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Article

Studies on the Leaf Nutrition Value in Different Mulberry (*Morus alba* L.) Cultivars

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ABSTRACT

Mulberry leaf exclusively assures the growth and development of the silkworm larvae, being considered a complete value nutrient, so that the knowledge of its nutritional status is of great interest. The experiment was made to study the mulberry leaf protein value of the mulberry varieties: V1, M5, S13, S36, and Anantha. The content of the nutritional management in mulberry leaves, considered as the first step in the study of nutritive value and varied according to the mulberry cultivars leaf taken apical, medium, and coarse leaves, moisture 75–82%; crude protein 24–56%; ash (minerals) 7–8%; and carbohydrate 12–20%. The mulberry leaf reaffirms as a valuable nitrogenous source. The mulberry variety V1 showed the highest nutritional content followed by S36, M5, S13, and Anantha, comparatively among the five mulberry cultivar.

Key words: Mulberry, Macronutrients, Micronutrients, *Morus alba* L.

1. INTRODUCTION

The nutritional management of mulberry is a very difficult one unlike other crops. It is fascinating to note that plant leaf tissue analysis has revealed the presence of some 16 elements in the leaf. Of these 16 elements, six elements, namely, nitrogen (N), phosphorus (P), and potassium (K), are primary nutrients. Calcium (Ca) and magnesium (Mg) are used in large quantities by plants as secondary nutrients, while other seven, namely, Zn, Fe, Cu, and Mn are called micronutrients [1-4].

Mulberry (*Morus alba* L.) is the only food plant of silkworm (*Bombyx mori* L.) on commercial scale with great economic importance to sericulture industry. Silkworm requires the maximum quantity of good quality mulberry leaf for successful cocoon production. Chemical composition of mulberry leaf is influenced by variety, spacing, irrigation levels, nitrogen levels, and seasons [4-7]. Different factors responsible for a successful cocoon crop are mulberry leaf (38.2%), climate (37%), rearing technique (9.3%), silkworm race (4.2%), silkworm eggs (3.0%), and other factors (8.2%) [8]. The crop responses are known to vary with levels of nitrogen, since the mulberry is being grown on variety of soils under different agro-climatic conditions.

India produced 16,319 MT of raw silk during 2003-04, of which 14,617 MT is being contributed by mulberry silkworm, which makes it the second-largest producer in the world next only to China. Tasar, Eri, and Muga contribute 284, 1316, and 102 MT, respectively. Karnataka state has 88903 ha under mulberry cultivation and producing 6760 MT of raw silk sharing 65.75% of Indian raw silk production [9].

2. MATERIALS AND METHODS

Mulberry (*M. alba* L.) cultivars, namely, V1, S13, S36, M5, and Anantha were obtained from Regional Sericulture Research Station, Ananthapuramu. The plant cutting of the above cultivars prepared from 9 months old, healthy hardwood branches which are at least half-inch in diameter. These were prepared for plantation according to Krishnaswamy *et al*. [6]. The stem cutting made of approximately five to six inches long with a minimum of 3–4 active buds, while preparing the cutting care was taken to see that the ends of the cutting were clean without split and bark peeling off. The cutting was brought to the laboratory and immediately planted in earthen pots (15–10″) containing 8 kg air-dried red loamy soil and farmyard manure in 3:1 proportion. The pots were maintained in the departmental botanic garden under photoperiod (10–12 h temperature 28 ± 4°C) by supplying water daily.

2.1. Mineral Nutrition

Mineral nutrition includes the supply, absorption, and utilization of essential nutrients for growth and yield of crop plants. Plants require 13 essential mineral nutrients to complete their full lifecycles.

2.1.1. Estimation of nitrogen

The total process of nitrogen estimation was down through three distinct steps, i.e., digestion of the leaf samples, distillation, and titration as per the Kjeldahl method.

2.1.2. Digestion of the leaf samples

A quality of 0.1 g of finely powdered mulberry leaf sample was taken in a digestion tube followed by adding 10 ml of concentrated H_2SO_4. A pinch of catalyst (K_2SO_4 and $CuSO_4$ in 5:1 ratio) was added to few microliters of 30% H_2O_2 to speed up the digestion. All the digestion tubes were kept in a digestion unit up to 300°C for 1.5 h when the solution became colorless.

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2.1.3. Distillation

All the digestion tubes were kept sequentially in Tecator kjeltec system distillation unit, which automatically added 10 ml of 40% NaOH, and the contents were distilled with alkali. Twenty-five microliters of 4% boric acid solutions were taken separately in conical flasks followed by adding two drops of mixed indicator (bromocrysal green 5 g + methyl red 0.1 g in 100 ml absolute alcohol) which became purple-red in color. Then, the solutions were kept in the distilling system for allowing the distillation vapor to get trapped in the boric acid solution. After the solution of distillation, the solution became bluish-green in color.

