immune function. *Moringa* has been used to improve feed efficiency, growth rate, and physiological performance when fed to insects and animals. Additionally, feeding silkworms *moringa* enhanced the growth of silk glands, hemolymph biochemistry, and cocoon properties (Pareek *et al.*, 2023) [12] (Abbas, 2013) [1]. An inventive and natural way to raise the nutritional value of silkworm feed is to add *moringa* leaf powder to castor leaves. The technique involved exogenous spraying of *moringa* solutions at varying concentrations (0.2%-1.0%) on castor leaves and feeding them to eri larvae during the fourth and fifth instars, the period of rapid metabolism and silk production. Not only does the combination diet provide essential nutrients, but it also improves the biochemical profiles of the silkworm, namely the protein and carbohydrate in the haemolymph, fat body, and silk glands. This biofortification method has been shown to greatly enhance commercial characteristics like larval weight, cocoon weight, shell weight, shell ratio, and silk yield (Mahanta *et al.*, 2023) [9]. Additionally, it offers a low-cost, environmentally acceptable alternative to chemical additives, which fits perfectly with the objectives of sustainable sericulture. The current study aims to examine the impact of *moringa*-enriched castor leaves on the biochemical composition and commercial characteristics of eri silkworms (*Samia ricini*, White-Zebra strain), hoping to increase productivity through natural food enhancement (Department of Botany, University of Science and Technology, Meghalaya, Ri-Bhoi, India *et al.*, 2017) [9].

This study explores a possible use of *moringa* leaf powder to supplement castor leaves to enhance the biochemical composition and commercial properties of eri silkworms. The aim is to create and develop a sustainable, organic process that includes larval health, cocoon production and, silk yield; this is also beneficial for sustainable sericulture and smallholder farmers.

Materials and Methods

Experimental Site and Duration

The experiment was carried out at the Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, during the period between February to June.

Experimental Design with Eri Silkworm Strain

The study utilized a Completely Randomized Design (CRD) with 7 treatments, each having 4 replications. The experiment used the White-Zebra eri silkworm strain, which is compatible with castor as a host plant and can yield commercially useful cocoons.

Host Plant and Supplements

The castor variety that was the study's only food source during the larval stage was Local Pink. *Moringa* powder was mixed with leaves to improve the nutritional quality. To make *Moringa* leaf powder solutions, commercially available powder was mixed with distilled water in various amounts. These solutions were sprayed onto castor leaves that had been freshly harvested and shade-dried to preserve the active compounds when the silkworms would consume them.

Table 1: Treatment details

Treatment	Concentration (%)
T_1	0.2
T_2	0.4
T_3	0.6
T_4	0.8
T_5	1.0
T_6	Distilled water control
T_7	Absolute control

Feeding Protocol
Eri silkworm larvae were fed *moringa*-enhanced castor leaves every alternate day during the four and five instars, the most nutritionally demanding instars. Each treatment group consisted of 50 larvae per replica.

Disinfection and rearing conditions
The rearing room and tools were disinfected on two separate occasions, with a 5% bleaching powder solution, and then on a second occasion with 4% formalin at a 1.5 L/m² application rate; the room was left under lock for 36 hours for effective sterilization. DFLs (disease-free layings) of Eri silkworms were obtained from the College of Sericulture, University of Agricultural Sciences, Chinthamani, and under controlled conditions (25±1 °C and 75±5% humidity), the eggs were incubated. Black-boxing was done with newly hatched larvae when the eggs were in the pinhead stage with black paper, to synchronize the hatching. Larvae were rubbed onto fresh Local Pink castor leaves. The brushing to spinning was kept to the rearing. The average temperature was 31.12 °C. The average relative humidity was 55.79%.

- **Economic parameters**: Throughout the spinning stage, the following economic parameters were noted in order to assess the commercial qualities of eri silkworms (Samia ricini):
  - The weight of matured larva (g).
  - The weight of the cocoon, in (g).
  - The weight of the pupa, in (g).
  - The weight of the shell, in grams (g).
  - The shell ratio, as a percentage (%).
  - The production of silk (kg/day).

