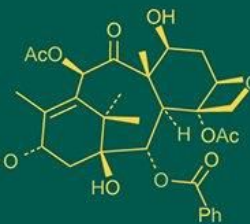
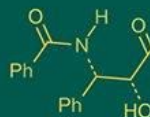


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 727-733
www.biochemjournal.com
Received: 20-11-2025
Accepted: 22-12-2025

Nitish Kumar LS
Ph. D. Scholar, Department of
Agricultural Entomology, CoA,
UAS, GKVK, Bangalore,
Karnataka, India

G Sugeetha
Associate Professor,
Department of Agricultural
Entomology, CoA, UAS, V. C.
Farm, Mandya, Karnataka,
India

J Mahadeva
Associate Professor,
Department of Forestry and
Environmental Science, CoA,
UAS, V. C. Farm, Mandya,
Karnataka, India

Corresponding Author:
Nitish Kumar LS
Ph. D. Scholar, Department of
Agricultural Entomology, CoA,
UAS, GKVK, Bangalore,
Karnataka, India

Comparative evaluation of biochemical constituents in mulberry varieties

Nitish Kumar LS, G Sugeetha and J Mahadeva

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1Si.7124>

Abstract

Mulberry (*Morus* spp.) leaves serve as the sole nutritional source for the mulberry silkworm, *Bombyx mori* L., and their biochemical composition plays a crucial role in silkworm growth, cocoon yield and silk quality. The present study evaluated variations in major primary and secondary biochemical constituents of mulberry leaves across different varieties and leaf maturity stages. Eight mulberry varieties viz., Mysore Local, Sahana, S36, DD, AR12, V1, G2 and G4 were cultivated under uniform agronomic conditions at the College of Agriculture, Mandya, Karnataka. Leaf samples were collected 45 days after pruning and analysed for total chlorophyll, crude protein, total carbohydrates, crude fibre, total phenols, proline and tannins using standard biochemical methods. The results revealed significant varietal and leaf maturity-dependent variations in all biochemical parameters. Chlorophyll content increased from 1.79-1.98 mg g⁻¹ in young leaves to 2.27-2.76 mg g⁻¹ in matured leaves, with Sahana, V1 and G4 recording the highest values. Crude protein content declined with maturity, ranging from 21.94-24.36% in young leaves to 18.22-20.16% in matured leaves, with V1 and G4 consistently exhibiting superior protein levels. Total carbohydrate content increased from 16.08-20.58% in young leaves to 18.14-22.02% in matured leaves, with V1 registering the maximum concentration. Crude fibre content increased with maturity (7.54-9.06% to 8.30-9.84%), though V1 and G4 maintained relatively lower fibre levels in young leaves. Secondary metabolites such as total phenols (3.02-4.32 mg g⁻¹), proline (8.92-12.18 µmol g⁻¹) and tannins (1.02-1.39 mg g⁻¹) were higher in young leaves and declined with maturity. Overall, the study underscores the importance of varietal selection and leaf maturity in determining mulberry leaf biochemical quality, with V1 and G4 emerging as superior varieties for enhancing silkworm performance and cocoon productivity.

Keywords: Mulberry (*Morus* spp.), *Bombyx mori*, biochemical constituents, leaf maturity, mulberry varieties

Introduction

Silk, widely regarded as the “Queen of Textiles” occupies a prominent place in the textile sector due to its superior lustre, strength and fineness. The production of silk through sericulture is a highly specialized agro-based enterprise that plays a crucial role in rural livelihood security and economic development, particularly in India (Raghavendra *et al.*, 2017) [37]. The efficiency and sustainability of sericulture are intrinsically dependent on the biological performance of the mulberry silkworm, *Bombyx mori* L., which is entirely governed by the quality and biochemical composition of its exclusive food plant, mulberry (*Morus* spp.).

The mulberry silkworm is a holometabolous insect undergoing complete metamorphosis through egg, larval, pupal and adult stages. Among these, the larval stage is of paramount importance, as it is the sole feeding phase during which nutrients required for growth, silk gland development and cocoon formation are accumulated. Since *B. mori* feeds exclusively on mulberry leaves, the biochemical composition of the foliage directly influences larval metabolism, nutrient assimilation efficiency, silk protein synthesis and cocoon productivity (Datta, 1992) [15]. Variations in leaf biochemical constituents among mulberry varieties and leaf maturity stages are therefore reflected in silkworm growth rate, survivability and silk yield. Mulberry leaves contain a complex array of primary biochemical constituents such as chlorophyll, proteins, carbohydrates and crude fibre, along with secondary metabolites including phenols, proline and tannins. Chlorophyll content is closely associated with

photosynthetic efficiency and biomass accumulation, thereby indirectly influencing nutrient availability in leaves. Proteins and carbohydrates serve as major nutritional components for silkworm larvae, contributing to body tissue formation, energy metabolism and silk protein synthesis. High protein content, particularly nitrogenous compounds, enhances silk gland growth and cocoon shell weight, whereas excessive crude fibre reduces leaf digestibility and nutrient utilization efficiency (Bongale *et al.*, 1997; Sannappa *et al.*, 2003) [9, 43].

