ISSN Print: 2664-6064
ISSN Online: 2664-6072
NAAS Rating (2026): 4.69
IJAN 2026; 8(8): 119-128
www.agriculturejournal.net
Received: 15-05-2026
Accepted: 20-06-2026

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Synergistic effect of vancomycin and honey supplemented with mulberry leaves on economic traits of the silkworm hybrid fc2 × fc

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DOI: https://www.doi.org/10.33545/26646064.2026.v8.i8b.742

Abstract
The present investigation was undertaken to evaluate the synergistic efect of vancomycin and honey supplemented through mulberry leaves on the economic traits of the silkworm hybrid $FC_2 \times FC_1$. The experiment was conducted at the Department of Studies in Sericulture Science, University of Mysore. The study comprised four treatment groups: T_0 (control with untreated mulberry leaves), T_1 (1% vancomycin), T_2 (1% honey with 1% vancomycin), and T_3 (2% honey with 1% vancomycin). Treatments were administered through foliar application on mulberry leaves during the fifth instar. Economic parameters including cocoon weight, shell weight, shell ratio, pupal weight, filament length, filament weight, denier, and rendita were evaluated. The results revealed that all three treatment groups showed notable improvements in cocoon weight compared to control, with T_2 (1% honey + 1% vancomycin) recording the highest cocoon weight of 2.68±0.067 g, representing a 28.35% increase over control. Shell weight remained consistent across all treatments (0.50 g). Filament length was highest in T_2 (1425±11.783 m), representing a 10.12% improvement over control. Denier values improved across all treatments, with T_2 and T_3 both recording 7.69% improvement. The synergistic combination of vancomycin and honey at optimal concentrations enhanced the overall economic performance of the $FC_2 \times FC_1$ hybrid, with T_2 (1% honey + 1% vancomycin) showing the best overall performance. These findings suggest that antibiotic-honey supplementation through mulberry leaves can serve as an efective strategy for improving cocoon productivity in commercial sericulture.

Keywords: *Bombyx mori*, vancomycin, honey, mulberry supplementation, economic traits, fc2 × fc1, sericulture

1. Introduction
The silkworm, *Bombyx mori* L., has been domesticated for silk production for over 4,000 years and remains an insect of paramount economic importance to Asian countries such as China, India, Thailand, and numerous other developing nations. Silk, often referred to as the "queen of textile fibre," is valued worldwide for its smoothness, lustre, and unique properties as a natural fibre. Sericulture, the science of rearing silkworms for commercial production of raw silk, encompasses all the operations required for the production of silk fibre (Krishnaswami *et al.*, 1973) [1]. It is estimated that more than 3,000 silkworm strains are available globally owing to various ongoing breeding programmes (Thangavelu *et al.*, 2003) [2]. The maintenance of pure silkworm genetic resources has become critically important for meeting the desired objectives of breeders for immediate and long-term utilization in silkworm seed production and race improvement (Yamaguchi, 2003) [3]. The economic productivity of sericulture is fundamentally governed by the health, growth, and development of silkworm larvae, which in turn are influenced by the intricate interplay of nutritional status, environmental conditions, and disease management. The silkworm is highly susceptible to infections caused by major pathogenic groups — viral (grasserie), bacterial (flacherie), fungal (muscardine), and protozoan (pebrine) —

which collectively account for estimated annual crop losses of 30-40% in India (Priyadharshini *et al.*, 2008) [4]. Therefore, improvement of silk quality and productivity necessarily involves both the enrichment of feed nutrition and the maintenance of larval health through efective disease management strategies. Antibiotics are bioactive molecules that kill or inhibit the growth of microorganisms, including both bacteria and fungi. In the context of sericulture, antibiotics serve four approved purposes: disease treatment, disease prevention, disease control, and health maintenance or growth promotion (Phillips *et al.*, 2004) [5]. Diferent antibiotics have been extensively used as growth-stimulating factors to enrich the nutrition of silkworms and enhance their productivity. Rahmathulla *et al.* (2003) [6] observed that antibiotic administration at diferent concentrations significantly improved economic parameters including larval weight, cocoon weight, shell weight, shell ratio, filament length, denier, and reelability. The improved performance was atributed to enhanced feed consumption and growth stimulation through metabolic processes within the silkworm, coupled with reduced disease incidence.

