Original Research Article

Estimation of biochemical constituents in leaves of elite mulberry hybrids

ABSTRACT

Mulberry silkworm *Bombyx mori* nourishes its nutrition from its sole food plant *Morus* spp. Various biochemical techniques have facilitated the sericulture's in identifying the most nutritive mulberry variety for obtaining eminent silk. A deficiency of certain nutrients or an imbalance of nutrients in leaves causes some changes in the composition or metabolic activity of larval body. Eight mulberry hybrids and two commercial check varieties were analysed for Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulphur, Chlorophyll, Ash, Crude Fibre, Carbohydrates, Proteins, Phenol, Sugar and Amino acid contents. The result revealedthat hybrids, ME-65 × V1 recorded highest nitrogen content (3.47 %), Potassium content (2.15%), calcium content (0.64%), Magnesium content (0.45%), Sulphur content (0.30%),Ash content (12.92 %), Phenol (3.99 mg) and sugar content of (12.66 %) compared to check variety V1 and S-36. WhereasME-146 × MI-66 recorded highest total amino acid(8.37 mg/g).Similarly,biochemical constituents *viz.*, phosphorous (0.55 %), Crude Protein (18.81%), Carbohydrate (22.8%),crude fibre (9.89 %)were recorded highest in the check variety V1.

Key words: Mulberry, Leaf quality, Hybrids, Biochemical constituents

1. Introduction

"Mulberry is a perennial woody plant having fast growth and short proliferation period, belongs to family Moraceae and genus *Morus"*(Yang *et al*., 2010). "It is the primary host plant of silkworm *Bombyx mori* L. which is exploited on commercial scale for production of silk in the country. There are about 68 species in the genus *Morus* and most of them are situated in Asia" (Datta, 2000). "In China there are over thousand varieties under cultivation which originated from four main species *viz*., *Morus alba, Morusmulticaulis*, *Morusbombycis* and *Morusatropurpurea"(*Yongkang, 2000). "In India, *Morus* is divided into four species *viz.*, *Morus alba* L., *Morusindica* L., *Moruslaevigata* Wall. and*Morusserrata*Roxb". (Tikader and Dandin, 2005)."In sericulture industry, more than 60% of total cost of cocoon production goes towards mulberry production alone. Hence, in recent years maximum attention has been given for the improvement of mulberry both in terms parameter of quality and quantity. Growth and development of silkworm *Bombyx mori* L. and cocoon crop are mainly influenced by yield and nutritional quality of mulberry leaf used as feed" (Vijaya*et al*., 2009). "Quality of mulberry leaf was highly influenced by varieties, cultivation practices, preservation techniques, age and position of leaf and leaf quality was determined based on moisture content. Nutritive value of mulberry (*Morus* spp.) leaf is a key factor besides environment and technology adoption for better silkworm cocoon crop" (Yogananda Murthy *et al.*, 2013). "Higher moisture content of mulberry leaves has a direct effect on growth and development of silkworm by favouring the ingestion, digestion and assimilation of nutrients. Mulberry leaves containing more water, total sugar and soluble carbohydrate and less mineral are best relished by silkworms. Nutritive requirement of silkworm larvae varies with the

maturity of leaves fed. It is essential that the leaf quality provided to the silkworm must agree with the requirement of them. As young worms require leaves of more water content for easy ingestion, thus they should be fed with young leaves. While late age silkworms fed with the mature leaves as they have strength to cut and ingest the hard leaves. Too much mature leaves do not have sufficient protein and water for the growth of either young or mature worms and hence should not be given to the any instars" (Das and Sikadar,1970). To select best hybrid which is having good quality and leaf yield. The present investigation was conducted.

2. MATERIALS AND METHODS

2.1 Experimental Site and Environment

The study was conducted during 2022- 2023 at the Department of Sericulture, UAS, Gandhi KrishiVignana Kendra, Bangalore. The type of soil is clay loam. The field is located at a latitude of 13˚08' north, the longitude of 78˚08'east and at an altitude of 918 m above mean sea level in the Eastern Dry Zone (Zone-5) of Karnataka.

2.2 Experimental Material

The experimental material for the present study comprised of eight mulberry hybrids and two commercial check varieties (Table 1).The mulberry hybrids were developed by following Line × Tester mating design during 2019, which were maintained as bush type at the Department of Sericulture, University of Agricultural Sciences, Gandhi KrishiVignana Kendra, Bangalore-65. The eight crosses were selected as elite mulberry hybrids based on their growth and yield parameters. The hybrids were planted with a spacing of 90 x 90 cm with Randomized Block Design (RBD) with three replications and observations was recorded during 2022-2023. These mulberry hybrids were allowed to grow tall with a crown height of two and half feet from the ground level and side branches were removed. The experimental plot is maintained as per the recommended package of practices adopted for rain-fed mulberry cultivation (Dandin and Giridar, 2014).