In addition to primary nutrients, secondary metabolites play a significant regulatory role in silkworm nutrition. Phenolic compounds and tannins, though involved in plant defense mechanisms, may act as antinutritional factors when present in higher concentrations by interfering with protein digestion and enzyme activity in silkworms (Murthy *et al.*, 1990) [33]. Conversely, proline functions as an important osmoprotectant and stress-related amino acid, contributing to stress tolerance in mulberry plants and influencing silkworm feeding behaviour and physiological adaptability (Jha *et al.*, 2016) [25]. The balance between beneficial and inhibitory biochemical components is therefore critical for determining leaf quality and silkworm performance.

The biochemical composition of mulberry leaves is influenced by several factors, including genetic makeup of the variety, agronomic practices, environmental conditions and leaf maturity stage. Young leaves are generally rich in moisture and carbohydrates, supporting early larval instars, whereas matured leaves tend to accumulate higher levels of proteins and fibre, catering to the nutritional demands of later instars involved in silk synthesis (Mahesh *et al.*, 2017; Yang *et al.*, 2018) [29, 53]. Understanding these biochemical variations is essential for selecting suitable mulberry varieties and optimizing feeding strategies in sericulture.

Considering the pivotal role of mulberry leaf biochemistry in determining silkworm growth, cocoon yield and silk quality, systematic evaluation of primary and secondary biochemical constituents across mulberry varieties and leaf stages becomes imperative. Such studies provide valuable insights for improving mulberry cultivation practices, varietal selection and overall sericultural productivity.

2. Materials and methods

2.1 Experimental Site and Environment

The present investigation was conducted at the “A” Block, College of Agriculture, Vishweshwaraiah Canal Farm, Mandya, is situated in Southern dry region (Zone-6) of Karnataka between 120 32' N latitude, 760 53' E longitude and 690 m above mean sea level. The average rainfall is 751 mm confined to monsoon from June to November with occasional showers in pre-monsoon period (March-May). The maximum rainfall is received in September and October. The mean maximum temperature and minimum temperature varies between 28-35°C and 24-26°C, respectively.

2.2 Experimental Material

The present study was conducted using eight mulberry (*Morus* spp.) varieties, namely Mysore Local, Sahana, S36, DD, AR12, V1, G2 and G4. The experiment was laid out near the silkworm rearing house under uniform management conditions. Mulberry plants were maintained following the recommended package of practices for rainfed mulberry cultivation as described by Dandin and Giridhar (2014) [13]. The recommended dose of fertilizers comprising N:P:K at

350:40:140 kg ha⁻¹ year⁻¹ and farmyard manure at 20 t ha⁻¹ year⁻¹ was applied (Dandin *et al.*, 2003) [12]. Irrigation was provided at 10-day intervals and intercultivation was carried out 30 days after pruning. Leaves were harvested 45 days after pruning for biochemical analysis.

2.3 Experiment Methodology

Leaf samples from each mulberry variety were collected at 45 days after pruning. The samples were first air-dried under shade and subsequently oven-dried at 60 °C for 18 h to achieve constant weight. Dried samples were finely powdered using a laboratory grinder and stored in airtight containers until further biochemical analysis. The processed leaf samples were analyzed for the following key biochemical constituents using standard and well-established procedures.

Total chlorophyll content (mg g⁻¹) was estimated by extracting fresh leaf tissue in 80 per cent acetone, following the method proposed by Arnon (1949) [6]. Absorbance was recorded at 645 and 663 nm using a UV-visible spectrophotometer and total chlorophyll content was calculated using standard equations.

Crude protein content (%) was determined using the method described by Lowry *et al.* (1951) [28]. Protein extraction was carried out using appropriate buffers, followed by reaction with Folin-Ciocalteu reagent. The intensity of the blue colour developed was measured at 650 nm and protein concentration was calculated using bovine serum albumin as the standard.

Total carbohydrate content (%) was estimated following the phenol-sulphuric acid method of Dubois *et al.* (1956) [17]. The absorbance of the coloured complex formed was measured at 490 nm and carbohydrate content was quantified using glucose as the reference standard.

Crude fibre content (%) was determined gravimetrically after sequential digestion of leaf samples with dilute acid followed by dilute alkali, as per standard analytical procedures. The residue obtained after digestion, drying and ignition was used for calculating crude fibre content.