When antibiotics are administered to silkworms, a shift in nitrogen metabolism occurs in favour of increasing body weight and silk output (Murthy and Sreenivasaya, 1953) [7]. Studies on auromycin and chloromycetin demonstrated that their addition to the mulberry diet resulted in heavier caterpillars with increased nitrogen metabolism (Verma and Atwal, 1963) [8]. Shyamala and Bhat (1962) [9] reported that chloromycetin supplementation enhanced the oxygen uptake of the gut of the silkworm, suggesting that these antibiotics exerted a beneficial influence by controlling the intestinal flora. Ahmad *et al.* (2001) [10] found that food assimilated, assimilation rate, assimilation eficiency, and conversion eficiencies were significantly higher in antibiotic-treated batches. Furthermore, antibiotic feed supplementation demonstrated both prophylactic measures to prevent bacterial infections and enhanced nutrition and economic parameters in *B. mori* (Sheebha *et al.*, 2008) [11].

Vancomycin is a glycopeptide antibiotic used to treat a range of bacterial infections, particularly those caused by Gram-positive organisms. It was first introduced in 1954 and is listed on the WHO′ s essential medicines list as one of the most efective and safe medicines. Hamamoto *et al.* (2004) [12] demonstrated that when vancomycin (200 µg) was injected into silkworm larvae after bacterial inoculation, at least 90% of the larvae survived for 4 days, confirming its therapeutic eficacy in the silkworm model system.

Honey is a natural sweetener and multifactorial nutrient produced by honeybees from floral nectar (Council of European Union, 2002) [13]. It is a rich nutrient source containing a variety of sugars, proteins, enzymes, vitamins, minerals, and bioactive compounds, with its chemical composition varying depending on geographical, environmental, and botanical origin (Ramirez, 2000; Falco *et al.*, 2003; David Ball, 2007) [14, 15, 16]. Beyond its nutritional value, honey possesses welldocumented antimicrobial, antiviral, anti-inflammatory, antioxidant, and antimutagenic properties (Taormina, 2001; Cooper *et al.*, 2002; Eileen De Mars, 2003) [17, 18, 19]. Honey contains hydrogen peroxide, which accounts for a significant portion of its antibacterial properties. The potential of honey as a dietary supplement in sericulture has been explored in several studies, with Thulasi and Sivaprasad (2014) [20] demonstrating that 2% honey-enriched mulberry diet significantly improved larval growth and economic parameters.

While the individual efects of antibiotics and honey on silkworm productivity have been investigated by various researchers, their synergistic efect when administered in combination through mulberry leaves remains relatively unexplored. The present investigation was therefore designed to evaluate the combined efect of vancomycin and honey supplemented through mulberry leaves on the economic traits of the commercially important silkworm hybrid $FC_2 \times FC_1$

2. Materials and Methods

2.1 Experimental Design and Silkworm Rearing

The present experimental analysis was carried out at the Department of Studies in Sericulture Science, University of Mysore, Manasagangothri, Mysuru. The commercially popular silkworm hybrid $FC_2 \times FC_1$ was selected for the investigation. Five disease-free layings of the hybrid were obtained from the NSSO, Mysuru. The silkworm eggs were incubated at 25±1°mathsfC temperature and 80-85% relative humidity for approximately 10-12 days until hatching. Plastic trays of 90×70×10 cm dimensions were washed with water, dried, and disinfected with 2% formalin solution and covered with parafin paper of corresponding size. The layings were blackboxed at the blue egg stage to obtain uniform hatching, and the hatched larvae on the 10th day were brushed into labelled plastic trays.

The silkworm larvae were reared under standard conditions following the methodology of Rajan and Himantharaj (2005), with two feeding schedules. Chawki worms (early instars) were fed with S_{36} variety of mulberry leaves, while later instars received V_1 variety of mulberry obtained from the garden of the Sericulture Department. Selected leaves were chopped to suitable sizes according to the larval stage. Silkworm beds were cleaned after the second moult and daily thereafter. The entire batches of silkworm were reared on normal untreated mulberry leaves until the completion of the fourth instar. Treatments were initiated from the fifth instar onwards, with treated leaves administered only during the morning feeding.

2.2 Treatment Groups

The experimental layout consisted of four treatment groups designated as follows: T_0 (Control) — untreated mulberry leaves; T_1-1% vancomycin with mulberry leaf; T_2-1% honey with 1% vancomycin with mulberry leaf; T_3-2% honey with 1% vancomycin with mulberry leaf.

