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Biochemical Variations in Mulberry Leaves: An Analysis Across Growth Stages and Farming Practices

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ABSTRACT: This research examined the variations in the biochemical composition of mulberry leaves, which serve as the primary sustenance for silkworms, by examining leaves across different growth stages and under diverse farming conditions in the Mysuru district. Our findings reveal that while the moisture content of the leaves largely remained consistent irrespective of the growth stage, significant differences were noted in protein, carbohydrate, and chlorophyll concentrations among leaves from different farms. These variations can be attributed to a combination of the growth stage of the leaf and the specific agronomic practices employed by each farmer. The study underscores the profound impact of farming practices on the quality of mulberry leaves, highlighting their importance not just in sericulture but also in potential applications within the food and pharmaceutical industries.

Keywords: Mulberry leaves, biochemical composition, sericulture, agronomic practices, protein content, carbohydrate content, chlorophyll, silkworm feed.

INTRODUCTION

Mulberry leaf forms the basic food material for silkworm (*Bombyx mori* L.). The silkworm requires specific quality of leaf during different phases of its growth and thus it reflects on the importance of mulberry cultivation practices. Leaf quality and quantity not only influence the silkworm growth and development, but also the cocoon production, quantity and quality of raw silk. Nearly, 70% of silk protein produced by silkworm is derived directly from proteins of mulberry leaves. According to Miyashita (1986), the contributing factors for successful cocoon crop production are mulberry leaf (38.2%), climate (37.0%), rearing techniques (9.3%), silkworm breed (4.2%), silkworm seed (3.1%) and other factors (8.2%).

The mulberry leaf yield and quality depends on the soil type, varieties and available plant nutrients in soil, agronomical practices and agro-climatic conditions. Hence, native soil fertility alone cannot be relied upon for quantity and quality of mulberry leaf productivity, unless the soil is replenished with external sources through manures and fertilizers. Therefore, package of practices for application of manure and fertilizer schedule is vital for obtaining higher leaf yield with superior quality (Anonymous, 2002). Mulberry leaves, commonly recognized as the primary food source for silkworms, have been valued across various cultures not just for their economic significance in sericulture but also for their potential nutritional and medicinal properties. Beyond the realm of traditional usage, in recent years, there has been growing scientific interest in the precise bio-chemical composition of these leaves. Investigations have delved into understanding the variety of compounds present, including proteins, fibers, sugars, amino acids, and phenols.

Mulberry leaves, derived from various mulberry varieties, have long been a subject of research for their notable biochemical composition. In a comprehensive study by Adeduntan and Oyerinde (2009), three distinct mulberry leaves, namely S36, S54, and K2, displayed significantly elevated crude protein content, registering at 21.66%, 21.55%, and 21.24% respectively. Delving deeper into the intrinsic components, mulberry is known to contain ash, crude protein, crude fiber, and a slew of other elements in the leaves (Kandylis *et al.*, 2009). Other than high crude protein, dry matter mulberry leaves also exhibits minimal low non-digestive factor content (Deshmukh *et al.*, 1993). Furthermore, a native black mulberry fruits outperforms other berries in terms of total sugar, crude fat, and crude protein content (Koyuncu *et al.*, 2014). Building on this, research by Jyothi *et al.* (2014) uniformly indicated elevated levels of sugars, proteins, amino acids, and phenols in specific mulberry varieties. Collectively, this body of research underscores the rich and varied biochemical composition of mulberry leaves, making them a valuable subject for further study.

Our study highlighted variations in the biochemical composition of mulberry leaves based on growth stages and farming conditions. Specifically, differences were observed in moisture, protein, and chlorophyll levels among farms. Such explorations offer insights not only into the potential health benefits of mulberry leaves but also into optimizing their utility in various sectors, from food and nutrition to pharmaceuticals. With a rich tapestry of compounds, the estimation of the biochemical composition of mulberry leaves has become paramount in uncovering the untapped potentials they harbor. As we embark on this exploration, we will be navigating a confluence of traditional knowledge and modern scientific findings, unveiling the intricate biochemical makeup of the mulberry leaf.

