International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Analysis of Elite Mulberry Hybrids (*Morus* spp.) for Biochemical Composition of Leaf and their Feeding Impact on Rearing Performance of Silkworm, *Bombyxmori* L.

Sapna J S[1], Dr. Chikkalingaiah[2], Dr. Kalpana B[3]

[1]PhD Scholar, Department of Sericulture, College of Agriculture, University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India
Email id: *sapnashivanna[at]gmail.com*
Contact no: 8197501768

[2]Professor, Department of Sericulture, College of Agriculture, University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India

[3]Professor, AICRP on PHET, College of Agriculture, University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India

Abstract: ***The twenty one F1 mulberry hybrids were developed using line x tester mating design using seven females and three male mulberry genotypes were planted in the field in RCBD design with three replications during the year 2019-20 at the Department of Sericulture UAS, GKVK, Bengaluru. The six best performing mulberry crosses along with check variety V1 were selected on the basis of combining ability studies with high sca effects which was found significant for leaf yield and growth parameters were selected and estimated for biochemical constituents of leaves and further bioassay studies of silkworm (Bombyxmori L.) was conducted. Results revealed that the hybrids, M. cathyana× C-776 recorded highest nitrogen content (3.70 %) and crude protein content (23.10 %), MI- 47 × MI- 66 recorded highest potassium (1.71 %), MI-47 × V1 recorded highest crude fibre (9.51 %) and ash content (9.79 %) when compared to check variety V1. Whereas, biochemical constituents viz., phosphorous (0.58 %), moisture content (69.65 %), chlorophyll (2.36 mg/g), carbohydrates (17.77 %) and phenol (3.52 %) were recorded highest in the check variety V1. Bioassay studies of silkworm revealed that rearing parameters were significantly more in the silkworms fed with the leaves of MI-47 × V1 and M. cathyana× C-776 hybrid. MI-47 × V1 recorded maximum cocoon weight, pupal weight, shell weight and silk productivity when compared to check variety V1. But none of the hybrids out yielded the check variety V1 for maximum larval weight, shell ratio, ERR and minimum larval duration.***

Keywords: Mulberry leaf quality, Mulberry hybrid, Biochemical constituents, Bioassay studies, Silkworm

1. Introduction

It is well-established fact that, in sericulture, 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 of quality and quantity. To enhance the productivity in sericulture, the mulberry leaf production per unit area has to be increased. This is made possible by developing new varieties with higher leaf yield and better adaptability through plant breeding. Silkworm (*Bombyxmori* L.) is essentially monophagous insect feeds solely on mulberry leaves (*Morus* spp.). Leaf quality is an important parameter used for evaluation of varieties aimed at selection of superior varieties for rearing performance (Yokoyama, 1963; Bongale et al., 1997). 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. 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 (Krishnaswami et al., 1971). Nutritive value of mulberry (*Morus*spp.) leaf is a key factor besides environment and technology adoption for better growth and development of the silkworms and cocoon production (Purohit and Pavankumar, 1996). Leaves of superior quality enhance the chances of good cocoon crop (Ravikumar, 1988). It is a confirmed fact that, leaf quality differs among mulberry varieties which in turn responsible for the difference in silkworm rearing performances (Bongale et al., 1997). Growth and development of silkworm *Bombyxmori* L. is known to vary depending on the quality and quantity of mulberry leaf used as food source, which in turn indicated by commercial characteristics of cocoon crop (Opender Koul *et al.*, 1979; Thangamani and Vivekanandan, 1984; Bari et al., 1989; Nagaraju, 2002). Superiority of different mulberry varieties used as food for silkworm larvae greatly affects the economy of sericulture industry (Das and Sikdar, 1970). Keeping in view, the importance of nutritional value of mulberry leaves, present study aims to evaluate better performing mulberry varieties through estimation of biochemical parameters and bioassay studies of silkworm to identify the well suited mulberry variety for rearing.

2. Materials and Methods

The mulberry hybrids were developed by following line x tester mating design for seven females and three male mulberry genotypes. These hybrids were planted in the field in RCBD design with three replications during the year 2019-20 at the Department of Sericulture UAS, GKVK, Bengaluru-65. These hybrids were maintained with all the recommended practices like weeding, fertilizer application as per the package of practices for rainfed mulberry. The best performing mulberry crosses were selected on the basis of combining ability studies with high sca effects which was found significant for leaf yield and growth parameters were selected. Six crosses showing superior quantitative traits were selected and estimated for biochemical constituents of leaves and further bioassay studies of silkworm (*Bombyxmori* L.) was conducted by feeding the leaves of individual selected mulberry crosses during winter and summer seasons in three replications to evaluate the best cross among them. The hybrids used in the study are as follows (Table 1).