Total phenol content was estimated using the Folin-Ciocalteu reagent method as described by Bray and Thorpe (1954) [10]. The results were expressed as milligrams of gallic acid equivalents (mg GAE 100 g⁻¹ fresh weight), based on a standard calibration curve prepared using gallic acid. Proline content (μmol g⁻¹ fresh weight) was determined by the acid-ninhydrin method outlined by Bates *et al.* (1973) [7]. The chromophore developed was extracted in toluene and absorbance was recorded at 520 nm. Proline concentration was calculated using a standard curve prepared with known concentrations of proline. Tannin content was estimated using the Folin-Denis method as described by Sadasivam and Manickam (1996) [41]. Absorbance was measured at 700 nm and tannin content was expressed as milligrams of tannic acid equivalents (mg TA 100 g⁻¹ fresh weight) using tannic acid as the standard.

2.4 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) as described by Gomez and Gomez (1984) [19] and Hoshmand (1988) [20]. Treatment means were compared using Tukey's Honest Significant Difference (HSD) test at a 5% significance level (Tukey, 1965) [51].

3. Results and Discussions

The biochemical constituents of young and matured leaves of mulberry varieties varied significantly across the eight varieties.

3.1 Chlorophyll (mg/g)

Chlorophyll concentration serves as a key indicator of photosynthetic efficiency and overall foliage quality in mulberry. In the present investigation, chlorophyll content in young leaves varied from 1.79 mg/g in DD to 1.98 mg/g in V1. A marked and significant increase was observed in matured leaves, with values ranging from 2.27 mg/g in G2 to 2.76 mg/g in Sahana. Among the evaluated varieties, V1 and G4 exhibited the highest chlorophyll content in young leaves (1.98 and 1.96 mg/g, respectively). In matured leaves, Sahana recorded the maximum chlorophyll concentration (2.76 mg/g), followed closely by V1 and G4, both registering 2.61 mg/g. Conversely, Mysore Local exhibited the lowest chlorophyll content in matured leaves (2.41 mg/g), despite maintaining a moderate level during the young leaf stage (1.85 mg/g).

These findings are in close agreement with earlier reports. Yadhav *et al.* (2022) [54] documented maximum total chlorophyll content in S146 mulberry leaves. Similarly, Raghavendra *et al.* (2017) [37] reported the highest chlorophyll concentration in V1 (2.429 mg/g) and the lowest in S30 (1.728 mg/g). Manjunatha and Krishnamurthy (2006) [30] further reported a comparatively higher chlorophyll content of 2.96 mg/g in V1 bush mulberry, reinforcing the superiority of this variety in photosynthetic pigment accumulation.

3.2 Proteins (%)

Protein content, a crucial determinant of leaf nutritive value, exhibited notable variation among mulberry varieties and leaf stages. In young leaves, protein levels ranged from 21.94% in S36 to 24.36% in V1. As leaves matured, a consistent decline in protein content was observed across all genotypes, with values ranging from 18.22% in G2 to 20.16% in G4. Among the tested varieties, V1, Sahana and G4 recorded the highest protein concentrations in young leaves (24.36%, 24.20% and 24.16%, respectively) and maintained relatively higher protein levels even at the matured stage. In contrast, S36 registered the lowest protein content in young leaves (21.94%) and only moderate levels in matured leaves (18.56%).

These observations corroborate earlier findings. Bongale and Chaluachari (1993) [8] reported significant varietal differences in protein content among mulberry varieties including S54, C1, MR2, Kitchili, Roso, Kosen, Japanese and M5. Raghavendra *et al.* (2017) [37] also recorded the highest protein content in V1 (25.367%) and the lowest in

M5 (24.21%). Additionally, Ramamoorthy *et al.* (2018) [38] reported high crude nutrient values in V1 and G4, further supporting the nutritional superiority of these genotypes.

3.3 Carbohydrates (%)

Carbohydrate content showed a progressive increase with leaf maturity across all mulberry varieties studied. In young leaves, carbohydrate levels ranged from 16.08% in G2 to 20.58% in G4. In matured leaves, carbohydrate concentration increased considerably, varying from 18.14% in G2 to 22.02% in V1. Among the varieties, V1 (20.26% in young and 22.02% in matured leaves) and G4 (20.58% in young and 21.78% in matured leaves) consistently recorded the highest carbohydrate content at both growth stages. S36 and DD also exhibited relatively higher carbohydrate levels exceeding 20% in both young and matured leaves. In contrast, G2 consistently recorded the lowest carbohydrate content, indicating comparatively reduced photosynthate accumulation or translocation efficiency.

These results align with the findings of Hossain *et al.* (2016) [21], who reported the highest carbohydrate content in V1 mulberry leaves ($17.85 \pm 0.78\%$). Ramamoorthy *et al.* (2018) [38] similarly observed carbohydrate contents of 18.3% and 16.3% in V1 and G4 varieties, respectively, highlighting their superior nutritional quality. Further confirmation was provided by Sapna *et al.* (2022) [44], who recorded the maximum carbohydrate content in V1 (17.77%), reinforcing its enhanced carbohydrate accumulation potential.