MATERIALS AND METHODS

The study was carried out in well-maintained irrigated mulberry gardens (V1) located in the agricultural lands of farmers including Ramshetty, Nagamma, Jagaraju, Ramshetty, and Mahadevshetty in Kempaiaghna Hundi, T. Narasipura Taluk, within the Mysuru district (Table 1).

Table 1: Details of farmers in the study area.

Sr. No.	Details	Name of the farmer				
		Ramshetty	**Nagamma**	**Jagaraju**	**Ramshetty**	**Mahadevshetty**
1.	Passbook no.	112937	112443	101718	20595	107557
2.	Mobile no.	9611834946	9535458285	9945487912	9686660502	9141115963
3.	Age (years)	49	50	58	62	46
4.	Education	Illiterate	Illiterate	Illiterate	7th Std.	Illiterate
5.	Total land holding (acre)	1 .75	1 .50	5.00	2 .50	1 87
6.	Mulberry land holding (acre)	1.00	1.00	1 .87	2.00	1 87
7.	Dry land/ irrigated	Irrigated	Irrigated	Irrigated	Irrigated	Irrigated
8.	Soil type	Red	Red	Red	Red	Red
9.	Mulberry variety	V_1	V_1	V_1	V_1	V_1
10.	Age of Mulberry garden (years)	7	8	7	8	15
11.	Spacing in mulberry	3’×3’	3’×3’	2 .5’ × 2 .5’	3’×3’	3’×3’
12.	Manure (FYM) (kg)	120	150	-	130	130
13.	Fertilizers (urea & complex) (kg)	30	50	140	50	-
14.	Irrigation method	Furrow	Furrow	Furrow	Drip	Drip
15.	Harvesting method	Shoot	Shoot	Shoot	Shoot	Shoot
16.	Method used for leaf preservation	Wet gunny cloth	Wet gunny cloth	Wet gunny cloth	Wet gunny cloth	Wet gunny cloth
17.	Pruning (in a year)	10	8	6-7	10	6-7
18.	Rearing house	Sheet	Sheet	Sheet	RCC	Sheet
19.	Disinfectants used	-	Ankush	-	-	-
20.	DFLs	150	150	150	150-200	125
21.	Direct brushing/ chawki worms	Chawki worms	Chawki worms	Chawki worms	Chawki worms	Chawki Worms
22	Silkworm breed	$CSR_2 \times CSR_4$	$CSR_2 \times CSR_4$	$CSR_2 \times CSR_4$	$CSR(FC_1 \times FC_2)$	$CSR(FC_1 \times FC_2)$
23.	Bed disinfectant	Lime, Vijetha	Lime, Vijetha	Lime, Vijetha	Lime, Vijetha	Lime, Vijetha
24.	Type of mountage	Plastic collapsible	Plastic collapsible	Plastic collapsible	Plastic collapsible	Plastic collapsible
25.	Cocoon harvesting (on which day)	7-8th day	6-7th day	7 day	7th day	7th day
26	Crop raised in a year	10	8	6-7	10	7-8

a) Moisture

Moisture content of the leaf was estimated through gravimetric method by taking the difference between fresh and dry weights and expressed in percentage on fresh weight basis (A.O.A.C., 1970).

$$\text{Leaf moisture}\,(\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

b) Protein

Total protein content of the leaf was estimated as per the procedure of Lowry *et al.* (1951) and expressed in mg/g of leaf on oven dry weight basis.

c) Carbohydrate

The total carbohydrate content of the leaf was estimated following the procedure of Dubios *et al*. (1956) and expressed in mg/g of leaf on oven dry weight basis.

d) Chlorophyll

Chlorophyll content of the leaf was estimated by following the procedure outlined by Hiscox and Israelstam (1979) at the wavelength of 645 and 663 nm using a spectrophotometer.