Table 1: Mulberry hybrids used in study

T1	MI-47 × V1
T2	BC-259 × MI-66
T3	*M. laevigata* × MI-66
T4	MI-47 × MI-66
T5	*M. indica* × C-776
T6	*M. cathyana*× C-776
T7 (Check)	V1

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, ash, crude fibre, carbohydrates, proteins, chlorophyll and phenol contents and further statistical analysis of results was done using RCBD (Table 2).

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

Estimation (%) of parameters	Method
Nitrogen (%)	Microkjeldhal method (Piper, 1966)
Phosphorous (%)	Spectrophotometric method (Piper, 1966)
Potassium (%)	Flame photometer method (Piper, 1966)
Crude fibre	AOAC method (1955)
Crude protein	Factor method multiplied by Nitrogen % × 6.25 (Jackson, 1973)
Ash content	AOAC method (1955)
Chlorophyll content	SPAD meter
Phenols	Spectrophotometric method by Folin Ciocalteu Reagent (Piper, 1966)
Total carbohydrates	Dubios *et al* (1956)

$$\text{Crude fiber}\left(\frac{\text{g}}{100\text{g}}\right) = \frac{[100 - (\text{Moisture} + \text{Fat content of the sample})] \times \text{We} - \text{Wa}}{\text{Weight of the sample taken}}$$

$$\text{Moisture content of leaf} = \frac{\text{Fresh wt (g)} - \text{Dry wt (g)}}{\text{Fresh wt (g)}} \text{X } 100$$

Total phenol content (mg gallic acid equivalents/100g) =
OD700 nm × Standard value (µg/OD) × Total volume of extract × 100
Assay volume × Weight of tissue (g) × 1000

Bioassay studies of silkworm

Silkworm (*Bombyxmori* L.) was reared by feeding selected best mulberry crosses separately along with check variety V1 during two favorable seasons *viz.*, winter and summer seasons in three replications with 50 worms per replication. To identify the crosses with superior leaf quality, bioassay study was conducted with the double hybrid silkworm FC1 × FC2 in two different seasons and average of two seasons were used for the study. The commercial bivoltine double hybrid, Krishnaraja {FC1 (CSR6 x CSR 26) x FC2 (CSR2 x CSR27)} was used in the study. The II in star disease free worms were obtained from the registered chawki rearing centre, Vijayapura for the experiment. Two rearingwere conducted during December to January 2021 and April 2021 respectively. Observations were recorded for total larval duration (days), larval weight (g), cocoon weight(g), pupal weight (g), shell weight (g), shell ratio (%), effective rate of rearing (%) and silk productivity (cg/day) were calculated using different formulas and further statistical analysis was done using randomized complete block design (RCBD).

$$\text{Shell ratio (\%)} = \frac{\text{Cocoon shell weight (g)}}{\text{Cocoon weight(g)}} \times 100$$

$$\text{ERR(\%)} = \frac{\text{Co. of cocoons harvested}}{\text{No. of worms brushed}} \times 100$$

$$\text{Silk productivity} = \frac{\text{Shell weight (g)}}{\text{V instar larval duration (days)}}$$

The experimental data were statistically analyzed by RCBD for field experiment and CRD for bioassay studies.

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, chlorophyll, phenols, carbohydrates and moisture content were estimated in leaves from different mulberry crosses used for rearing and results are presented in table 3 and 4.

Nitrogen (%)
The mulberry hybrid *M. cathyana*× C-776 recorded slightly more nitrogen content of 3.70 per cent when compared to check V1 (3.62 %) (Table 3). The present results are corroborating with the findings of Sori and Gebreselassie (2016), observed highest nitrogen content of 4.29 per cent in Kumbi genotype.

Phosphorous (%)
The check variety V1 recorded highest phosphorous content (0.58 %) closely followed by *M. indica*× C-776 (0.57 %) (Table 3). The present results are in concurrence with the findings of earlier workers. Sori and Gebreselassie, 2016, observed highest phosphorous content of 0.45 per cent in Kumbi genotype and Lalfelpuii Ruth *et al*. (2019), also reported highest phosphorous content (1.672 ± 0.06) in Jorhat mulberry compared to other varieties.