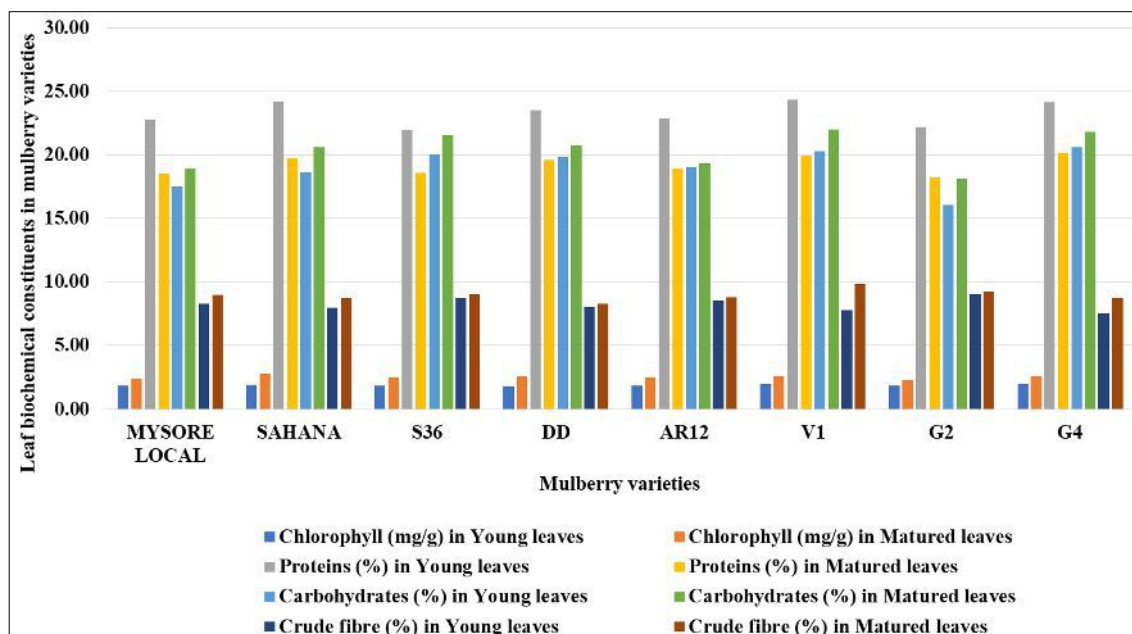
3.4 Crude fibre (%)

Crude fibre content varied significantly among mulberry varieties and increased with leaf maturity. In young leaves, crude fibre content ranged from 7.54% in G4 to 9.06% in G2, whereas in matured leaves it increased to values between 8.30% in DD and 9.84% in V1. Among the varieties, G2 and S36 recorded the highest crude fibre content in young leaves (9.06% and 8.74%, respectively), while V1 exhibited the maximum fibre content in matured leaves (9.84%). Conversely, G4 and V1 showed the lowest crude fibre content in young leaves (7.54% and 7.76%, respectively), indicating better digestibility during early leaf stages.

These findings are consistent with previous studies. reported maximum carbohydrate and fibre content in V1 followed by G4. Sushmitha (2024) [48] observed that V1 recorded the highest crude fibre content (9.89%). Earlier studies by Maribashetty *et al.* (1999) [31] and Manjunatha and Krishnaswamy (2006) [30] also reported higher fibre content in V1 mulberry leaves. Ramamoorthy *et al.* (2018) [38] similarly documented crude fibre values of 8.8% in both V1 and G4 varieties.

Table 1: Biochemical composition of young and matured leaves of different mulberry varieties

Sl. No.	Mulberry varieties	Young leaves				Matured leaves			
		Chlorophyll (mg/g)	Proteins (%)	Carbohydrates (%)	Crude fibre (%)	Chlorophyll (mg/g)	Proteins (%)	Carbohydrates (%)	Crude fibre (%)
1.	Mysore Local	1.85 ^c	22.74 (28.47) ^d	17.50 (24.72) ^g	8.26 (16.70) ^d	2.41 ^f	18.52 (25.48) ^e	18.92 (25.77) ^f	9.00 (17.45) ^c
2.	Sahana	1.90 ^b	24.20 (29.46) ^b	18.62 (25.55) ^f	7.94 (16.36) ^e	2.76 ^a	19.70 (26.34) ^{bc}	20.62 (27.00) ^d	8.74 (17.19) ^d
3.	S36	1.82 ^d	21.94 (27.92) ^f	20.04 (26.58) ^c	8.74 (17.19) ^b	2.49 ^d	18.56 (25.51) ^e	21.56 (27.66) ^c	9.06 (17.51) ^c
4.	DD	1.79 ^e	23.52 (29.00) ^c	19.82 (26.43) ^d	8.06 (16.49) ^e	2.58 ^c	19.62 (26.28) ^c	20.72 (27.07) ^d	8.30 (16.74) ^e
5.	AR12	1.81 ^{de}	22.86 (28.55) ^d	19.02 (25.85) ^e	8.56 (17.01) ^c	2.46 ^e	18.92 (25.77) ^d	19.36 (26.09) ^e	8.76 (17.21) ^d
6.	V1	1.98 ^a	24.36 (29.56) ^a	20.26 (26.74) ^b	7.76 (16.17) ^f	2.61 ^b	19.90 (26.48) ^b	22.02 (27.98) ^a	9.84 (18.27) ^a
7.	G2	1.82 ^d	22.16 (28.07) ^e	16.08 (23.63) ^h	9.06 (17.51) ^a	2.27 ^g	18.22 (25.26) ^f	18.14 (25.20) ^g	9.26 (17.71) ^b
8.	G4	1.96 ^a	24.16 (29.43) ^b	20.58 (26.97) ^a	7.54 (15.93) ^g	2.61 ^b	20.16 (26.67) ^a	21.78 (27.81) ^b	8.72 (17.17) ^d
	SE m ±	0.01	0.02	0.02	0.03	0.01	0.02	0.03	0.04
	CD @p=0.05	0.02	0.06	0.07	0.09	0.02	0.06	0.09	0.09