The chlorophyll 'a', 'b' and total chlorophyll were computed using the standard formulae (Arnon, 1949).

Chlorophyll 'a' (mg/g fresh weight) = 12.7 (O.D. 663) – 2.69 (O.D. 645) × V/1000 x W

Where, O. D. = Optical difference

V = Volume made up (ml)

W = Weight of leaf sample (g)

Chlorophyll 'b' (mg/g fresh weight) = 22.9 (O.D. 645) – 4.68 (O.D. 663) × V/1000 x W

Total Chlorophyll (mg/g fresh weight) = 20.2 (O.D. 645) + 8.02 (O.D. 663) × V/1000 × W

RESULTS

1. Moisture

Tender: In tender leaves, moisture content was found non-significant. More being in Farmer-1 (82.87%) followed by Farmer-2 (82.00 %), Farmer-5 (80.25%), Farmer-4 (79.40 %) and moisture content was less in Farmer-3 (76.44%).

Medium: In medium leaves, moisture content was higher in Farmer-2 (85.78%) followed by Farmer-1 (81.68%), Farmer-5 (81.26%), Farmer-4 (80.31%). However, moisture content was lower in Farmer-3 (79.26%).

Matured: In coarse leaves, moisture content was higher in Farmer-1 (80.62%) followed by Farmer-3 (78.08%), Farmer-5 (76.91%), Farmer-4 (71.49%) and it was lower in Farmer-2 (68.76%).

Mean: The mean value of moisture content was highest in Farmer-1 (81.72%) followed by Farmer-5 (79.47%), Farmer- 2 (78.85%), Farmer-3 (77.93%) and less in Farmer-4 (77.07%) (Table 2 & Fig. 1).

2. Protein

Tender: Protein content in tender leaf was non-significant, being highest in Farmer- 2 (25.18 mg/g) followed by Farmer-4 (24.23 mg/g), Farmer-3 (22.49 mg/g) and Farmer- 5 (22.36 mg/g) but least was found in Farmer-1 (22.10 mg/g).

Medium: Protein content in medium leaf was highest in Farmer-5 (31.56 mg/g) followed by Farmer-2 (30.64 mg/g), Farmer-1 (30.00 mg/g), and Farmer-4 (28.85 mg/g) and less protein content was recorded in Farmer-3 (27.59).

Matured: In the matured leaf, protein content was highest in Farmer-5 (41.13 mg/g) followed by Farmer-1 (38.28 mg/g), Farmer-4 (36.71 mg/g), Farmer-3 (33.72 mg/g) and Farmer-2 (31.75 mg/g).

Mean: The mean of tender, medium and matured leaves with respect to protein content was highest in Farmer-5 (31.68 mg/g) followed by Farmer-1 (30.13 mg/g), Farmer-4 (29.93 mg/g) and Farmer-2 (29.19 mg/g) but protein content was less in Farmer-3 (27.93 mg/g) (Table 2 & Fig. 2).

3. Carbohydrate

Tender: Carbohydrate content in the tender leaf was highest in Farmer-5 (42.49 mg/g) followed by Farmer-4 (42.41 mg/g), Farmer-2 (38.66 mg/g) and Farmer-1 (38.65 mg/g) while least protein content was recorded in Farmer-3 (37.66 mg/g).

Medium: In medium leaf, carbohydrate content was more in Farmer-4 (64.47 mg/g) followed by Farmer- 3 (61.05mg/g), Farmer-2 (59.15 mg/g) and Farmer-1 (57.82 mg/g) accordingly, less was found in Farmer-5 (54.53 mg/g).

Matured: Carbohydrate content in matured leaf was better in Farmer-5 (59.70 mg/g) followed by Farmer-4 (55.44 mg/g), Farmer-3 (52.53 mg/g) and Farmer-2 (49.33 mg/g). However, least protein content was recorded in Farmer-1 (42.94 mg/g).