2.3 Preparation of Treatment Solutions

For the preparation of vancomycin stock solution, 500 mg of vancomycin was weighed and diluted with 20 ml of distilled water. The individual treatment solutions were prepared as follows: Treatment 1 (T_1) consisted of 1 ml of vancomycin stock dissolved in 99 ml of distilled water to yield 1% vancomycin solution. Treatment 2 (T_2) comprised 1 ml of vancomycin and 1 ml of honey dissolved in 98 ml of distilled water to yield 1% vancomycin with 1% honey. Treatment 3 (T_3) comprised 1 ml of vancomycin and 2 ml of honey dissolved in 97 ml of distilled water to yield 1% vancomycin with 2% honey. The mulberry leaves were soaked in the respective treatment solutions and dried under cool weather conditions before feeding.

Treatment was administered for 6 consecutive days during the fifth instar until the larvae entered the ripening stage. The ripened silkworms were then mounted on bamboo mountages for spinning. On the 7th day of mounting, the hard and compact cocoons were harvested.

2.4 Assessment of Economic Parameters
The harvested cocoons were subjected to evaluation of seven economic characters: cocoon weight (average weight of 10 randomly selected cocoons in grams), shell weight (weight of cocoon shell after removal of pupa, in grams), shell ratio (shell weight/cocoon weight ×100 9 expressed as percentage), filament length (total length of silk filament reeled from a single cocoon using an epprouvete, calculated as L=R×1.125, where R is the number of revolutions and 1.125 is the circumference of the epprouvete in meters), filament weight (total weight of silk filament from a single cocoon in grams), denier (filament weight/filament length × 9000, representing the thickness of the silk filament), and rendita (cocoon weight/raw silk weight, representing the quantity of cocoons required to produce one kilogram of raw silk).

2.5 Statistical Analysis
The experimental data were subjected to statistical analysis for test of significance. Percent change over control was calculated for each parameter across all treatment groups to assess the relative eficacy of each treatment.

3. Results
The economic parameters of the silkworm hybrid $FC_2 \times FC_1$ supplemented with vancomycin and honey through mulberry leaves during the fifth instar are presented in Table 1. The results demonstrate that the synergistic application of vancomycin and honey exerted a discernible influence on several cocoon quality parameters.

3.1 Cocoon Weight
The cocoon weight exhibited substantial variation across the treatment groups. The control batch (T_0) recorded a mean cocoon weight of 1.92±0.061 g. Among the treated groups, T_2 (1% honey + 1% vancomycin) registered the highest cocoon weight of 2.68±0.067 g, representing a notable increase of 28.35% over control. T_3 (2% honey + 1% vancomycin) recorded 2.40±0.046 g, representing a 20.00% increase. T_1 (1% vancomycin alone) showed a modest increase to 1.97±0.181 g (2.53% increase). These results clearly indicate that the combination of honey with vancomycin produces a synergistic enhancement in cocoon weight, with the 1% honey concentration proving most efective.

3.2 Shell Weight and Shell Ratio
Shell weight remained remarkably consistent across all treatment groups at 0.50 g, indicating that neither vancomycin nor honey supplementation significantly altered shell weight deposition. However, the shell ratio exhibited a positive trend in the treated groups. While the control recorded 20.13%, T_1 showed 20.00% (0.65% change), T_2 recorded 19.82% (1.56% change), and T_3 showed 19.90% (1.15% change).

3.3 Pupal Weight
The pupal weight demonstrated marked increases across all treatments compared to control. The control batch recorded 1.25±0.087 g, while T_1 recorded 1.57±0.045 g (20.38% increase), T_2 recorded 1.67±0.015 g (25.14% increase), and T_3 recorded 1.65±0.028 g (24.24% increase). The highest pupal weight was observed in T_2, consistent with the trend observed for cocoon weight.

3.4 Filament Characters
The filament length showed consistent improvement across all treated groups. The control batch recorded 1299±26.033 m, while T_1 recorded 1395±20.817 m (6.88% increase), T_2 recorded the highest filament length of 1425±11.783 m (10.12% increase), and T_3 recorded 1354±28.199 m (4.06% increase). Filament weight also showed positive trends across treatments: control (1.47±0.046 g), T_1 (1.54±0.021 g, 4.54% increase), T_2 (1.58±0.019 g, 6.96% increase), and T_3 (1.49±0.018 g, 1.34% increase).