Figures in the parenthesis indicate arcsine transformed values; Values in the column followed by common letters are non-significant at $p=0.05$ as per Tukey's HSD (Tukey, 1965) ^[51]

Fig 1: Biochemical constituents (Chlorophyll (mg/g), Proteins (%), Carbohydrates (%) and Crude fibre (%)) observed in leaves of mulberry varieties

Table 2: Secondary metabolites (Total Phenols, Proline and Tannins) content present in young and matured leaves of mulberry varieties

Sl. No.	Mulberry Varieties	Young Leaves			Matured Leaves		
		Total phenols (mg/g)	Proline ($\mu\text{mol/g}$)	Tannins (mg/g)	Total phenols (mg/g)	Proline ($\mu\text{mol/g}$)	Tannins (mg/g)
1.	Mysore Local	4.32 ^a	9.84 ^e	1.12 ^e	3.02 ^d	7.24 ^f	0.82 ^c
2.	Sahana	3.54 ^d	10.56 ^e	1.24 ^e	2.96 ^e	8.16 ^c	0.93 ^b
3.	S36	4.26 ^a	8.92 ^h	1.02 ^h	3.64 ^a	6.52 ^h	0.74 ^d
4.	DD	3.98 ^b	10.24 ^d	1.18 ^d	3.16 ^c	7.84 ^d	0.89 ^c
5.	AR12	3.24 ^e	9.62 ^f	1.10 ^f	2.84 ^e	7.54 ^e	0.86 ^c
6.	V1	3.96 ^b	11.46 ^b	1.31 ^b	3.36 ^b	8.64 ^b	0.90 ^b
7.	G2	3.74 ^c	9.24 ^g	1.05 ^g	2.74 ^f	6.84 ^g	0.78 ^d
8.	G4	3.02 ^f	12.18 ^a	1.39 ^a	2.56 ^g	9.24 ^a	1.05 ^a
	SE m $\pm$	0.03	0.04	0.01	0.02	0.03	0.01
	CD @ $p=0.05$	0.09	0.12	0.02	0.06	0.10	0.02

Figures in the parenthesis indicate arcsine transformed values; Values in the column followed by common letters are non-significant at $p=0.05$ as per Tukey's HSD (Tukey, 1965) ^[51]