Mean: The mean of carbohydrate content was recorded in Farmer-4 (54.11 mg/g) followed by Farmer-5 (52.24 mg/g), Farmer-3 (50.42 mg/g) and Farmer-2 (49.05 mg/g) but carbohydrate content was less in Farmer-1 (46.47 mg/g) (Table 2, Fig. 2).

4. Chlorophyll 'a'

Tender: Chlorophyll 'a' content in tender leaves was significantly higher in Farmer-2 (1.085 mg/g) followed by Farmer-4 (0.977 mg/g), Farmer-3 (0.932 mg/g) and Farmer-1 (0.930 mg/g). However, lower chlorophyll 'a' content was found in Farmer-5 (0.820 mg/g).

Medium: Chlorophyll 'a' content in medium leaves registered significantly more in Farmer- 3 (1.113mg/g) followed by Farmer-4 (1.091 mg/g), Farmer-2 (1.007 mg/g) and Farmer-1 (0.855 mg/g) and less were in Farmer-5 (0.760 mg/g).

Matured: In mature leaf, chlorophyll 'a' content was significantly higher in Farmer-4 (1.275mg/g) followed by Farmer-3 (1.158 mg/g), Farmer-2 (1.101 mg/g) and Farmer- 1 (0.978mg/g) and was lower in Farmer-5 (0.787 mg/g).

Mean: In the mean of all three types of leaves, chlorophyll 'a' content was significantly highest in Farmer-4 (1.115mg/g) followed by Farmer-3 (1.068 mg/g), Farmer-2 (1.064 mg/g) and Farmer-1 (0.921mg/g). However, least was recorded in Farmer-5 (0.789 mg/g) (Table 3, Fig. 3).

5. Chlorophyll 'b'

Tender: Chlorophyll 'b' content in tender leaves was significantly more in Farmer-1 (0.365 mg/g) followed by Farmer-4 (0.251mg/g), Farmer-3 (0.238 mg/g) and Farmer-5 (0.220 mg/g) and less was found in Farmer-2 (0.184 mg/g).

Medium: Chlorophyll 'b' content in medium leaves was non-significant, higher in Farmer-4 (0.358 mg/g)

followed by Farmer-3 (0.296 mg/g), Farmer-5 (0.272 mg/g) and Farmer-1 (0.264 mg/g) and lower was recorded in Farmer-2 (0.233 mg/g).

Matured: Chlorophyll 'b' content in matured leaf was significantly higher in Farmer-3 (0.446 mg/g) followed by Farmer-1 (0.382 mg/g) and Farmer-4 (0.332 mg/g) and Farmer-2 (0.314 mg/g) and lower chlorophyll 'b' content was found in Farmer-5 (0.218 mg/g).

Mean: In all three types of leaves, mean chlorophyll 'b' content was significantly higher in Farmer-1 (0.337 mg/g) followed by Farmer-3 (0.327 mg/g), Farmer-4 (0.313 mg/g) and Farmer-2 (0.243 mg/g) and less was observed in the Farmer-5 (0.237 mg/g) (Table 3 Fig. 3).

6. Total chlorophyll

Tender: Total chlorophyll content in tender leaves registered significantly higher in Farmer-1 (1.296 mg/g) followed by Farmer- 2 (1.270 mg/g), Farmer-4 (1.228 mg/g) and Farmer- 3 (1.170 mg/g) and less was found in Farmer-5 (1.041 mg/g).

Medium: In medium leaf, total chlorophyll content was higher in Farmer-4 (1.449 mg/g) followed by Farmer-3 (1.410 mg/g), Farmer-2 (1.240 mg/g), Farmer-1 (1.119mg/g) and total chlorophyll content was lower in Farmer-5 (1.032 mg/g).