3.5 Denier and Rendita
The denier values showed an interesting trend with improvement across all treatments compared to control. The control recorded 2.38±0.140, while T_1 recorded 2.25±0.003 (5.77% change), and both T_2 and T_3 recorded 2.21 (7.69% change each). The rendita values were: control (6.55±0.052), T_1 (6.388±0.037, 2.66% change), T_2 (6.56±0.024, −0.15% change), and T_3 (6.86±0.019, −4.51% change).

Table 1: Performance of economic parameters of $FC_2 \times FC_1$ hybrid supplemented with vancomycin and honey

Treatment	Cocoon wt (g)	Shell wt (g)	Shell ratio (%)	Pupal wt (g)	Filament length (m)	Filament wt (g)	Denier (d)	Renditta (kg)
Control	1.92±0.061	0.50±0.012	20.13±0.012	1.25±0.087	1299±26.033	1.47±0.046	2.38±0.140	6.55±0.052
T1 (1% Vancomycin)	1.97±0.181	0.50±0.010	20.00±0.010	1.57±0.045	1395±20.817	1.54±0.021	2.25±0.003	6.388±0.037
% Change T1	(-2.53)	(0)	(0.65)	(-20.38)	(-6.88)	(-4.54)	(5.77)	(2.66)
T2 (1% Honey + 1% Vancomycin)	2.68±0.067	0.50±0.016	19.82±0.016	1.67±0.015	1425±11.783	1.58±0.019	2.21±0.000	6.56±0.024
% Change T2	(-28.35)	(0)	(1.56)	(-25.14)	(-10.12)	(-6.96)	(7.69)	(-0.15)
T3 (2% Honey + 1% Vancomycin)	2.40±0.046	0.50±0.015	19.90±0.015	1.65±0.028	1354±28.199	1.49±0.018	2.21±0.024	6.86±0.019
% Change T3	(-20.00)	(0)	(1.15)	(-24.24)	(-4.06)	(-1.34)	(7.69)	(-4.51)

4. Discussion
The impact of exogenous nutrients on the growth, metabolism, and development of *Bombyx mori* has been a subject of considerable research interest in sericulture (Laskar and Data, 2000; Kanafi *et al.*, 2007) [21, 22]. Such studies have largely focused on enriching the silkworm diet with exogenous nutrients including proteins, carbohydrates, amino acids, vitamins, minerals, hormones, and antibiotics, and assessing their influence on desired parameters of *B. mori* (Sanappa *et al.*, 2002; Bhatacharya and Kaliwal, 2004, 2005a; Chakrabarty and Kaliwal, 2011; Kavitha *et al.*, 2012; Thulasi and Sivaprasad, 2013, 2014) [23, 24, 25, 26, 27, 20, 28]. The present investigation extends this body of work by examining the synergistic efect of a glycopeptide antibiotic (vancomycin) combined with honey as a natural nutrient supplement.

The substantial increase in cocoon weight observed in the honey-vancomycin combination treatments (28.35% in T_2 and 20.00% in T_3) represents a significant finding with practical

implications for commercial sericulture. This enhancement can be atributed to the dual mechanism of action: vancomycin's role in controlling intestinal pathogenic bacteria and honey's contribution as a multifactorial nutrient that stimulates metabolic processes. The honey, being a rich source of sugars, proteins, enzymes, vitamins, and minerals (David Ball, 2007; Garcia *et al.*, 2005) [16, 29], provides the nutritional substrate necessary for enhanced growth, while vancomycin ensures optimal gut health by suppressing pathogenic microflora. This synergistic mechanism explains why the combination treatments consistently outperformed vancomycin alone (T_1) across most economic parameters.

The consistent improvement in filament length across all treatments, with the highest increase of 10.12% recorded in T_2, aligns with earlier findings by Rahmathulla *et al.* (2003) [6], who reported significant improvements in filament parameters following antibiotic administration.