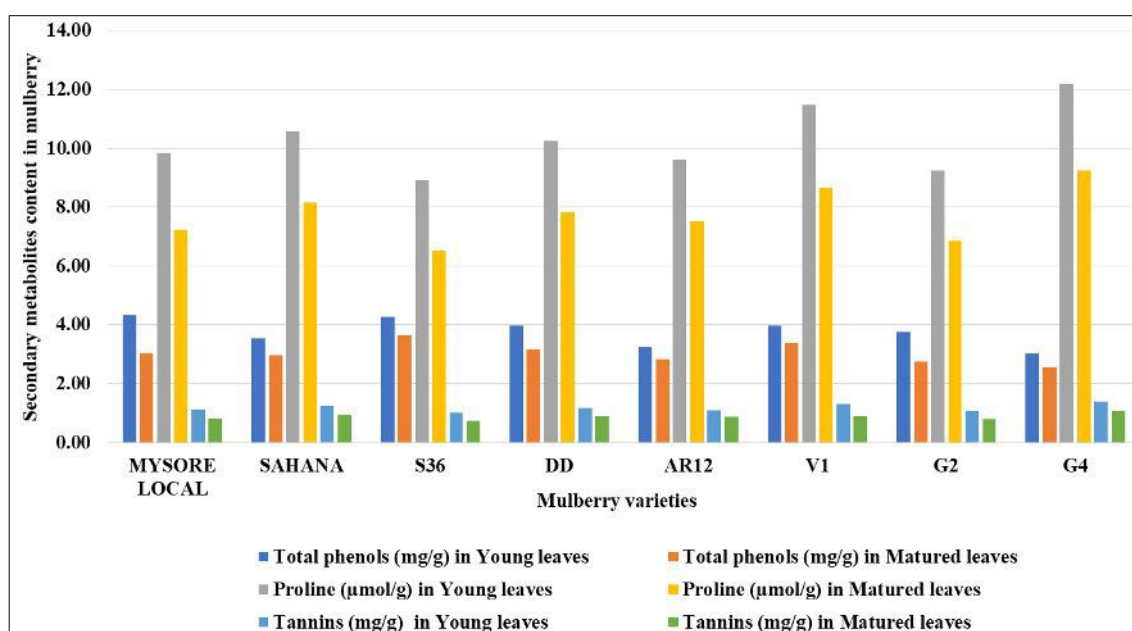


Fig 2: Secondary metabolites (Total Phenols, Proline and Tannins) observed in young and matured leaves of mulberry varieties

3.5 Total Phenols (mg/g)

Total phenol content varied considerably among mulberry varieties and declined with leaf maturity. In young leaves, phenol content ranged from 3.02 mg/g in G4 to 4.32 mg/g in Mysore Local. In matured leaves, a noticeable reduction was observed, with values ranging from 2.56 mg/g in G4 to 3.64 mg/g in S36. Mysore Local and S36 recorded the highest phenol concentrations in young leaves (4.32 and 4.26 mg/g, respectively), whereas AR12, Sahana and G4 exhibited comparatively lower levels. Interestingly, S36 was the only variety that showed a marginal increase in phenol content at the matured stage, while all other varieties displayed a declining trend.

These results are in agreement with earlier findings. Tewary *et al.* (2008) ^[50] reported higher phenol content in tree mulberry (3.18%) compared to bush mulberry (2.90%). Vanitha (2018) ^[52] also recorded significantly higher phenol content in tree mulberry (3.22 ± 0.03 mg). Sushmitha *et al.* (2023) ^[49] further reported a phenol content of 3.82 mg in V1 mulberry leaves, supporting the present observations.

3.6 Proline (μmol/g)

Proline content showed significant varietal differences and declined with leaf maturity. In young leaves, proline concentration ranged from 8.92 μmol/g in S36 to 12.18 μmol/g in G4. In matured leaves, values decreased to a range of 6.52 μmol/g in S36 to 9.24 μmol/g in G4. Among the varieties, G4 and V1 consistently recorded the highest proline levels at both leaf stages. In contrast, S36 exhibited the lowest proline concentration in both young and matured leaves. The observed decline in proline content with leaf age may be attributed to reduced osmotic stress and metabolic demand in older tissues.

These findings are supported by Ramamoorthy *et al.* (2018) ^[38], who reported higher proline levels in V1 (11.8 μmol/g) and G4 (12.1 μmol/g), identifying these varieties as more drought tolerant with superior osmotic adjustment ability. Jha *et al.* (2016) ^[25] also highlighted that higher amino acid content, particularly proline, is positively associated with improved leaf palatability, digestibility and cocoon shell weight in silkworms.

3.7 Tannins (mg/g)

Tannin content exhibited a declining trend with leaf maturity across all mulberry varieties. In young leaves, tannin concentration ranged from 1.02 mg/g in S36 to 1.39 mg/g in G4, while in matured leaves it declined to values between 0.74 mg/g in S36 and 1.05 mg/g in G4. G4 and V1 consistently recorded higher tannin content at both stages, whereas S36 maintained the lowest tannin levels throughout. The reduction in tannin content with advancing leaf age may be attributed to decreased synthesis and polymerization of phenolic compounds during leaf maturation and lignification.

These results are in close agreement with Ramamoorthy *et al.* (2018) ^[38], who reported the highest tannin content in G4 (1.36 mg/g) followed by V1 (1.30 mg/g), with minimum levels in S36 and G2. Sapna *et al.* (2022) ^[44] also observed a decline in tannin content with leaf maturity, influencing leaf palatability and silkworm feeding behaviour. Murthy *et al.* (1990) ^[33] reported that excessive tannins act as antinutritional factors by binding to proteins and digestive enzymes, thereby reducing nutrient availability and assimilation in silkworms. Rathore and Chakraborty (1995)

^[39] further demonstrated that silkworms fed on tannin-rich mulberry leaves exhibited reduced silk gland weight and overall silk yield, due to impaired digestion and metabolic activity.

4. Conclusion

The present investigation clearly demonstrates that the biochemical composition of mulberry leaves varies significantly among varieties and between young and matured leaf stages and these variations have direct implications for leaf quality and suitability for silkworm rearing. Primary biochemical constituents such as chlorophyll, proteins, carbohydrates and crude fibre, along with secondary metabolites including total phenols, proline and tannins, collectively determine the nutritional value and physiological suitability of mulberry foliage.