Matured: In the matured leaf, total chlorophyll content was significantly higher in Farmer-4 (1.607 mg/g) followed by Farmer-3 (1.604 mg/g), Farmer-2 (1.414 mg/g), Farmer-1 (1.360 mg/g) and it was lower in Farmer-5 (1.005 mg/g).

Mean: In all three types of leaf, the total chlorophyll was significantly higher in Farmer-4 (1.428mg/g) followed by Farmer- 3 (1.395mg/g), Farmer-2 (1.308mg/g) and Farmer-1 (1.259mg/g) and least was recorded in Farmer- 5 (1.026 mg/g) (Table 3, Fig. 3).

Table 2: Moisture, protein and carbohydrate contents in mulberry leaf under farmers condition

Farmer	Moisture (%)				Protein (mg/g)				Carbohydrate (mg/g)			
	Tender	Medium	Mature	Mean	Tender	Medium	Mature	Mean	Tender	Medium	Mature	Mean
F1 = Ramshettty	82.87± 0.648	81.68± 1.007	80.62± 2.313	81.72± 1.200	22.10± 0.534	30.00± 1.140	38.28± 2.165	30.13± 0.558	38.65± 2.297	57.82± 2.946	42.94±2.722	46.47± 1.017
F2 =Nagamma	82.00± 1.426	85.78± 2.383	68.76± 3.299	78.85± 0.829	25.18± 0.096	30.64± 1.222	31.75± 1.823	29.19± 0.342	38.66± 1.505	59.15± 2.675	49.33±4.149	49.05± 1.340
F3 = Jagaraju	76.44± 8.254	79.26± 2.434	78.08± 4.533	77.93± 3.709	22.49± 1.378	27.59± 0.313	33.72± 3.452	27.93± 1.444	37.66± 2.636	61.05± 4.063	52.53±3.850	50.42± 1.825
F4=K.L. Ramshetty	79.40± 0.820	80.31± 1.329	71.49± 3.157	77.07± 0.461	24.23± 0.776	28.85± 1.570	36.71± 2.629	29.93± 1.246	42.41± 2.245	64.47± 7.257	55.44±1.460	54.107± 2.515
F5=Mahadevshetty	80.25± 1.143	81.26± 2.909	76.91± 0.347	79.47± 1.175	22.36± 1.172	31.56± 1.362	41.13± 1.399	31.68± 0.557	42.49± 3.380	54.53± 4.276	59.70±1.284	52.24± 0.206
F - value	0.433^{NS}	1.350^{NS}	2.555^{NS}	0.895^{NS}	2.207^{NS}	1.666^{NS}	2.376^{NS}	2.149^{NS}	0.853^{NS}	0.661^{NS}	4.645^{NS}	2.476^{NS}

NS = Non-significant

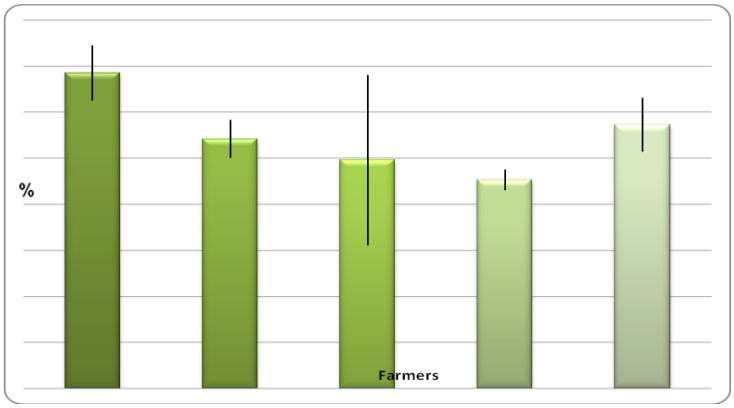

Fig. 1. Moisture content of mulberry leaf under farmers condition.