The enhanced filament output can be atributed to improved nitrogen metabolism and protein synthesis in the silk gland under antibiotic treatment (Murthy and Sreenivasaya, 1953) [7]. Thulasi and Sivaprasad (2015) [30] demonstrated that under the impact of 2% honey, silk gland protein profiles increased significantly during fifth instar development, predominantly in the anterior and posterior silk gland regions. The honey stimulates protein synthesis in all three segments of the silk gland but particularly enhances fibroin synthesis, which directly translates to improved filament parameters.

The observation that shell weight remained constant at 0.50 g across all treatment groups suggests that the exogenous supplementation afects the pupal body development more profoundly than the shell deposition process. This finding is consistent with the marked increase in pupal weight (20-25% across treatments), indicating that the nutritional and antimicrobial benefits of the treatments are primarily channeled toward larval and pupal body growth rather than silk shell formation. The positive changes in denier values (5.77-7.69% improvement) across all treatments indicate that the treatments enhance the thickness and quality of the silk filament, which is a commercially desirable trait.

The role of antibiotics in enhancing silkworm productivity has been well documented. A selected group of antibiotics functions by destroying or inhibiting undesirable bacteria in the gut, thereby preventing suboptimal absorption of food and allowing the silkworm to derive maximum benefit from its diet (Phillips *et al.*, 2004; Baig *et al.*, 1990) [5, 31]. Fortification of mulberry leaves with antibiotics is recognized as one of the most efective methods to enrich the silkworm diet (Radha *et al.*, 1981) [32]. The oral administration of antibiotics along with mulberry leaves to healthy silkworms has been shown to boost growth, fecundity, and silk content (Tayade *et al.*, 1988) [33] while simultaneously reducing disease incidence (Rai and Devaiah, 1988) [34].

The honey component of the present treatment formulation contributes through multiple mechanisms. Firstly, honey acts as a potent modulator of growth and silk production in *Bombyx mori*, as demonstrated by Thulasi and Sivaprasad (2014) [20]. At optimal concentrations, honey not only stimulates silk protein synthesis in the silk gland but also mobilizes appropriate protein reserves from the fat body and fine-tunes silk output. Honey confers a two-fold advantage: it enhances silk productivity and quality while simultaneously reducing floss output, which is treated as sericultural wastage. The nutritional impact of honey is atributable to its constituent vitamins and minerals (Bhatacharya and Kaliwal, 2005a; Rajabi *et al.*, 2006a; Kavitha *et al.*, 2012) [28, 35, 26].

The finding that T_2 (1% honey + 1% vancomycin) outperformed T_3 (2% honey + 1% vancomycin) in most parameters is noteworthy. This suggests that the optimal concentration of honey in combination with vancomycin is 1%, beyond which the additional honey may not confer proportional benefits. This observation is consistent with the dose-response relationship reported by Alagumanikumaran and Prema (2016) [36], who found that the efective concentration of honey for silkworm supplementation varies depending on the formulation and route of administration.

The antimicrobial properties of both vancomycin and honey provide complementary protection against silkworm diseases. Vancomycin is particularly efective against Gram-positive bacteria, while honey's antibacterial action, mediated through hydrogen peroxide and other bioactive compounds, provides broader antimicrobial coverage. Hamamoto *et al.* (2004) [12] established that vancomycin has quantifiable therapeutic efects in silkworms infected with human pathogenic microorganisms. The combination of these two antimicrobial agents may thus provide superior disease protection compared to either agent alone, contributing to the improved economic parameters observed in the combination treatments.

The present findings are further supported by the body of literature on dietary supplementation in sericulture. Krishnaswami *et al.* (1973) [1] and Santha *et al.* (2007) [37] reported significant improvements in cocoon parameters when silkworms were reared on mulberry leaves enriched with diferent antibiotics. The growth and development of silkworms and their economic characters are influenced to a great extent by the nutritional content of mulberry leaves (Shivakumar, 1995) [38]. Antibiotics improve feed consumption and growth by stimulating metabolic processes within the silkworm as well as reducing the occurrence of diseases, which causes immense loss to the sericulture industry.