- **Biochemical estimation**

Biochemical samples, in the form of haemolymph, fat body, and silk gland, were obtained from fifth-day, fifth-instar larvae. Samples were stored at -20°C and then analyzed using a spectrophotometer. The total protein and total carbohydrate contents were analysed using Lowry's method (Lowry *et al.*, 1951) [8] and the Anthrone method (DuBois *et al.*, 1956), respectively. And were expressed in mg/ml for haemolymph and mg/g of tissue for the fat body and silk gland.

Statistical analysis
The data were analyzed statistically using Fisher's Analysis of Variance (ANOVA) with Cochran and Cox (2000) procedures. The levels of significance were determined using the SPSS software package.

Results
The analysis of the different concentration outcomes of *Moringa oleifera* (T_1-T_5) and distilled water (T_6) sprayed on castor leaves, and key rearing parameters of the eri silkworm (*Samia ricini*) (such as matured larval weight, cocoon weight, pupal weight, shell weight, shell ratio, and silk productivity,) can be seen in Figure 1. Over nearly all parameters, T_5 (1.0% *moringa*) outperformed other groups.
The supplementation of *Moringa oleifera* leaf powder in castor leaves had a significant influence on the economic indicators of eri silkworms in all parameters examined. The larvae fed with 1.0% *moringa* (T_5) leaves reached a developed larval weight of greater than 22 g, cocoon weight of statistically significant (~14 g), and pupal weight (~9 g), indicating improved digestion of nutrients and overall health.
Particularly, T_3 (0.6% *moringa*) had the highest shell ratio, indicating the ideal cocoon to shell mass ratio at a moderate enrichment level. The effect of *moringa* supplementation on optimal silk biosynthesis was highlighted by the noticeably higher silk productivity in T_5. However, T_6 (distilled water control) continuously displayed the lowest values for every trait, demonstrating the significant contribution of nutritional enrichment caused by *moringa*. This indicates that adding *moringa* to castor leaves at a rate of 1.0% was very beneficial for eri silkworm larval growth and commercial silk characteristics.

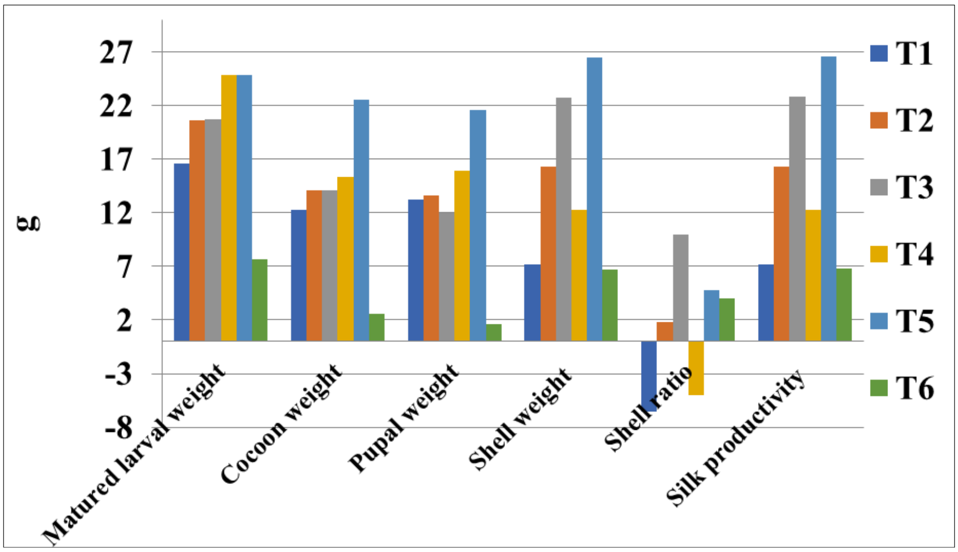

Fig 1: Percent change in economic parameters of eri silkworm reared on castor leaf supplemented with '*moringa*' over absolute control

Biochemical analysis
The impact of supplementing the eri silkworm on tissues, such as the haemolymph, fat body, and silk gland, with *Moringa oleifera* leaf powder was assessed through a battery of biochemical tests.