Table 1: List of mulberry hybrids used in the study

Sl. No.	Mulberry hybrids
1	MI-47(*M. indica)* × MI-66 (*M. indica*)
2	MI-79(*M. laevigata*) × MI-66 (*M. indica)*
3	ME-03(*M. cathyana*) × MI-66 (*M. indica)*
4	ME-146 (*M. indica*) × MI-66(*M.indica)*
5	ME-65 (*M. alba*) × V1(*M. indica)*
6	ME-67 (*M. alba*) × V1(*M. indica*)
7	ME-02(*M. cathyana*) × MI-66 (*M. indica*)
8	ME-95 (*M. rotandifolia)* x V1(*M. indica*)
9	V1(Check variety)
10	S36(Check variety)

2.3 Statistical Analysis and Estimation of biochemical constituents of leaves

Composite samples of thirty leaves were collected from selected mulberry crosses and were air dried, then further dried in hot-air oven at 60 °C for 18 hours. The samples were then powdered and stored in polythene covers. These samples were analysed for Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulphur, Chlorophyll, Ash, Crude Fibre,

Carbohydrates, Proteins, Phenol, Sugar and Amino acid contents and further statistical analysis of results was done using OPSTAT Software(Table 2) [28].

Table 2: Methods used for estimation of biochemical constituents of mulberry leaf [28]

Elements	Analysis Procedure
Nitrogen (%)	Microkjeldhal method (Piper, 1966)
Phosphorus (%)	Spectrophotometric method (Piper, 1966)
Potassium (%)	Flame photometer method (Piper, 1966)
Calcium (%)	Di-acid digestion and Versanate titration
Magnesium (%)	Di-acid digestion and Versanate titration
Sulphur (%)	Di-acid digestion and Turbidometry method
Crude protein(%)	Factor method multiplied by Nitrogen % × 6.25 (Jackson, 1973)
Total carbohydrates (%)	Dubios*et al* (1956)
Crude fibre (%)	AOAC method (1955)
Ash Content (%)	AOAC method (1955)
Phenols	Spectrophotometric method by FolinCiocalteu Reagent (Piper, 1966)
Sugar content (%)	Dubois *et al*., 1956
Amino acid content (mg/g)	Ninhydrin method (Spies, 1955).

3. RESULTS AND DISCUSSION

"Nutritional quality of mulberry leaves plays an important role in growth and development of silkworm and commercial characters of the cocoons. All the nutrients in balanced proportion are necessary for growth of silkworm to produce superior quality of cocoons. In the present investigation, the parameters *viz.*, nitrogen, phosphorus, potassium, crude protein, crude fibre, ash content, phenols, carbohydrates sugar and amino acid were estimated in leaves from different mulberry hybrids" [28].

3.1 Nitrogen (%)

Among the eight mulberry hybrid ME-65 × V1 mulberry hybrid recorded maximum nitrogen content of (3.47%) followed by V1 (3.35%) and ME-03× MI-66 (3.28 %). Whereas minimum nitrogen content of (2.80 %) was recorded in ME-146 × MI-66 (Table 3). The present results are in line with the findings with the earlier studies, where Tewary (2008) found that leaves harvested from tree mulberry contained 3.90 per cent nitrogen. Sahida (2015) also estimated the nitrogen content (2.1%) in V1 mulberry. Waktole and Wosene (2016) noticed that the Kumbi genotype had the highest nitrogen content of 4.29 %. Sapna *et al*. (2022) also recorded the maximum nitrogen content in the leaves of hybrid *M. cathyana* × C-776 (3.70 %), whereas minimum nitrogen content was observed in hybrid BC-259 × MI-66 (2.16 %).Karthick Mani Bharathi *et al*., 2024 reported in MR2, mulberry leaves have found nitrogen content (3.32%).

3.2 Phosphorous (%)

The phosphorous content was maximum in the check variety V1 (0.55 %), followed by the hybrid ME-65 × V1(0.50%) and minimum was recorded in the leaves of hybrid ME-03 × MI-66 (0.36 %) (Table 3). The present results are corroborative with the earlier works by

Waktole and Wosene (2016) who had recorded the highest phosphorous content (0.45 %) in the Kumbi genotype. Ruth *et al.* (2019) also found that Jorhat mulberry had the highest phosphorus content (1.672 ± 0.06 %) when compared to other varieties. Sapna *et al.* (2022) also reported the highest phosphorous content in V1 check (058 %) followed by *M. indica*× C-776 (0.57 %).Karthick Mani Bharathi *et al.*, 2024 reportedin MR2, mulberry leaves have found phosphorous content (0.28%).