Potassium (%)
The MI-47 × MI-66 mulberry crosses recorded maximum potassium content of (1.71 %) when compared to the check variety V1 (1.17 %) (Table 3). The present results were corroborative with the findings of Ruth *et al*. (2019), also reported highest potassium content (1.952 ± 0.072) was observed in Tr-10 mulberry compared to other varieties.

Moisture content (%)
Moisture content of mulberry leaf was recorded maximum in the variety V1 (69.65 %) followed by mulberry hybrid MI-47 × MI-66 (68.82 %). Whereas, least moisture content was recorded in BC-259 × MI-66 (63.67 %) compared to other mulberry hybrids (Table 3).

Chlorophyll (mg/g)
The check variety V1 (2.36 mg/g) recorded highest chlorophyll content of followed by *M. indica*× C-776 (1.82 mg/g) (Table 3). The present results are in agreement with the studies of Manjunatha and Krishnamurthy (2006) recorded highest chlorophyll content of 2.96 mg/g in V1 bush mulberry.

Crude protein (%)
The hybrid *M. cathyana*× C-776 (23.10 %) was noticed with higher crude protein content followed by V1 (22.63 %) (Table 4). These results are in accordance with findings of Tewary, 2005, reported higher crude protein content of 24.38 % in tree mulberry when compared to bush mulberry.

Crude fibre (%)
The MI-47× V1 (9.51 %) recorded highest crude fibre followed by V1 (9.33 %) Whereas *M. laevigata*× MI-66 recorded lesser crude fibre (8.50 %) compared to other elite hybrids (Table 4). Similar findings were reported by Manjunatha and Krishnamurthy (2006) reported crude fibre content of (12.50 %) in V1 bush mulberry.

Ash content (%)
The MI-47× V1 recorded significantly highest ash content of 9.79 % followed by V1 (9.64 %) and lowest was recorded in *M. laevigata*× MI-66 (8.51 %) compared to other elite hybrids (Table 4). These results were in line with the studies of Mangammal, (2012) who reported ash content was significantly highest in bush mulberry leaves of TB-21 (12.65 %) followed by MI-79 (12.57%).

Carbohydrates (%)
The check variety V1 recorded highest carbohydrate content of 17.77 % followed by *M. indica*× C-776 (16.78 %). The present results are in concurrence with the findings of Shakhawat *et al*. (2016), who reported tree mulberry leaves recorded highest carbohydrate content of 17.85 ± 0.78 % compared to bush and low-cut system of cultivation (Table 4).

Phenols (mg/100g)
The variety V1 recorded highest phenol content (3.52 mg/100g) followed by *M. indica*× C-776 (3.33 mg/100 g) (Table 4). The present results are in concurrence with the findings of earlier workers. Tewary *et al*. (2008), reported highest phenol content of 3.18 per cent 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.

Table 3: Biochemical constituents in leaves of selected mulberry crosses

Sl. No.	Crosses	Nitrogen (%)	Phosphorous (%)	Potassium (%)	Moisture content (%)	Chlorophyll (mg/g)
1	MI-47 × V1	2.74	0.45	1.37	66.54	1.74
2	BC-259 × MI-66	2.19	0.49	1.25	63.67	1.49
3	*M. laevigata* × MI-66	3.14	0.43	1.33	67.86	1.72
4	MI-47 × MI-66	2.48	0.41	1.71	68.82	1.60
5	*M. indica* × C-776	2.69	0.57	1.28	64.13	1.82
6	*M. cathyana* × C-776	3.70	0.42	1.16	66.90	1.65
7	V1	3.62	0.58	1.17	69.65	2.36
	C.D. (5%)	0.148	0.071	0.088	6.058	0.058
	F-test	*	*	*	NS	*
	SE. m ±	0.048	0.023	0.029	1.966	0.019

Table 4: Biochemical constituents in leaves of selected mulberry crosses

S. No.	Crosses	Crude Protein (%)	Crude fibre (%)	Ash (%)	Carbohydrates (%)	Phenols (mg/100g)
1	MI-47 × V1	17.15	9.51	9.79	16.58	3.21
2	BC-259 × MI-66	13.67	8.92	8.65	15.55	2.33
3	*M. laevigata* × MI-66	19.60	8.50	8.51	16.35	2.65
4	MI-47 × MI-66	15.48	8.65	8.74	15.62	2.60
5	*M. indica* × C-776	16.80	9.30	9.38	16.78	3.33
6	*M. cathyana*× C-776	23.10	8.85	8.79	16.56	3.24
7	V1	22.63	9.33	9.64	17.77	3.52
	C.D. (5%)	0.927	0.051	0.204	0.189	0.059
	F-test	*	*	*	*	*
	SE.m ±	0.301	0.017	0.066	0.061	0.019