Protein content
The total amount of protein in tissues is displayed in Figure 2. The graph shows that T_2 (0.4%) and T_3 (0.6%) had the highest protein concentrations in their haemolymph, with values that were nearly 85 mg/ml and 75 mg/ml,

respectively. Higher *moringa* concentrations were associated with increased metabolic storage and silk gland activity, as evidenced by the peak protein accumulation in the fat body and silk gland in T_5 (1.0%). On the other hand, the lowest protein levels were found in all tissues in the absolute control (T_7) and distilled water control (T_6).

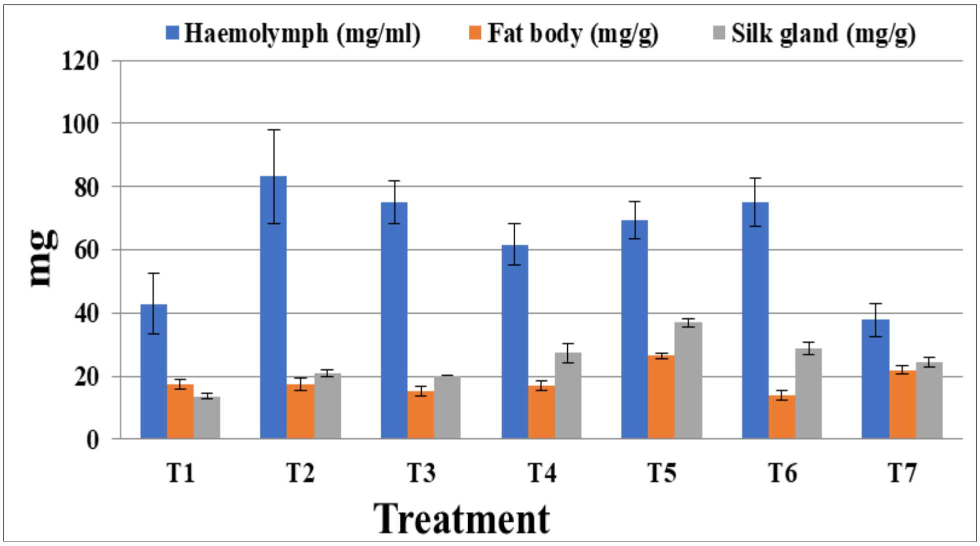

Fig 2: Protein content in different tissues of eri silkworm reared on castor leaf supplemented with '*moringa*'

Carbohydrate-Content

Carbohydrate concentrations in the tissues (Haemolymph, fat body, and silk glands) are displayed in Figure 3. T_5 (1.0%) outperformed other treatments and showed a notable increase, especially in the fat body (~28 mg/g). This indicates a strong response to energy storage at elevated levels of *moringa*. T_5 had the highest carbohydrate in its haemolymph and silk glands, followed by T_4 and T_3. These results indicate that supplementing castor leaf with *moringa* enhances the metabolism of carbohydrates in a dose-dependent way.

