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Role of green tea on larval development and cocoon parameters of mulberry silkworm, *Bombyx mori* (L.)

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Abstract
Bombyx mori (L) is a fragile, sensitive, monophagous lepidopteron insect that produces silk. The current study used FC1×FC2 (bivoltine double hybrid) to conduct the experiment. Green tea was introduced to the V1 mulberry leaves of the silkworm, *B. mori,* at concentrations of 1%, 2%, and 3% to see how it affected larval development and cocoon characteristics. Fresh mulberry leaves were sprayed with each concentration of green tea and fed to silkworms ranging from IV to V instar, twice each day. The current investigation was conducted at Zoology Research Lab, University College of Science, Tumkur University. Among them oral supplementation of 3% green tea resulted in significant increase in larval parameters such as larval weight, larval duration, growth index and cocoon parameters such as cocoon weight, pupal weight, shell weight, shell ratio and silk productivity as compared to absolute control.

Keywords: Bivoltine*, bombyx mori*, economical parameters, green tea, mulberry

Introduction
Mulberry silkworm is the most domesticated of all silk-producing insects (Divya Naik *et al.,* 2017) [5]. Silkworm a crucial economic insect, is used to convert mulberry leaf protein into silk (Simi Simon *et al.,* 2024a) [18]. Sericulture is a labour-intensive, land-based industry in India that employs many people and delivers significant financial advantages to rural communities (Ravikiran *et al.* 2024a) [20]. The FC1×FC2 double hybrid bivoltine outperforms single hybrid bivoltine in terms of crop balancing and weather resistance (Simi Simon *et al.,* 2024b) [18]. The mulberry silkworm (*B. mori*) is a lepidopteran insect that only devours mulberry leaves (Ravikiran *et al.,*2024b) [14]. Mulberry leaf quality and nutritional content have a substantial impact on silkworm larvae growth, development, and future silk output (Krishnaswami *et al.*, 1973) [10]. Compared to oolong or black tea, green tea has more catechins and is a "non-fermented" tea. Green tea contains minerals including manganese, aluminum, and potassium, as well as vitamins like ascorbic acid and chlorogenic acid (Graham, 1984) [6]. Proteins (15–20% dry weight), minerals and trace elements (5% dry weight), carbohydrates (5-7% dry weight), trace amounts of vitamins B, C, and E, xanthic bases and lipids (linoleic and linolenic acid), pigments (carotenoids, chlorophyll), and volatile substances (alcohols, lactones, aldehydes, esters, and hydrocarbons) are also present in green tea. Depending on the age and size of tea leaves, different minerals such as calcium, selenium, magnesium, fluoride, and zinc are present in varying proportions (Cabrera, Artacho & Giménez, 2006) [3]. According to reports, a number of green tea leaf chemicals, including polyphenols, have anticancer (Shimizu *et al.,* 2008) [17], antioxidant, and anti-diabetic qualities (Cabrera *et al.,* 2006) [3]. Green tea has anti-oxidative, anti-inflammatory, anti-arthritic, anti-stress, hypolipidemic, hypocholesterolemic, skin/collagen protective, hepatoprotective, anti-diabetic, anti-microbial, anti-infective, anti-parasitic, anti-cancerous, inhibition of tumorigenesis and angiogenesis, anti-mutagenic, and bone health and memory (Alagawany *et al.,* 2020) [1]. The procedure involved fortifying green tea solutions at varying concentrations (1%, 2%, and 3%) on V1 mulberry leaves and feeding them to FC1×FC2 larvae during the fourth and fifth instars, which are optimal for high metabolism and silk production. Aside from providing essential nutrients, the combination meal improves the silkworm's biochemical profiles, particularly the protein and carbohydrate levels in its fat body, silk glands, and haemolymph. Because they directly affect the profitability and efficiency of sericulture activities, commercial standards are important in the production of silkworms (Simi Simon *et al.,* 2024c) [14]. This study proposes using *green tea* leaf powder to supplement V1 mulberry leaves, potentially improving the biochemical composition and commercial qualities of FC1×FC2 silkworms. The objective is to create an organic, sustainable technique that will aid small-scale farmers and sustainable sericulture by taking into account cocoon development, larval health, and silk yield.