Among the evaluated varieties, V1 and G4 consistently exhibited superior biochemical profiles. These varieties recorded higher chlorophyll content, indicating greater photosynthetic efficiency and biomass accumulation. They also showed elevated levels of proteins and carbohydrates in both young and matured leaves, which are critical for larval growth, silk gland development and silk protein synthesis. Although crude fibre content increased with leaf maturity in all varieties, V1 and G4 maintained relatively lower fibre levels at the young leaf stage, enhancing leaf digestibility for early instar silkworms.

Secondary metabolites showed a general decline with leaf maturity. Phenol and tannin contents were higher in young leaves and decreased in matured leaves, suggesting reduced antinutritional effects in older foliage. G4 and V1 recorded comparatively higher proline levels, indicating better stress tolerance and metabolic adaptability, which may contribute to improved leaf palatability and silkworm performance. In contrast, varieties such as S36 and G2 exhibited lower protein and carbohydrate contents and relatively higher fibre or phenolic concentrations, indicating comparatively inferior nutritional quality.

Overall, the study highlights that varietal differences and leaf maturity stages play a crucial role in determining mulberry leaf biochemical quality. The superior performance of V1 and G4 suggests their suitability for commercial sericulture, particularly for achieving enhanced larval growth and improved cocoon productivity. The findings emphasize the importance of selecting nutritionally superior mulberry varieties and adopting stage-specific leaf harvesting strategies to optimize silkworm performance and sustain higher silk yield.

5. References

1. Ahalya M, Chikkalingaiah C. Biochemical evaluation of mulberry varieties in relation to leaf quality and silkworm performance. *Journal of Sericultural Science*. 2022;57(2):134-140.
2. Ahalya BN, Chikkalingaiah, Sushmitha C, Ravikumar A, Harish M, Manjunatha N. Comparative assessment of biochemical constituents in tree mulberry genotypes. *International Journal of Advanced Biochemical Research*. 2025;9(1):610-614.
3. Alipanah H, Rahimi M, Zamani A. Sericulture and rural development: An integrated approach toward sustainable livelihoods. *International Journal of Agricultural Economics*. 2020;8(4):225-232.

4. Anonymous. Annual report of the Central Sericultural Research and Training Institute. Mysore: Central Sericultural Research and Training Institute; 1975.
5. Anonymous. Central Silk Board Annual Report 2023-24. Bangalore: Ministry of Textiles, Government of India; 2024.
6. Arnon DI. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. Plant Physiology. 1949;24(1):1-15.
7. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-207.
8. Bongale UD, Chaluvachari P. Evaluation of mulberry varieties for leaf yield and quality. Indian Journal of Sericulture. 1993;32(1):33-40.
9. Bongale UD, Chaluvachari P, Narasimhanna MN. Nutritional status of mulberry leaves and its relation to silkworm growth. Sericologia. 1997;37(2):219-228.
10. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. Methods of Biochemical Analysis. 1954;1:27-52.
11. Chandrasekhar S, Dandin SB, Giridhar K. Studies on biochemical changes in mulberry varieties influencing silkworm performance. Indian Journal of Sericulture. 2010;49(1):74-79.
12. Dandin SB, Jayaswal J, Giridhar K. Handbook of sericulture: Technologies and management. Bangalore: Central Silk Board; 2003.
13. Dandin SB, Giridhar K. Mulberry cultivation and leaf quality management. Bangalore: Central Silk Board; 2014.
14. Das BC, Krishnaswamy S. Studies on the nutritive value of mulberry leaves. Indian Journal of Sericulture. 1969;8(2):45-53.
15. Datta RK. Improvement of mulberry through breeding in India. Sericologia. 1992;32(3):327-342.
16. Doss SG, Ramesh B, Bongale UD. Influence of nitrogen on mulberry leaf quality and cocoon yield. Sericologia. 1991;31(2):203-209.
17. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956;28(3):350-356.
18. Ghosh MK, Murthy BN, Raghuraman R. Role of micronutrients in mulberry leaf quality and silkworm performance. Indian Journal of Sericulture. 2006;45(2):150-155.
19. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed. New York: John Wiley and Sons; 1984.
20. Hoshmand AR. Statistical methods for agricultural scientists. Westport: Praeger Publishers; 1988.
21. Hossain MA, Islam MN, Rahman MM. Varietal differences in carbohydrate accumulation in mulberry leaves. Bangladesh Journal of Sericulture. 2016;5(1):22-28.
22. Islam T, Bhat SA, Malik FA, Khan FA, Mir SA, Nazir N, *et al.* Rearing of silkworm, *Bombyx mori* L. on different mulberry genotypes and its impact on post-cocoon parameters. Plant Archives. 2022;22(2):380-382.
23. Ito T. The nutrition of the silkworm. Japan Agricultural Research Quarterly. 1972;6(2):55-60.
24. Jackson ML. Soil chemical analysis. New Delhi: Prentice Hall of India; 1973.
25. Jha SK, Gupta RK, Singh A. Effect of water stress on proline accumulation and silk yield in *Bombyx mori*. Journal of Entomological Research. 2016;40(3):291-298.
26. Krishnaswami S. New technology of silkworm rearing. Bulletin No. 2. Mysore: CSRandTI; 1978.
27. Krishnaswami S, Kumararaj S, Vijayaraghavan K, Kasiviswanathan K. Silkworm rearing. Bulletin No. 15. Mysore: CSRandTI; 1971.
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951;193(1):265-275.
29. Mahesh M, Prakash NB, Mahadeva J. Seasonal influence on mulberry leaf biochemistry and silkworm productivity. Indian Journal of Sericulture. 2017;56(1):89-95.
30. Manjunatha MK, Krishnamurthy NB. Photosynthetic pigments in mulberry and their impact on cocoon yield. Karnataka Journal of Agricultural Sciences. 2006;19(2):355-360.
31. Maribashetty VG, Sannappa B, Dandin SB. Biochemical basis for varietal differences in mulberry. Sericologia. 1999;39(3):423-430.
32. Murthy BN, Ghosh MK, Raghuraman R. Mineral composition of mulberry leaves and its impact on silkworm nutrition. Indian Journal of Sericulture. 2013;52(1):45-51.
33. Murthy DN, Raju SS, Venkataraman NK. Influence of tannins on silkworm digestion and silk production. Sericologia. 1990;30(4):551-556.
34. Nagaraju J, Goldsmith MR. Silkworm genomics — progress and prospects. Current Science. 2002;83(4):415-425.
35. Narayanaswamy TK, Sannappa B, Rajeshwari G. Nutrient composition and quality evaluation of tree mulberry. Indian Journal of Sericulture. 2003;42(2):128-132.
36. Piper CS. Soil and plant analysis. New York: Inter Science Publishers; 1966.
37. Raghavendra B, Manjunatha MK, Shankar R. Comparative analysis of biochemical composition in mulberry varieties and its influence on silkworm performance. Indian Journal of Sericulture. 2017;56(2):212-220.
38. Ramamoorthy V, Senthilkumar N, Rajkumar K. Influence of biochemical composition of mulberry on silkworm growth and silk yield. Sericologia. 2018;58(1):97-106.
39. Rathore DS, Chakraborty M. Effect of tannins on protein digestion and silk synthesis in *Bombyx mori*. Indian Journal of Sericulture. 1995;34(1):54-58.
40. Ruth R, Ahalya M, Sahida A. Varietal differences in nutrient uptake and silk yield in mulberry. Journal of Sericultural Research. 2019;48(2):120-128.
41. Sadasivam S, Manickam A. Biochemical methods for agricultural sciences. New Delhi: Wiley Eastern; 1996.
42. Sahida A. Nutrient composition and biochemical evaluation of mulberry varieties in relation to silkworm growth [MSc thesis]. Bangalore: University of Agricultural Sciences; 2015.