3.3 Potassium (%)

The ME-65 × V1 mulberry hybrid recorded maximum potassium content of (2.15%) followed by ME-146 × MI-66 (2.04%). Whereas lowest potassium content (1.45 %) was recorded by ME-03× MI-66 hybrid compared to other hybrids (Table 3). The present results are in corroborative with the findings of Ruth*et al.* (2019) also reported highest potassium content (1.952 ± 0.072) in TR10 mulberry compared to other varieties. Sapna *et al.* (2022) recorded the highest potassium content of 1.71 percent in hybrid MI-47 × MI-66.Karthick Mani Bharathi *et al.*, 2024reportedin MR2, mulberry leaves have found potassium content (1.59%).

Table 3: Major nutrients (Nitrogen, Phosphorous and Potassium) content in leaves of elite mulberry hybrids

Sl. No.	Mulberry hybrids	Nitrogen (%)	Phosphorous (%)	Potassium (%)
1	MI-47 × MI-66	2.96	0.38	1.69
2	MI-79 × MI-66	3.23	0.41	1.58
3	ME-03× MI-66	3.28	0.36	1.45
4	ME-146 × MI-66	2.80	0.37	2.04
5	ME-65 × V1	3.47	0.50	2.15
6	ME-67 × V1	2.74	0.37	1.52
7	ME-05 x MI-66	3.11	0.37	1.78
8	ME-95x V1	2.94	0.38	1.64
9	V1	3.35	0.55	1.91
10	S36	3.03	0.46	1.83
	F-test	*	*	*
	S.Em±	0.169	0.010	0.046
	CD@5%	0.655	0.037	0.179
	CV	11.150	5.969	6.807

*Significance at 5 % level

3.4 Ca, Mg and S content

Significantly higher calcium (0.64 %) content was noticed in mulberry leaves obtained from the mulberry hybrid ME-65 × V1 followed by V1 (0.59 %) and S36 (0.56 %). Lowest calcium (0.44 %) content was noticed in ME-67 × V1(Table 4; Fig. 1). The magnesium (0.45 %) content was significantly higher in mulberry leaves obtained from the ME-65 ×V1mulberry hybrids on par with MI-79 × MI-66 (0.45 %) and followed by V1 (0.42 %) and lowest magnesium (0.28 %) content was observed in MI-79 × MI-66 mulberry hybrid

(Table 4; Fig. 1).There was significant difference among the mulberry hybrids with respect to sulphur content at 45th day after treatment. Highest sulphur (0.30 %) content was found in ME-65 × V1 followed by V1(10.26 %) and S36 (0.25 %). Whereas lowest sulphur (0.18 %) was found in ME-67 × V1 (Fig. 1).

The present results were corroborative with the findings of Vanitha (2018) who has studied the biochemical constituents of tree mulberry and bush mulberry. The calcium (1.92 and 2.11%), magnesium (0.49 and 0.54%) and sulphur (0.12 and 0.14%) were significantly maximum in tree mulberry.Shyla (2012) reported the Ca (1.71 %), Mg (0.39 %), S (0.26 %) on 75th DAP (days after pruning) in V1 mulberry garden cultivated by customized fertilizers in bush system of plantation.