Materials and Methods
Silkworm Rearing
Prior to conducting the research, the lab was sterilized using limestone powder. The study was carried out at Zoology Research Lab, University College of Science, Tumkur University, Tumakuru. DFL silkworms (II instar larvae) of the FC1×FC2 breed were procured from the Chawki rearing centre in Honnudike, Tumakuru, and raised in a lab with a temperature 25±3℃, 72±2% relative humidity. The larvae were randomly divided up into three groups, each comprised of twenty larvae. The larvae were reared under standard rearing conditions (Krishnaswami., 1973) [10].

Supplementation of Green tea: Commercially available Green tea was made into different concentrations (1%, 2%, and 3%), and treated with V1 mulberry leaves and fed to silkworms twice a day from fourth instar first day till they reached spinning stage.

Economic parameter: The commercial characteristics of the *Bombyx mori* species FC1×FC2 were determined based on the subsequent parameters: Larval parameters [Larval weight, Larval duration, and Growth Index], Cocoon

Parameters [Cocoon weight, Shell weight, Pupa weight, Shell ratio and Silk productivity].

Larval parameters

1. **Larval weight:** Randomly picked 5 larvae from IV and V instar of FC1, FC2 and FC1×FC2 silkworms were measured using an electronic balance.
2. **Larval duration:** No. of days in IV and V instar larvae
3. **Growth Index:**

$$\frac{\text{final weight of the larvae (g)} - \text{Initial weight of the larvae (g)}}{\text{Initial weight of the larvae (g)}}$$ (Rateb S.H, 2015)

Cocoon Parameters

1. **Cocoon weight (g):** After 6 days of spinning randomly picked 5 cocoons from FC1, FC2 and FC1×FC2 breeds were measured using an electronic balance.
2. **Shell weight (g):** Cocoon weight- Pupa weight
3. **Pupa weight (g):** Cocoon weight – Shell weight
4. **Shell ratio (%):** The shell ratio will be calculated by using the formula, (Harendra Kumar *et al*., 2013) [7]

Shell ratio % =

$$\left\{\frac{\text{Cocoons shell weight (g)}}{\text{Cocoon weight (g)}}\right\} 100$$

5. **Silk productivity(cg/day): -** Silk productivity will be computed using the formula SP (cg/day) =

$$\frac{\text{Shell weight (centigram)}}{5^{th} \text{ instar larval duration (day)}}$$

Biostatical Analysis: A commercially accessible statistical program (Graph Pad Prism 8 for Windows) was used to evaluate the provided data using two-way analysis of variance (ANOVA) and Dunnett's Multiple Comparisons test. The findings were displayed as Mean±SD. Statistical significance was defined as P values < 0.001.

Table 1: Effect of different concentrations of *Green tea* on Larval development in FC1×FC2 race of *Bombyx mori*. L. The values are expressed in grams for larval weight and Growth index and days for larval duration during IV instar and V instar

Characteristics	Larval weight (g)		Larval Duration (days)		Growth Index (g)
	IV Instar	V Instar	IV Instar	V Instar	
Control	6.6±0.11	13.40±0.2	6.1±0.2	8.1±0.5	1.03±12
1% *Green tea*	7.47±0.01	15.85±0.32	5.3±0.5	7.2±0.7	1.12±0.21
2% *Green tea*	8.18±0.23	16.29±0.11	4.8±0.11	6.5±0.02	0.99±0.15
3% *Green tea*	8.49±0.12	16.58±0.52	4.4±0.32	6.1±0.02	0.95±0.22