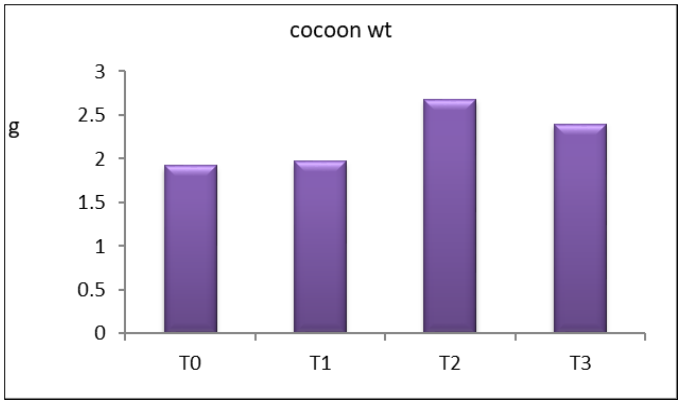

Fig 2: Effect of vancomycin and honey on cocoon weight of FC_2 X FC_1 hybrid

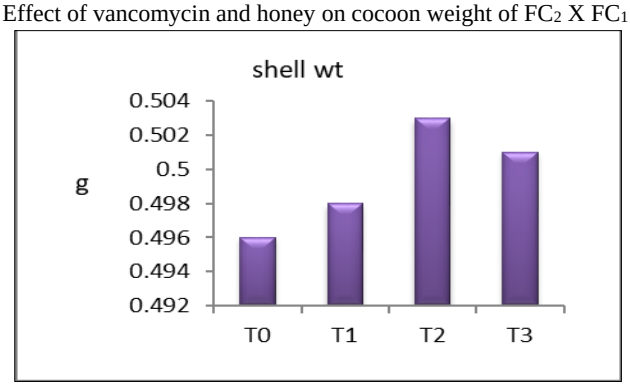

Fig 3: Effect of vancomycin and honey on pupal weight of FC_2 X FC_1 hybrid

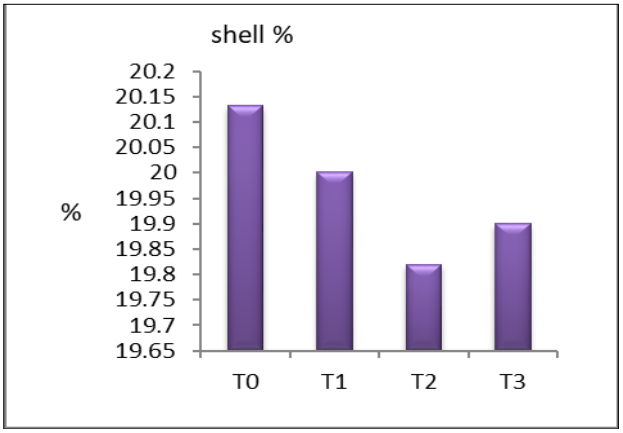

Fig 4: Effect of vancomycin and honey on shell weight of FC_2 X FC_1 hybrid

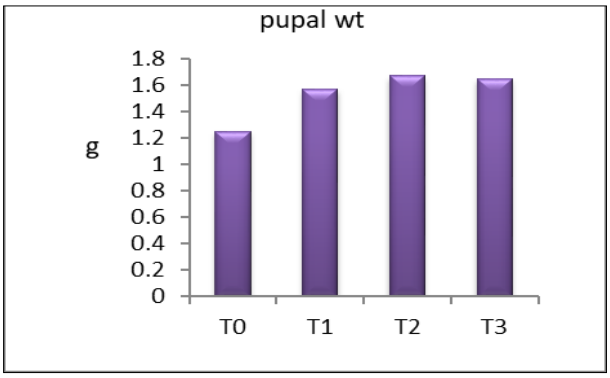

Fig 5: Effect of vancomycin and honey on shell percentage of FC_2 X FC_1 hybrid

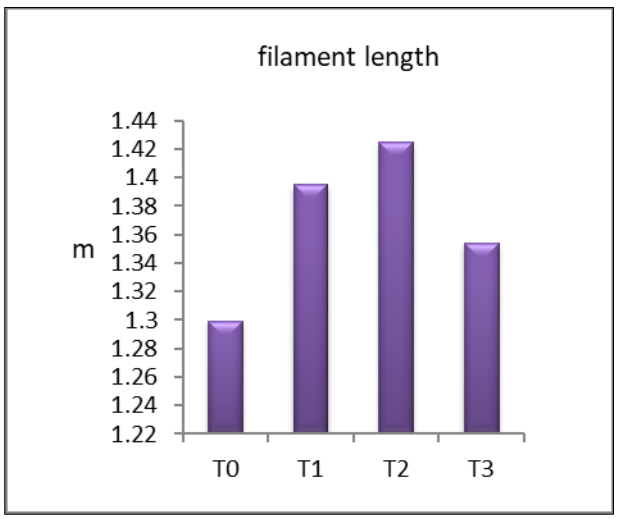

Fig 6: Effect of vancomycin and honey on filament length of FC_2 X FC_1 hybrid.