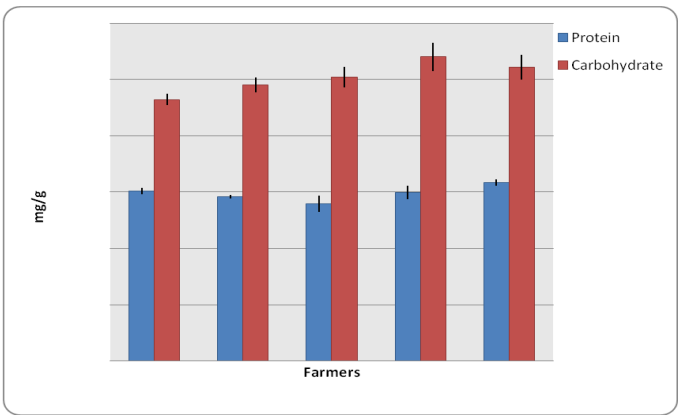

Fig. 2. Protein and carbohydrate contents in mulberry leaf under farmers condition.

Table 3: Chlorophyll content in mulberry leaf under farmers condition.

Farmer	Chlorophyll 'a' (mg/g)				Chlorophyll 'b' (mg/g)				Total chlorophyll (mg/g)			
	Tender	Medium	Mature	Mean	Tender	Medium	Mature	Mean	Tender	Medium	Mature	Mean
F1 = Ramshettty	0.930± 0.011	0.855± 0.074	0.978± 0.040	0.921± 0.831	0.365± 0.025	0.264± 0.031	0.382± 0.041	0.337± 0.028	1.296± 0.030	1.119± 0.065	1.360± 0.031	1.259± 0.025
F2 =Nagamma	1.085± 0.011	1.007± 0.018	1.101± 0.018	1.064± 1.035	0.184± 0.032	0.233± 0.017	0.314± 0.025	0.243± 0.005	1.270± 0.024	1.240± 0.005	1.414± 0.025	1.308± 0.011
F3 = Jagaraju	0.932± 0.014	1.113± 0.021	1.158± 0.009	1.068± 1.037	0.238± 0.019	0.296± 0.009	0.446± 0.029	0.327± 0.009	1.170± 0.008	1.410± 0.024	1.604± 0.031	1.395± 0.006
F4=K.L. Ramshetty	0.977± 0.023	1.091± 0.017	1.275± 0.058	1.115± 1.068	0.251± 0.025	0.358± 0.039	0.332± 0.025	0.313± 0.019	1.228± 0.007	1.449± 0.038	1.607± 0.037	1.428± 0.022
F5=Mahadevshetty	0.820± 0.007	0.760± 0.015	0.787± 0.026	0.789± 0.757	0.220± 0.023	0.272± 0.032	0.218± 0.018	0.237± 0.019	1.041± 0.025	1.032± 0.020	1.005± 0.041	1.026± 0.023
F – value	42.53*	16.87*	27.62*	69.54*	7.163*	2.739 NS	8.456*	6.678*	22.66*	23.82*	52.42*	66.01*

NS = Non-significant ; *(p≤0.05)

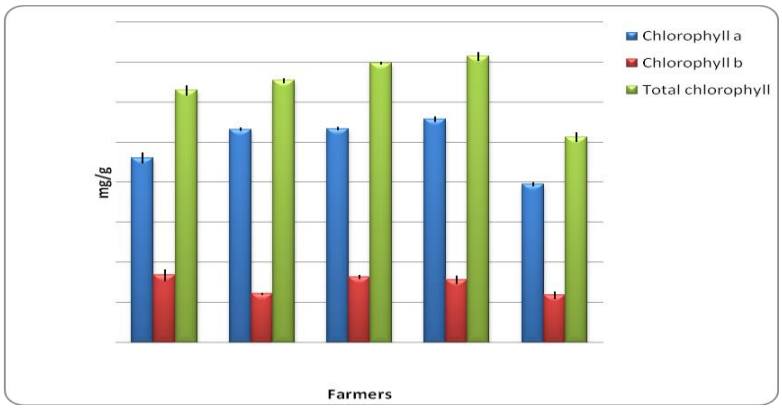

Fig. 3. Chlorophyll content in mulberry leaf under farmers condition.