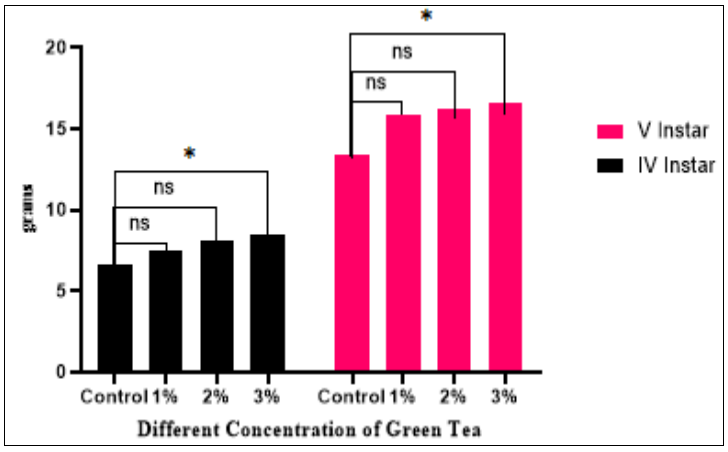
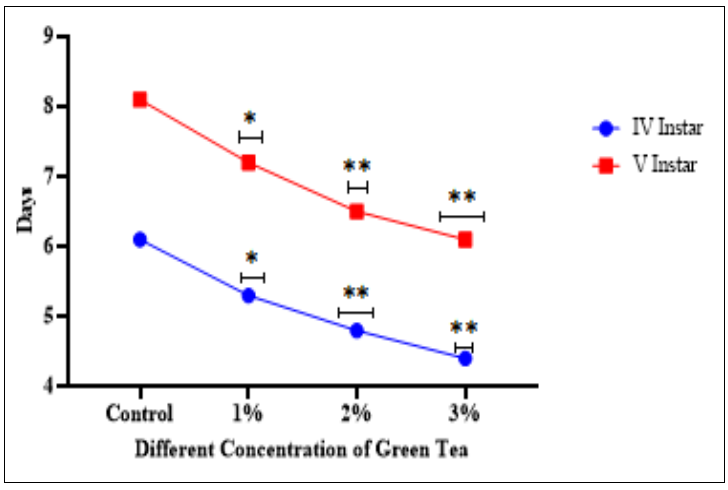


Fig 1(a):



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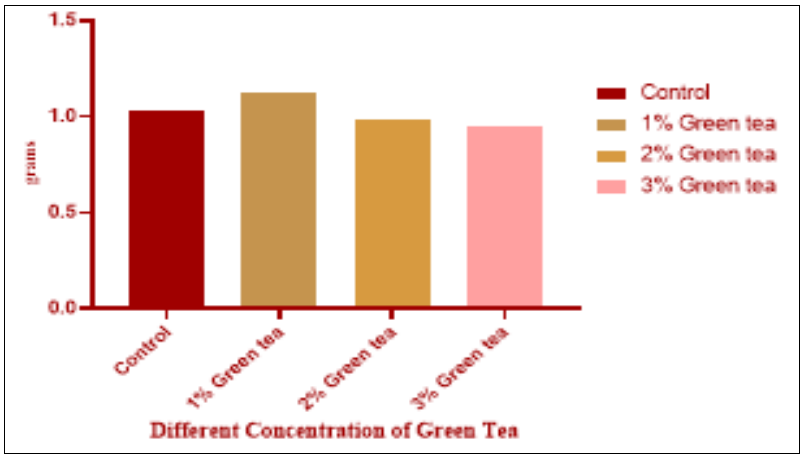

Fig 1(c):

Fig 1(a), (b), (c): Larval weight, Larval duration and Growth index in FC1×FC2 race of *Bombyx mori*. L fed V1 mulberry leaves treatment of *Green tea* during IV instar and V instar

Table 2: Effect of different concentrations of *Green tea* on cocoon parameters in FC1×FC2 race of *Bombyx mori*. L. The values are expressed in grams for cocoon weight, shell weight and pupa weight and percentage for ERR, shell ratio and Cg/day for Silk productivity

Characteristics	Control	1% *Green tea*	2% *Green tea*	3% *Green tea*
Cocoon weight (g)	5.2 ±0.01	6.01±0.12	6.52±0.22	7.28±0.02
Shell weight (g)	0.5±0.03	0.7±0.01	1.04±0.02	1.22±0.2
Pupa weight (g)	4.7±0.01	5.3±0.41	5.48±0.11	6.06±0.34
Shell ratio (%)	9.61±0.2	11.64±0.24	15.95±0.17	16.75±0.05
Silk productivity (Cg/day)	0.06±0.02	0.09±0.02	0.16±0.01	0.2±0.03