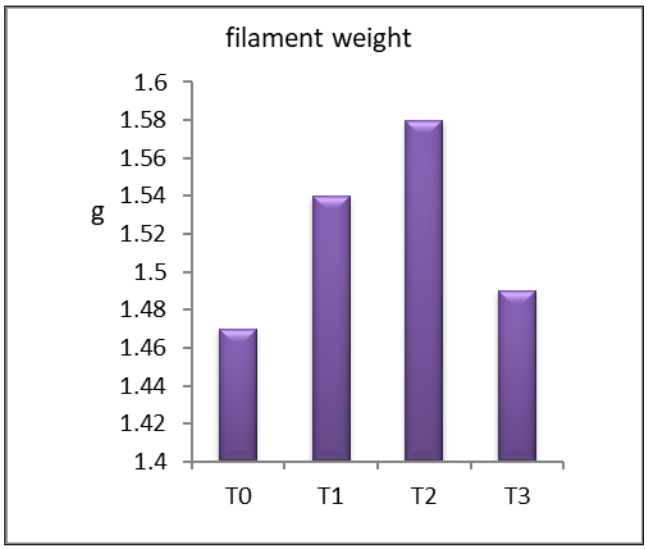

Fig 7: Effect of vancomycin and honey on filament weight of FC_2 X FC_1 hybrid.

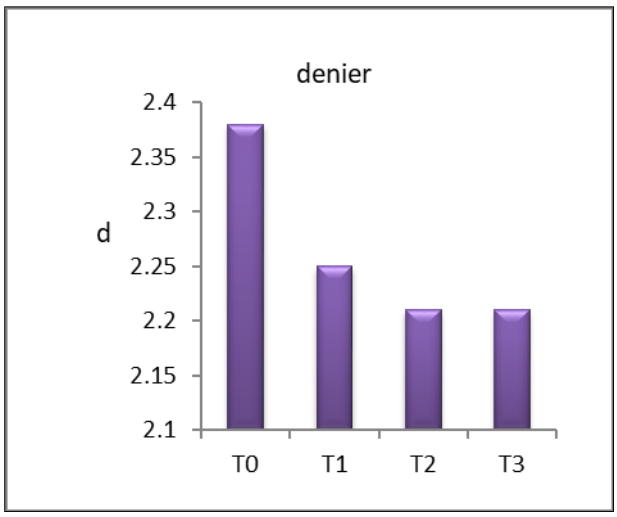

Fig 8: Effect of vancomycin and honey on denier of FC_2 X FC_1 hybrid.

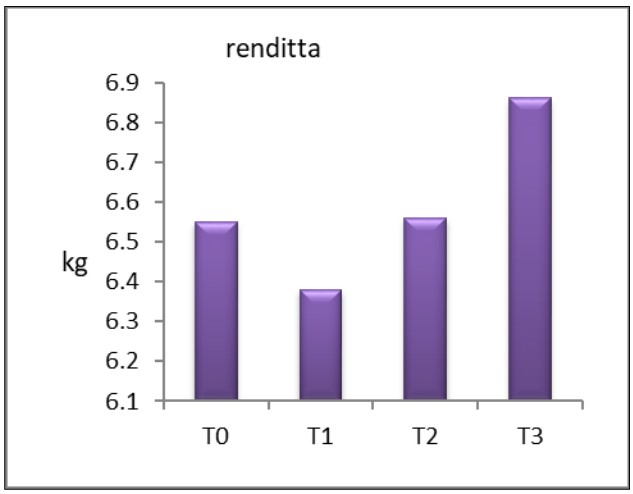

Fig 9: Effect of vancomycin and honey on renditta of FC_2 X FC_1 hybrid.