DISCUSSION

In tender, medium and matured leaves, moisture content was found non-significant. The mean value of moisture content was highest in Farmer-1 and less in Farmer-4. Protein content in tender leaves was highest in Farmer-2 but least was found in Farmer-1. In medium and matured leaves were highest in Farmer-5 and less protein content was found in Farmer-3. The mean of tender, medium and matured leaves with respect to protein content was highest in Farmer-5 but less was in Farmer-3. Carbohydrate content in the tender and matured leaves was highest in Farmer-5 while least protein content was recorded in Farmer-3. In medium leaves, more content was found in Farmer-4 and less was found in Farmer-5 in respect of carbohydrate content. The mean of carbohydrate content was recorded in Farmer-4 but less in Farmer-1. Nethra *et al.* (1999) recorded increased protein content in mulberry leaf with vermicompost applied plots possibly be due to higher availability of macro and micronutrients in vermicompost which enhances the synthesis in leaves.

Among the five farmers mean chlorophyll 'a'content was significantly higher in Farmer-4. However, least was recorded in farmer-5. Chlorophyll 'b' content was significantly more in Farmer-1 and less was found in Farmer-5. Total chlorophyll content among the farmers in all three types of leaves, significantly higher was registered in Farmer-4 and lower in Farmer- 5.

These observations are in conformity with the results of Quadir and Nisar (2004); Babu *et al.* (2013), who observed that supplementation of nutritional requirements for mulberry through different organic sources and biofertilizers enhanced the chlorophyll 'a' and 'b' through improvement in soil fertility. Babu *et al.* (2013) opined that increase in leaf moisture might be due to the enhancement of hydrogen ion concentration in plant sap due to accumulation of chloride and less moisture loss. Similar results have also been reported by Rashmi *et al.* (2006) ; Sunil (2005) in S_{36} and M_5 mulberry varieties. The increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency, thus it can be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin 2000).

SUMMARY

In tender, medium, and matured leaves, the moisture content showed no significant difference, with the highest mean values in Farmer-1 and the lowest in Farmer-4. Among the five farmers, Farmer-4 had the highest chlorophyll 'a' content average for all leaf categories, while Farmer-5 had the least. Chlorophyll 'b' content was most abundant in Farmer-1, with Farmer-5 having the least. In terms of total chlorophyll content, Farmer-4 was the highest and Farmer-5 the lowest. For protein content, tender leaves from Farmer-2 had the highest amounts, but Farmer-1 had the least. Medium and matured leaves from Farmer-5 contained the most protein, with Farmer-3 showing the lowest levels. On average, Farmer-5 had the highest protein content across all leaf categories, whereas Farmer-3 had the least. Regarding carbohydrates, tender and matured leaves from Farmer-5 had the highest levels, with the

lowest protein content in Farmer-3. Medium leaves from Farmer-4 had more carbohydrates, but Farmer-5 had the least. Overall, the mean carbohydrate content was highest in Farmer-4 and lowest in Farmer-1.

CONCLUSIONS

This study assessed the biochemical composition of mulberry leaves across various growth stages and farmer conditions. We found that while moisture content remained fairly consistent across leaf stages, other compositions like protein, carbohydrate, and chlorophyll varied across the sampled farms. These findings emphasize the impact of agronomic practices on leaf quality, crucial for sericulture and potential applications in the food and pharmaceutical sectors. The study underscores the need for continued exploration into the benefits of mulberry leaves, bridging traditional knowledge with modern research.

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Conflict of Interest. None.

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