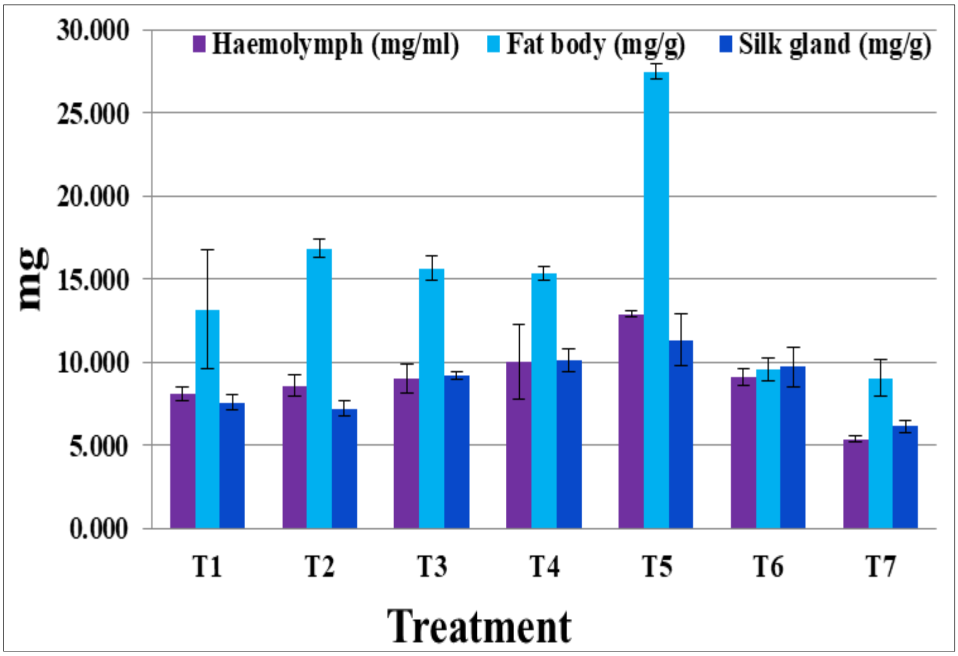

Fig 3: Carbohydrate content in different tissues of the eri silkworm reared on castor leaf supplemented with '*moringa*'

Percentage change compared to absolute control

The effect was measured by comparing the percentage changes in biochemical components to T_7, the absolute control (Figure 4). The highest positive increase in haemolymph protein was observed in T_2, which was closely followed by T_3 and T_4. Nonetheless, the fat body and silk gland in T_5 showed a notable decrease when expressed as percentages, most likely as a result of a higher baseline in T_7 or protein metabolic redistribution.

The amount of carbohydrates in the tissues of eri silkworms was significantly increased by adding *Moringa oleifera* to castor leaves, especially at 1.0% (T_5), as shown in Figure 5. The fat body showed the largest gain, followed by the haemolymph and silk gland, indicating increased energy storage and metabolic activity. Lower concentrations (T_1-T_3) showed moderate effects, but T_6 (distilled water control) had less of an impact. These results show how *moringa* enhances the metabolism of carbohydrates, which in turn promotes larval growth and silk production.

Protein and carbohydrate levels are visually compared side by side. With the highest or nearly highest values across all tissues, T_5 seemed to be the most well-rounded and ideal treatment. With the lowest biochemical reserves, T_6 and T_7 showed how beneficial *moringa* enrichment is for boosting metabolic vigor in eri silkworms. Overall, the graphs demonstrate that supplementing with *Moringa oleifera*, particularly at 1.0% (T_5), considerably raises the levels of protein and glucose in vital eri silkworm tissues. Larval growth is aided by higher haemolymph protein concentrations (0.4-0.6%), while better silk biosynthesis and energy storage are associated with fat body and silk gland content of 1.0%. The importance of *moringa* phytonutrients and amino acids in boosting nutrition assimilation, which enhances silkworm physiology and commercial traits, is supported by these results.

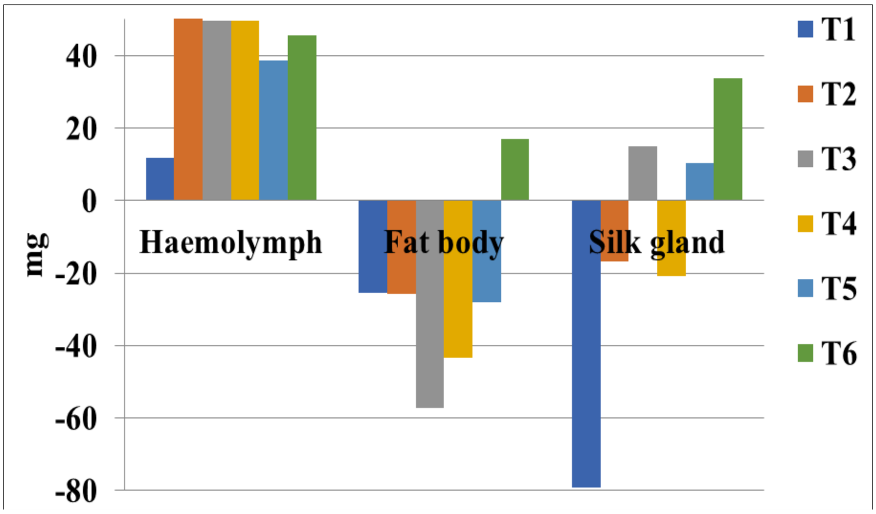

Fig 4: Percent change of protein content in different tissues of eri silkworm reared on castor leaf supplemented with '*moringa*' over the absolute control