Plate 1: Cocoons treated with vancomycin and honey

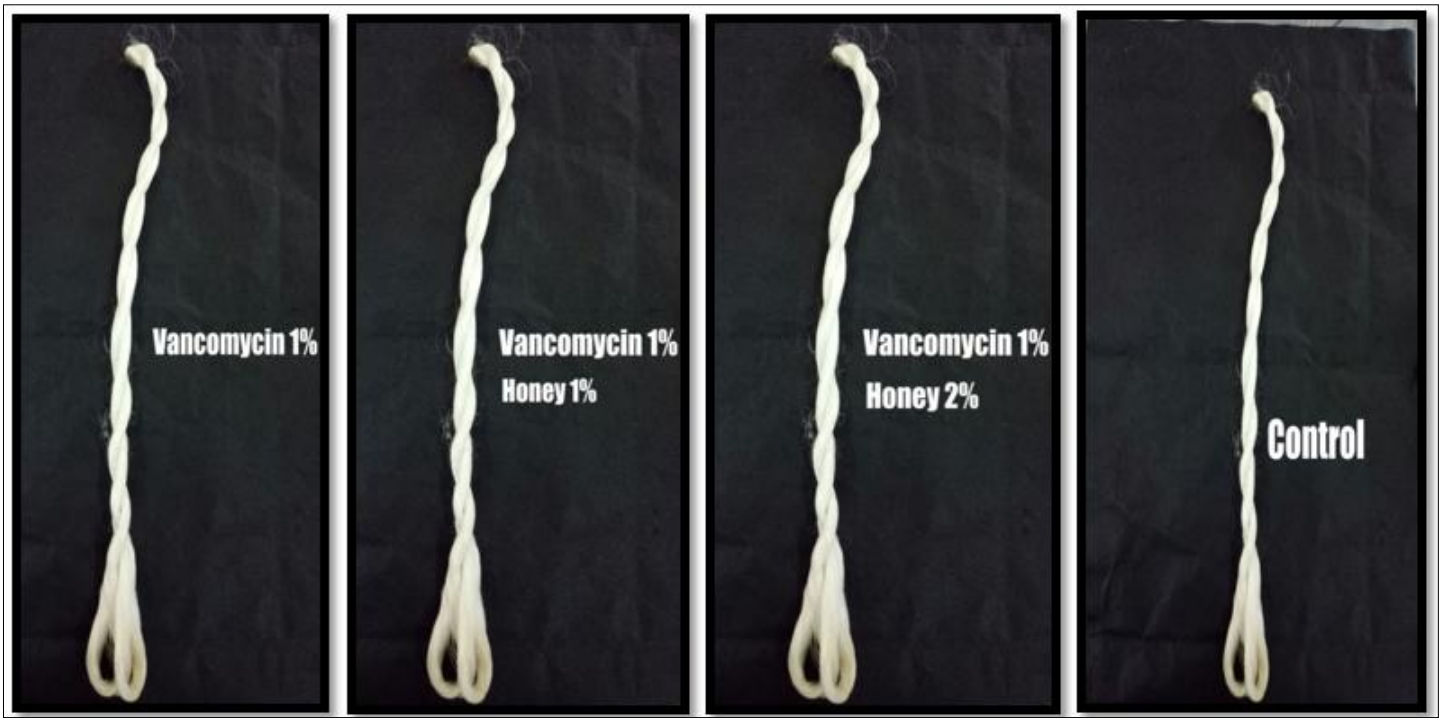

Plate 2: Silk filament reeled from treated cocoons

5. Conclusion

The present investigation has established that the synergistic supplementation of vancomycin and honey through mulberry leaves during the fifth instar exerts a positive influence on the economic traits of the silkworm hybrid $FC_2 \times FC_1$. Among the three treatment formulations tested, T_2 (1% honey combined with 1% vancomycin) demonstrated the best overall performance, recording the highest cocoon weight (2.68 g, 28.35% improvement), the highest filament length (1425 m, 10.12% improvement), the highest filament weight (1.58 g, 6.96% improvement), and the highest pupal weight (1.67 g, 25.14% improvement). Shell weight remained consistent at 0.50 g across all treatments. The denier values improved by 7.69% in both T_2 and T_3 treatments. The consistent improvement in cocoon weight, filament parameters, and pupal weight across all treatment groups confirms the beneficial efect of vancomycin-honey supplementation on silk production. The combination of an antibiotic that controls gut pathogens with a natural nutrient-rich supplement like honey represents a promising approach for enhancing sericulture productivity. However, extensive field trials under diferent agro-climatic conditions are recommended before adopting this supplementation strategy at the commercial level in sericulture operations.

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