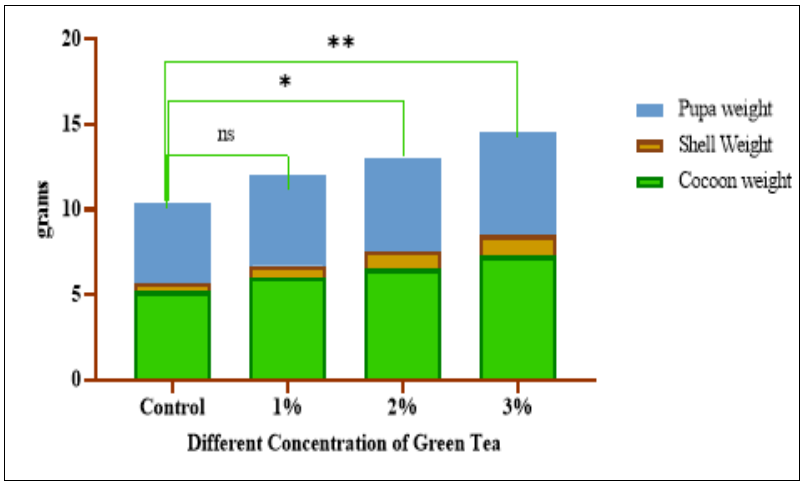

Fig 2(a):

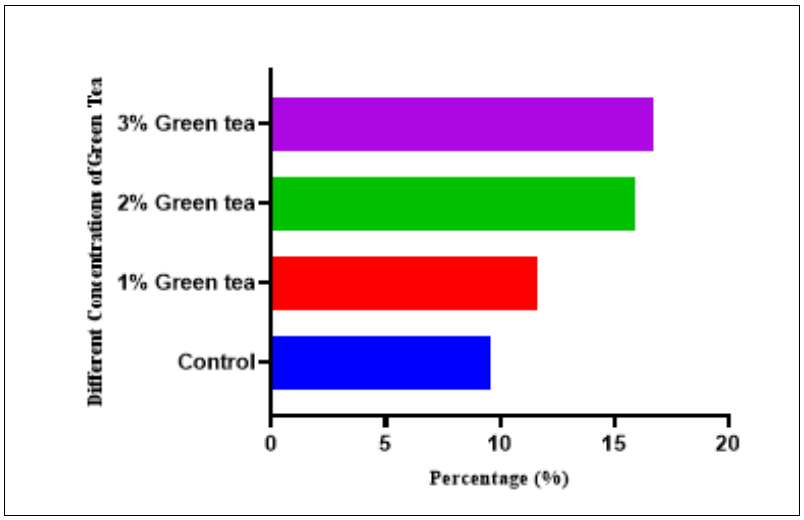

Fig 2(b):

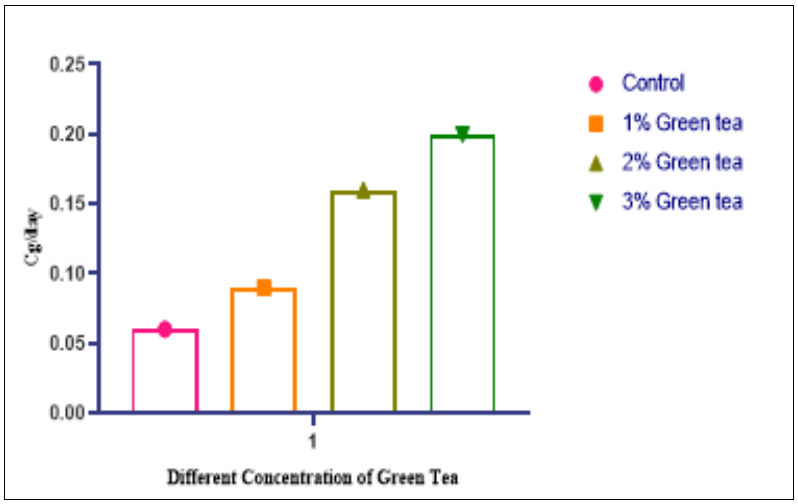

Fig 2(c):

Fig 2(a), (b), (c): Cocoon weight, Pupa weight, Shell weight, Shell ratio and Silk productivity in FC1×FC2 race of *Bombyx mori*. L fed V1 mulberry leaves treatment of *Green tea*

Results

The economic parameter was observed in FC1×FC2 breed of silkworm under study which includes larval weight, larval duration, growth index, cocoon weight, pupa weight, shell weight, shell ratio and silk production ratio. The economic parameter of FC1×FC2 breed of silkworm from 4th and 5th instar is given in Table 1, & 2 and figure 1 &2.