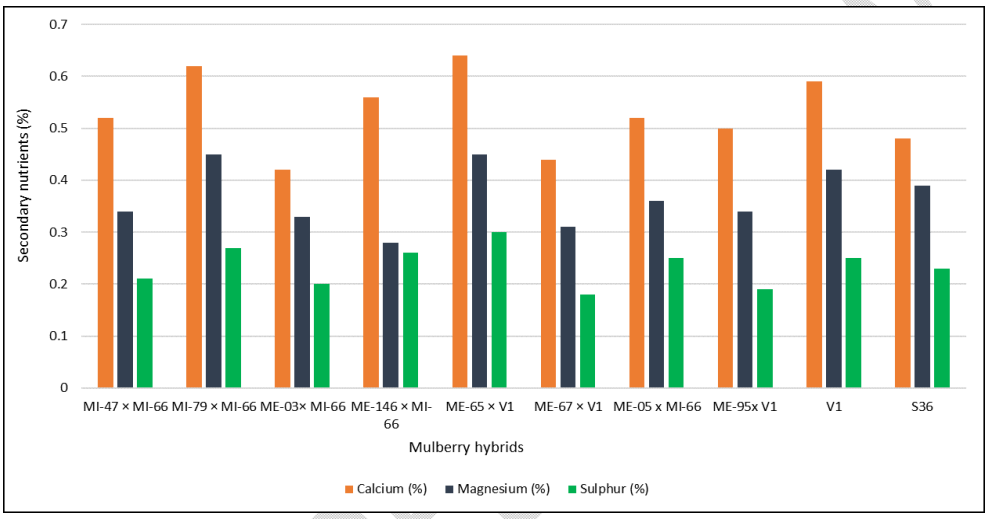

Fig 1. Response of mulberry hybrids with respect to sulphur content

3.5 Crude Protein (%)

The check variety V1had recorded significantly high crude protein content of 18.81 per cent followed by the hybrid ME-65 × V1 (18.51%) and ME-146 × MI-66 (18.44%). respectively. Whereas significantly low crude protein (14.50%) was noticed in the leaves of hybrid MI-47 × MI-66 (18.22 %) (Table 4). These findings support the results of Narayanaswamy*et al*. (2003), who reported that the highest crude protein content was 13.55 *per cent*. Crude protein was reported to be 17.91. mg/100 g by Qader*et al*. in 1991. Tewary*et al*. (2008), reported higher crude protein content (24.38 %) in tree mulberry. Additionally, Sapna *et al*. (2022) observed that the cross *M. cathyana* × C-776 recorded the highest crude protein of 23.10 per cent. Moreover Ramamoorthy*et al*. (2018) also recorded crude fibre content of 24.8 per cent and 24.6 per cent in V1 and G4 varieties of mulberry.Lohithashwa, 2023 reported Hybrid ME-65 × V1 recorded the highest crude protein (19.82 %).

3.6 Carbohydrates (%)

The check variety V1 (22.8 %) recorded the significant maximum carbohydrate content in the leaves, followed by the hybrid ME-65 × V1 (21.61 %) and ME-146 × MI-66 (20.73%). The hybrid MI-47 × MI 66 (15.65 %) registered the lowest carbohydrate content (Table 4).The findings are in accordance with Hossain *et al*. (2016) who recorded that V1 mulberry leaves had the highest carbohydrate content of 17.85 ± 0.78 per cent. Further Ramamoorthy*et*

al. (2018) also registered carbohydrate content of 18.3 per cent and 16.3 per cent in V1 and G4 varieties of mulberry. Sapna *et al.* (2022) also found that the V1 mulberry had the highest per centage of carbohydrate (17.77 %) and the hybrid BC-259 × MI-66 (15.55 %) had the lowest carbohydrate content.

3.7 Crude fibre (%)

The check variety V1 recorded highest crude fibre (9.89%) followed by hybrids ME-65 × V1 (9.79%) and ME-67 × V1 (9.26%). Whereas hybrid ME-95 x V1 recorded lesser crude fibre content (7.03%) compared to other hybrids (Table 4).Similar results were reported by Maribashetty*et al*. (1999), in their studies V1 mulberry leaves had more crude fibre (9.22 %) than other mulberry varieties. According to Manjunatha and Krishnaswami (1971), the crude fibre content of V1 mulberry was 12.50 per cent. The cross between *M. laevigata* × MI-66 (8.5 %) had registered minimum crude fibre content, as reported by Sapna *et al*. (2022). Also, Ramamoorthy*et al*. (2018) also noticed crude fibre content of 8.8 per cent and 8.8 per cent in V1 and G4 varieties of mulberry.Lohithashwa, 2023 reported Hybrid ME-65 × V1 recorded the highest crude fiber (9.68 %).

3.8 Ash content (%)

ME-67 × V1 recorded the highest ash content (12.92 %) among ten hybrids analysed, followed by ME-65 × V1 (12.45%), which is significantly higher than the check variety V1. The hybrid MI-79 × MI-66 had a lower ash content of 7.96 per cent (Table 4).The findings are consistent with Mangammal (2012) who has found that ash content was significantly higher in bush mulberry leaves of TB-21 (12.65 %) than in MI-79 (12.57 %). Additionally, Ramamoorthy*et al*. (2018) also observed ash content of 11.0 per cent and 11.2 per cent in V1 and G4 varieties of mulberry. Sapna *et al.* (2022) had also reported the lowest ash content in the hybrid *M. laevigata* × MI-66 (8.51 %).