Mean rearing performance of different elite mulberry hybrids on silkworm double hybrid, FC1 × FC2

Mean rearing performance of two seasons on different elite mulberry hybrids on silkworm double hybrid, FC1 × FC2 studies revealed that among the hybrids, silkworms fed with the leaves of MI-47 × V1 hybrid recorded maximum fifth instar larval weight (26.18 g), cocoon weight (1.93 g), pupal weight (1.44 g), shell weight (0.39 g), shell ratio (20.40 g), ERR (90.67 %) and higher silk productivity (4.06 %). Minimum larval duration (26.87 days) was recorded in the silkworms fed with the leaves of *M. cathyana*× C-776 hybrid. MI-47 × V1 mulberry hybrid recorded maximum cocoon weight, pupal weight, shell weight and silk productivity when compared to check variety V1. But none of the crosses out yielded the check variety V1 for maximum larval weight (30.41 g), shell ratio (20. 57 %), ERR (93.33 %) and minimum larval duration (26.85 days) (Table 5).

In the present investigation, it is indicated that among the hybrids rearing parameters were significantly more in silkworms fed with the leaves of MI-47 × V1 and *M. cathyana*× C-776 hybrid.

The results are on-par with the findings of Tayade *et al*. (1988), with significant difference in weight of the larvae and total larval duration which may be due to the maximum food consumption and growth rate during fifth instar and difference in temperature during rearing when fed with different mulberry varieties. These results are in conformity with the findings of Sujathamma *et al.* (2001).

Table 5: Mean rearing performance of different elite mulberry hybrids on silkworm double hybrid, FC1 × FC2

Hybrids	Larval weight (g)	Total Larval duration (days)	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)	ERR (%)	Silk productivity (cg/day)
MI-47 × V1	26.18	27.03	1.93	1.44	0.39	20.40	90.67	4.06
BC-259 × MI-66	23.78	27.05	1.81	1.38	0.32	17.89	88.33	3.35
M. laevigata× MI-66	25.21	26.96	1.76	1.31	0.34	19.58	88.00	3.55
MI-47 × MI-66	23.54	26.91	1.89	1.43	0.37	19.30	88.00	3.79
M. indica× C-776	23.35	26.95	1.86	1.41	0.35	18.80	85.67	3.65
M. cathyana× C-776	24.85	26.87	1.91	1.43	0.38	19.76	88.33	3.95
V1	30.41	26.85	1.85	1.37	0.38	20.57	93.33	3.93
F-test	*	*	*	*	*	*	*	*
SE.m ±	0.315	0.030	0.016	0.017	0.005	0.344	0.807	0.054
C.D. (5%)	0.956	0.091	0.047	0.053	0.015	1.042	2.447	0.163

References

[1] Association OfOffical Agricultural Chemists (AOAC). 1955. *Official methods of analysis. 8th edition*, Washington. **99**: 118-119.

[2] Bari, M. A., Qaiyyum, M. A. and Ahmed, S. U. 1989. Correlation studies in mulberry (Morus alba L.). *Indian J. Seri*.**28**(1): 11-16.

[3] Bongale, U. D. and Chaluvachari. 1995. Evaluation of eight mulberry germplasm varieties by leaf biochemical and bio-assay moulting studies. *Sericologia*.**35**(1): 83-94.

[4] Bongale, U. D., Chaluvachari, Mallikarjunappa, R. S., Narahari Rao, B. V., Anantharaman, M. N. and Dandin, S. B. 1997. Leaf nutritive quality associated with maturity levels in fourteen important varieties of mulberry (*Morus*spp.). *Sericologia*. **37**(1): 71-81.

[5] Das, B. C. and Sikdar, A. K. 1970. Evaluation of some improved strains of mulberry by feeding experiment. *Indian J. Seric*. **9**(1): 26-30.

[6] Dubois, M., Giller, K. A., Hamilton. K., Relers, P. A. and Smith, F. 1956. Studies on biochemical constituents. *Anal. Chem*. **28**: 350-356.