Larval parameter: The table 1 and figure 1(a) summarizes larval weight gained by silkworms treated with 3% *green tea* shows high value in 4th and 5th instar larvae and least in control. Figure 1(b) shows the larval duration of FC1×FC2 breed of silkworm feeding on mulberry leaves supplemented with *green tea*. Larval duration shows reduced in both 4th and 5th instar larvae treated with 3% *green tea* and the high in control. The Growth index gained by silkworms treated with 3% *green tea* shows low value in and high in control.

Cocoon parameter: Data presented in table 2 and figure 2(a) indicates the cocoon weight shows highest in silkworms treated with 3% *green tea* and least in control. Pupa weight is highest in silkworms treated with 3% *green tea* least in control. Shell weight is highest in silkworms treated with 3% *green tea* least in control. The figure 2(b) summarizes the shell ratio shows maximum in 3% *green tea* treated and least in control and figure 2(c) indicates Silk production maximum in 3% *green tea* treated and least in control.

Discussion

The study found that incorporating *green tea* to V1 mulberry leaves for silkworm *Bombyx mori* FC1×FC2 had a significant impact on economic parameters such as larval development (larval duration, weight, and growth index) and cocoon parameters (cocoon weight, shell weight, pupa weight, shell ratio, and silk productivity). This finding demonstrates that mulberry leaves supplemented with *green tea* produce a better outcome in every stage of silkworm development. The nutritional value of V1 mulberry leaves and supplements had a significant impact on larval parameters. Mulberry leaves supplemented with 3% *green tea* demonstrate increased larval weight than the control, which could be owing to *green tea's* high protein, amino acid, and mineral content (Cabrera, Artacho & Giménez, 2006) [3]. The highest larval weight results in better silk production (Venugopal Reddy Bovilla, 2015) [21]. When mulberry leaves were supplemented with *green tea*, larval duration was significantly shorter, implying that the worms' nutritive requirements were met in a shorter period of time due to feed supplementation, as compared to unsupplemented mulberry leaves (Roy Choudhary *et al.,* 1994). The reduced larval duration in treated silkworms may be attributed to the antioxidant properties of phenolic compounds like chlorogenic acid found in green tea. This boosts tissue strength and improves larval health, allowing for a shorter duration of feeding (Divya Naik *et al.,* 2017) [5]. The ideal concentration of feed additive may have a stimulating impact on silkworms, leading to increased food consumption. Feed supplements contain carbohydrates, vitamins, minerals, and phenolic substances that can significantly impact larval growth at specific concentrations. Cocoon weight, shell weight, and pupa weight all indicate enhanced nutrition digestion and overall silkworm health (Anusha *et al.,* 2025) [2]. The shell ratio indicates the ideal cocoon to shell mass ratio at a moderate enrichment level; silk production is determined by the rearing conditions, quality, nutritive elements, and quantity of mulberry leaves supplied during rearing; the higher the weight, the approximate quantity of raw silk output (Simi Simon *et al.,* 2024c) [14]. These improvements in cocoon parameters might be due to nutritional composition of green tea extract especially chlorogenic acid. In a comparable context, Patil and Chandrashekhar (2013) [13] found that silkworms fed 5% roasted coffee bean extract grew consistently and well. Jeypaul *et al*. (2003), who investigated the effect of *Coffea arabica* leaf extract, Weight of the cocoon, shell, pupal, and shell the 1:25 concentration ratio was shown to be effective. The current study supports earlier studies on the use of *green tea* in sericulture, demonstrating that feeding silkworms' *green tea* improved silkworm growth and cocoon qualities.

Conclusion

Green tea supplementation during the V instar stage of the bivoltine double hybrid FC1×FC2 is a practical and bio-rational method to current sericulture. It turns a naturally

strong silkworm breed into a commercial high-performer. It exhibits good yield in economic indicators such as larval weight, duration, growth index, cocoon weight, pupa weight, shell weight, silk production, and shell ratio. FC1×FC2, a bivoltine double hybrid breed, excels in seed crop output, recovery, and stability. The current study examines the effect of *Green tea* supplementation on silkworm economic characteristics.

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Declaration of competing interest: The author's states that they have no known challenging financial interests that could have seemed to impact the work reported in this paper.

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