43. Sannappa B, Bongale UD, Dandin SB. Relationship between mulberry leaf quality and cocoon yield. *Sericologia*. 2003;43(1):101-108.
44. Sapna K, Ahalya M, Chikkalingaiah C. Secondary metabolites in mulberry and their implications for silkworm nutrition. *Indian Journal of Sericulture*. 2022;61(1):49-56.
45. Sehna F, Akai H. Insect silk glands: Their types, development and function. *International Journal of Insect Morphology and Embryology*. 1990;19(2):79-132.
46. Shyla D. Biochemical studies on mulberry genotypes in relation to their productivity [MSc thesis]. Bangalore: University of Agricultural Sciences; 2012.
47. Sori M, Ahalya M, Vanitha C. Comparative analysis of macronutrient composition in mulberry leaves. *Karnataka Journal of Agricultural Sciences*. 2016;29(3):410-417.
48. Sushmitha C. Evaluation of mulberry genotypes for biochemical composition and silkworm performance [MSc thesis]. Bangalore: University of Agricultural Sciences; 2024.
49. Sushmitha C, Ahalya M, Ruth R. Phenolic content variation in mulberry leaves and its effect on silkworm nutrition. *Journal of Sericultural Science*. 2023;60(2):145-151.
50. Tewary PK, Mohan B, Saha AK. Phenolic variation in mulberry leaves and their influence on silkworm growth. *Sericologia*. 2008;48(3):239-247.
51. Tukey JW. The analysis of variance and multiple comparisons. New York: Wiley; 1965.
52. Vanitha C. Biochemical and physiological evaluation of mulberry varieties and their influence on silkworm growth [PhD thesis]. Bangalore: University of Agricultural Sciences; 2018.
53. Yang C, Zhang Y, Liu X. Nutrient variation in mulberry leaves during silkworm growth stages. *Journal of Applied Entomology*. 2018;142(9):823-831.
54. Yadhav SP, Raghavendra B, Manjunatha MK. Chlorophyll dynamics in mulberry and its relation to silk productivity. *Sericologia*. 2022;62(1):33-40.