[7] Jackson, M. L. 1973. Soil chemical analysis: *Prentice Hall (India) Pvt. Ltd.* New Delhi. Pages 260.

[8] Krishnaswami, S., Kumararaj, S., Vijayaraghavan, K. and Kasiviswanathan, K. 1971. Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacing and nitrogen fertilization, *Indian J. Seric*. **10**: 79-90.

[9] Manjunatha, A. and Krishnamurthy. 2006. Evaluation of improved mulberry genotypes for production of leaf suitable for young age silkworm rearing with annual 12 leaf harvest schedule. *Indian J. Seric*. **39** (2): 122-126.

[10] Mangammal, P. 2012. Evaluation of mulberry germplasm for morphological characters and silkworm (*Bombyxmori L.*) rearing parameters. *Ph.D. (Seri) Thesis*, UAS, Bangalore. Pages 1-99.

[11] Nagaraju, J. 2002. Application of genetic principles in improving silk production. *Current Science.* **83**(4): 409-415.
[12] OpenderKoul, Tikku, K., Saxena, B.P. & Atal, C.K. 1979. Growth and silk production in *Bombyxmori* L. fed on three different varieties of mulberry. *Indian J. Seric.* **18**(1): 1-5.
[13] Piper, C. S. 1966. Soil and plant analysis-A laboratory manual of methods for the examination of soils and the determination of the inorganic constituents of plants. *Hans Publication, Bombay*. Pages 197-299.
[14] Purohit, K. M. and Pavankumar, T. 1996. Influence of various agronomical practices in India on the leaf quality in mulberry, A review. *Sericologia*. **36**(1): 27-39.
[15] Ravikumar, C. 1988. Western ghat as a bivoltine region prospects, challenges and strategies for its development. *Indian Silk*. **26**(9): 39-54.
[16] Ruth Lalfelpuii, Bidyuth Nath Choudhury, Gurusubramanian, G. and Senthil Kumar, N. 2014. Effect of different mulberry plant varieties on growth and economic parameters of the silkworm, *Bombyxmori* in Mizoram. *Sci. Vis*. **14**(1): 127- 134.
[17] Shakhawat Hossain, Saiful Islam and Moinul Haque. 2016. Influence of Different Cultivation Forms of mulberry variety BM-3 (*Morus alba*) on leaf nutrition, yield and economic traits of silkworm. *Elixir Applied. Chemistry*. **95**: 41169-41172.
[18] Sujathamma, P., Dandin, S. B. and Savithri. 2001. Quality evaluation of mulberry (*Morus*spp.) genotypes through bioassay under Rayalaseema conditions of Andhra Pradesh. *Indian J. Seric*. **40**(1): 27-34.
[19] Tayade, D. S., Jawale, M. D. and Unchegaonkar, P. K. 1988, Evaluation of some improved strains of mulberry by feeding experiment under Marathwada conditions. *Indian J. Seric*. **22**(2): 92-94.
[20] Tayade, D. S., Jawale, M. D. and Unchegaonkar, P. K. 1988. Evaluation of some improved strains of mulberry by feeding experiment under Marathwada conditions. *Indian J. Seric*. **22**(2): 92-94.
[21] Tewari, R. K. 2005. Antioxidant responses to enhanced generation of superoxide anion radical and hydrogen peroxide in the copper-stressed mulberry plants. *Springer*. **223**: 1145–1153.
[22] Tewary, P. K., Singh, M. K., Sinha, U. P. S. and Bajpai. 2008. Mulberry as small tree – a new approach for sustainable sericulture in Jharkhand. *Indian Silk*. **46**(7): 10-11.
[23] Thangamani, R. and Vivekanandan, M. 1984. Physiological studies and leaf nutrient analysis in the evaluation of best mulberry variety. *Sericologia.* **24**: 317-324.
[24] Vanitha. 2018. Assessment of productivity of tree mulberry, M*orus alba* L. *MSc. Thesis,* UAS, GKVK, Bengaluru. Pages 35-70.
[25] Waktole Sori and Wosene Gebreselassie. 2016. Evaluation of mulberry (*Morus*spp.) Genotypes for growth, leaf yield and quality traits under south west Ethiopian condition. *J. Agron.* 173-178.
[26] Yokoyama, T. 1963. Sericulture. *Annual Review of Entomol.* **8**: 287-306.