Table 4: Biochemical constituents (Protein (%), Carbohydrate (%) and Crude fibre (%), Ash (%) in leaves of elite mulberry hybrids

Sl. No.	Mulberry hybrids	Protein (%)	Carbohydrate (%)	Crude fibre (%)	Ash content (%)
1	MI-47 × MI-66	14.50	15.65	7.76	8.32
2	MI-79 × MI-66	15.37	18.49	6.95	7.96
3	ME-03× MI-66	15.17	17.84	7.33	8.19
4	ME-146 × MI-66	18.44	20.73	8.30	10.05
5	ME-65 × V1	18.51	21.61	9.79	12.45
6	ME-67 × V1	14.97	16.00	9.26	12.92
7	ME-05 x MI-66	15.94	19.22	8.73	9.20
8	ME-95x V1	16.44	19.86	7.03	9.67
9	V1	18.81	22.28	9.89	11.61
10	S36	18.40	20.28	9.04	10.72
	F-test	*	*	*	*
	S.Em±	0.232	0.335	0.137	0.196
	CD@5%	0.902	1.304	0.534	0.760
	CV	3.618	4.533	4.242	5.025

*Significance at 5 % level

3.9 Phenols (mg/100g)

The ME-65 × V1 recorded highest phenol content 3.99 mg followed by V1 (3.82mg) and ME-146 × MI-66 (3.55mg). Whereas lowest phenol content was recorded by MI-79 × MI-66 (2.07mg) compared to other hybrids (Table 5). The present results are in concurrence with the findings of earlier workers. Tewary*et al*. (2008) reported highest phenol content of 3.18 % was recorded in tree mulberry than 2.90 % bush mulberry. Vanitha, 2018, reported highest phenol content of 3.22±0.03 mg in tree mulberry compared to bush mulberry.

3.10 Total amino acid (mg/g)

The ME-146 × MI-66 recorded highest amino acid content of 8.37 mg/g followed by V1 (8.27 mg/g) and ME-65 × V1 (8.26 mg/g). Whereas MI-47 × MI-66 recorded lowest amino acid content of 6.95 mg/g compared to other hybrids (Table 5). The present results are in agreement with the findings of Jyothi, *et al*., 2014 who reported V1 recorded highest amino acid (9.88µg/g) compared to others.

3.11Soluble Sugar content (%)

The ME-65 × V1 recorded highest sugar content of (12.66 %) followed by V1 (12.47 %) and ME-146 × MI-66 (11.95 %). Whereas least sugar content (10.38 %) was recorded in MI-47 × MI-66 compared to other hybrids (Table 5). The present results agree with the findings of earlier reports. Manjunatha and Krishnaswami (1971) recorded soluble sugars (18.90 %) in V1 bush mulberry.Tewary*et al*. (2008) reported the highest sugar content of 11.17 % in tree mulberry.

3.12 Amino acid (mg/g)

The ME-146 × MI-66 recorded highest amino acid content of 8.37 mg/g followedbyV1 (8.27 mg/g) and ME-65 × V1 (8.26 mg/g). Whereas MI-47 × MI-66 recorded lowest amino acid content of 6.95 mg/g compared to other hybrids (Table 5). The present results are in agreement with the findings of Jyothi, *et al*., 2014 who reported V1 recorded highest amino acid (9.88µg/g) compared to others.

Table 5: Biochemical constituents (Phenol (mg/100g), Soluble Sugar (%), Amino acid (mg/g) in leaves of elite mulberry hybrids

Sl. No.	Mulberry hybrids	Phenol (mg/100g)	Soluble Sugar (%)	Amino acid (mg/g)
1	MI-47 × MI-66	3.00	10.38	6.95
2	MI-79 × MI-66	2.07	10.59	7.27
3	ME-03× MI-66	2.43	10.87	7.42
4	ME-146 × MI-66	3.55	11.95	8.37
5	ME-65 × V1	3.99	12.66	8.26
6	ME-67 × V1	3.23	11.23	7.79
7	ME-05 x MI-66	3.12	10.48	7.11
8	ME-95x V1	2.13	11.10	7.57
9	V1	3.82	12.47	8.27
10	S36	3.50	11.41	7.95
	F-test	*	*	*

	S.Em±	0.101	0.094	0.143
	CD@5%	0.394	0.365	0.5543
	CV	6.967	2.158	4.807

*Significance at 5 % level

4. CONCLUSION

Based on the investigation, it can be concluded that among the eight mulberry hybrid, ME-65 × V1 was found to be superior with respect to biochemical constituents followed by MI-79 × MI-66 compared to check varieties.The mulberry leaves which are rich in optimum level of nutrients are found to accelerate the healthy growth of silkworms which reflects in the better silk gland development and increased cocoon crop production. Therefore, the careful selection and development of new mulberry varieties with respect to the regions acquires importance for the overall developmet of the sericulture industry.

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