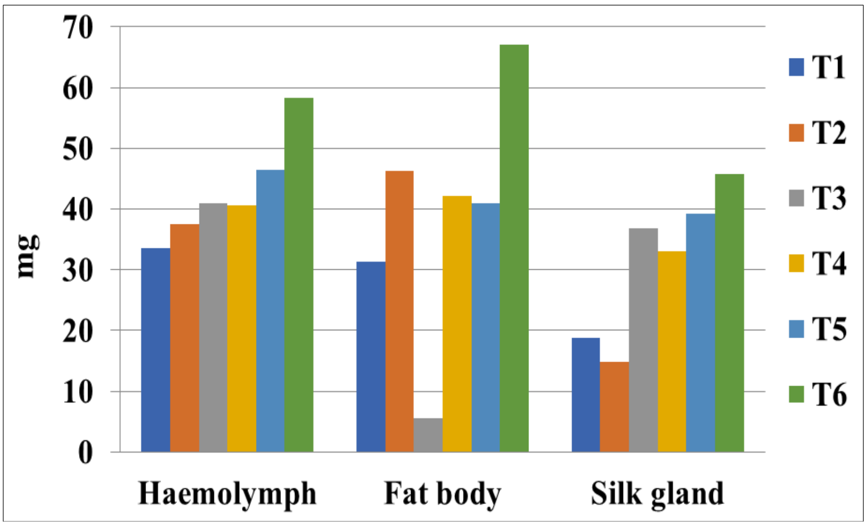

Fig 5: Percent change of carbohydrate content in different tissues of eri silkworm reared on castor leaf supplemented with '*moringa*' over the absolute control

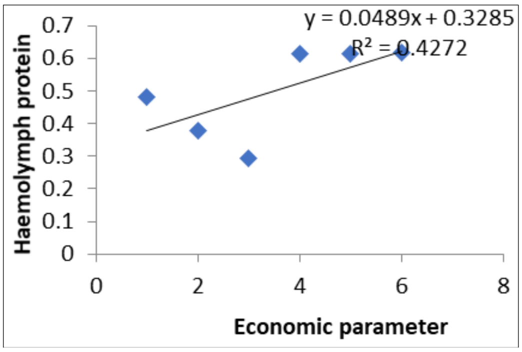

Fig 6 a: Matured larval weight

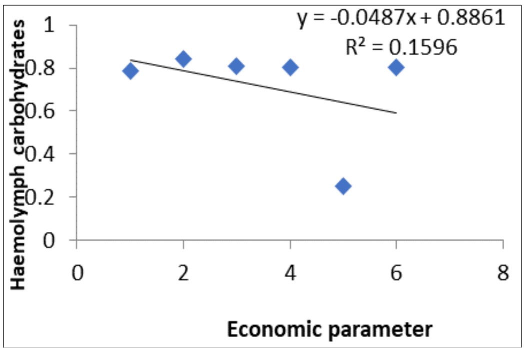

Fig 6 (b): Cocoon weight

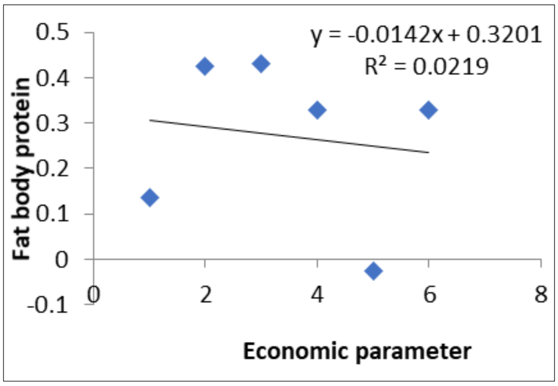

Fig 6 (c): pupal weight

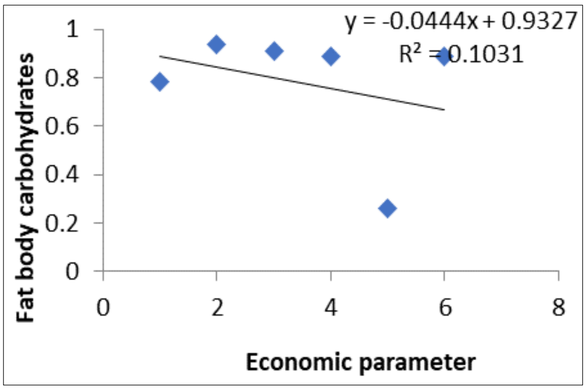

Fig 6 (d): Shell weight

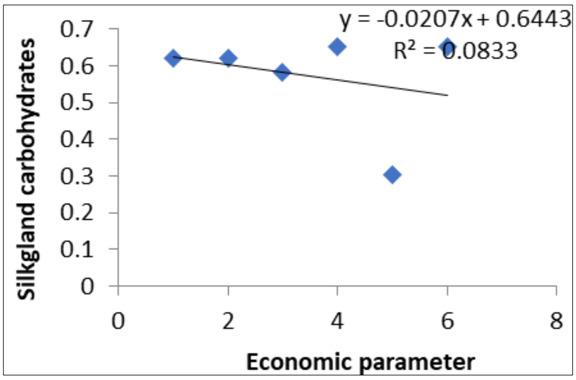

Fig 6 (e): Shell ratio

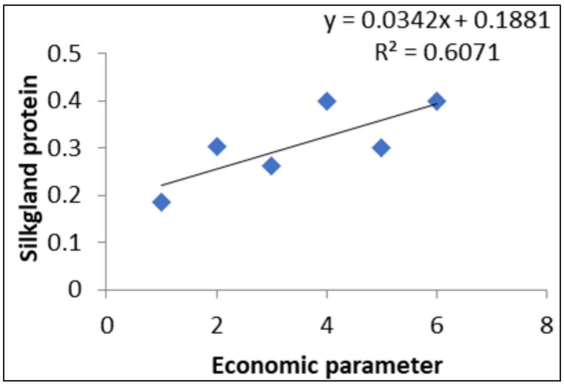

Fig 6 (f): Silk productivity

Fig 6: Correlation co-efficients between biochemical composition and economic parameters of eri silkworm

Correlation Analysis

A correlation analysis between key economic parameters of eri silkworms (*Samia ricini*), including mature larval weight, cocoon weight, pupal weight, shell weight, shell ratio, silk productivity, and biochemical traits (protein and carbohydrate content in haemolymph, fat body, and silk gland), are summarised here. The findings are graphically represented in Figure 6(a-f).

The amount of carbohydrates in haemolymph and fat body is positively correlated with most economic characteristics, according to typical statistical and graphical data. However, there is a negative correlation between the haemolymph and shell ratio of carbohydrates and the weight of the larvae (r=0.788*), cocoons (r=0.840**), pupae (r=0.811**), shells, and silk productivity (r=0.802**). In contrast, the weight of the larvae (r=0.785*), cocoon (r=0.939**), pupae (r=0.908**), shell, and silk productivity (r=0.886**) are strongly positively correlated with the carbohydrates in the fat body, while the shell ratio is negatively correlated with them.

However, the study found no significant correlation between any of the economic indicators and the amount of carbohydrates in the silk gland or the protein levels in all tissues (fat body, silk gland, and haemolymph). These observations indicate that proteins are needed to make structures, but the levels of proteins may not directly influence the commercial aspects. While carbohydrates in the fat body and haemolymph are the critical determinant force of larval growth and cocoon output. Therefore, economic aspects of eri silkworms can be improve with supplementation with *moringa*, as it improved carbohydrate levels in the fat body and haemolymph.

Summary

According to the study, the biochemical and commercial traits of eri silkworms (Samia ricini) are significantly enhanced when *Moringa oleifera* leaf powder is added to castor leaves, especially at a 1.0% concentration. The higher protein and carbohydrate content of the enriched diet in the haemolymph, fat body, and silk glands supported improved larval growth, cocoon development, and silk productivity. while T_3 (0.6%) showed the highest shell ratio, T_5 (1.0%) yielded the best results across most criteria. Strong positive correlations between the quantity of carbohydrates (especially in the fat body and hemolymph) and important economic characteristics were found by correlation analysis, suggesting that carbohydrates are necessary for the production of silk and energy storage.

These findings suggest that using *moringa* to biofortify castor leaves is a simple, economical, and environmentally friendly method of increasing eri silkworm productivity and cocoon yield. It is also compatible with eco-friendly and organic sericulture techniques.

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