2.1.4. Titration

The conical flasks were taken out from the Kjeltec system, and the contents were titrated with 0.1 N H_2SO_4 until the color became purple-red. A blank was also made with the distilled water, which was also titrated to calculate the total nitrogen percentage.

$$\text{Total N}(\%) = \frac{14 \times (\text{Sample titration value} - \text{Blank value}) \times \text{Normality of the acid}}{\text{Sample weight (g)} \times 1000} \times 100$$

2.2. Phosphorus Estimation

Phosphorus was determined according to the method described by Chapman and Parker using a spectrophotometer. The color of the sample was developed by adding 5 ml of dilute H_2SO_4 (1:6), 5 ml of 5% ammonium molybdate, and 5 ml of 0.25% ammonium vanadate. The standard curve was developed using different concentrations of potassium dihydrogen phosphate. The colored samples were fed in using IRMECO, UV-visible is spectrophotometer Model U2020, and transmittance at a wavelength of 420 nm was recorded, which was compared with that of the standard curve to find out the quantity of element in ppm which was then converted into percentage using the following formula.

$$\text{P (\%)} = \frac{\text{ppm on graph} \times \text{Dilution}}{10^6} \times 100$$

2.3. Potassium Estimation

Potassium was determined by flame photometer according to the method described by Chapman and Parker. Quantity of element was estimated in ppm by comparing the emission of flame photometer with that of the standard curve, which was then converted into percentage using the following formula.

$$\text{K (\%)} = \frac{\text{ppm on graph} \times \text{Dilution}}{10^6} \times 100$$

The organic matter present in the sample (1 g) was wet digested with 25 ml of diacid mixture (HNO_3: $HClO_4$ in 5:1) and kept overnight. Digestion was done on the next day by heating till clear white precipitates settle down at the bottom. The crystals were dissolved by diluting in double-distilled water. The contents were filtered through Whatman No. 42 filter paper. The filtrate was made up to the volume of 25 ml. The digested samples were analyzed for the determination of phosphorus, magnesium, and Iron by Counter Atomic Absorption 700 Apparatus.

2.4. Calcium

Calcium is precipitated as calcium oxalate. The precipitate is dissolved in hot dilute H_2SO_4 and titrated against standard potassium permanganate. Pipette an aliquot (25 ml) of the ash solution obtained by dry ashing to a 250 ml conical flask. Dilute to 150 ml with water. Add a few drops of methyl red indicator (0.5 g in 100 ml of 95% alcohol) and neutralize the mixture with ammonia (concentrated) till the pale pink color changes to yellow. Heat the solution to the boiling point, add 10 ml of ammonium oxalate (6%) and boil again for a few minutes. Add glacial acetic acids (concentrated) until the color of the mixture is distinctly pink. Allow to stand at room temperature for at least 4 h or preferably overnight. Filter through Whatman No. 42 paper and wash with warm water, till the filtrate is oxalate free. (Since HCl had been used for preparing the ash solution, it is convenient to test for the absence of chloride using $AgNO_3$). Add 5–10 ml of dilute H_2SO_4 (2N) on the filter paper, break the point of the filter paper with a pointed glass rod and transfer it to the conical flask. The solution is heated to 70°C and titrated against 0.01 N $KMnO_4$ to a permanent pale pink color.

Calculation: 1 ml of 0.01N $KMnO_4$ = 0.2004 mg of calcium

If the normality of the standard $KMnO_4$ solution is not exactly 0.01N, the following formula can be used.

$$\text{Ca mg/100 g} = \frac{\text{Titervalue} \times \text{Normality of } KMnO_4 \times 0.2004 \times \text{Total volume of ash solution} - \text{md}}{\text{ml of ash solution taken for estimation} \times \text{Weight of sample taken for ashing}} \text{ x } 100$$

2.5. Micro Minerals

Zinc, copper, magnesium, iron, and manganese were analyzed by atomic absorption spectrophotometer (AAS). In AAS, light radiation from a specific wavelength from a hollow cathode lamp (HCl-cathode made of specific metal to be assayed) passes through the flame to the detector. The ash solution is aspirated into the flame (Temperature = 2400°C). The sample is atomized in the flame, where the atoms of the element which are in the ground state, absorb energy from the hollow cathode lamp radiation and go to the excited state. The amount of radiation energy absorbed by the element is proportional to its concentration of metal under assay. Instrument parameters such as resonant wavelength, slit width, and air-acetylene flow rate that are appropriate for each element were selected (AOAC, 2000). The instrument was set up and calibrated as per the guidelines in the manual provided by the manufacturer. A calibration curve (concentration vs. absorbance) for each mineral to be determined was prepared using a range of working standards. The flame parameters were optimized in accordance with the instrument manufacturer's instructions. Micronutrients calculated ppm values.

The standard solutions were read before and after each group of 6–12 samples. The burner was flushed with water between samples, and zero was reestablished each time. Sui dilutions of the ash solutions were made to read the content of the minerals in the ash solution. In the case of sodium and potassium assay, the ash solutions as well as standard sodium and potassium should have a cesium content of 0.5% (w/v). Lanthanum chloride solution was added to the final dilution of each standard and test dilution to make 0.1% (w/v) lanthanum for the determination of magnesium only. The concentration of metals in ash solutions of samples as well as in blank solutions was read from the calibration curve and the concentration in the test sample calculated taking into account the dilutions and the weight of the sample taken.

Calculation:

$$\text{Concentration of metal in sample} = \frac{(CS - CB) \times V \times D}{W}$$

Where: CS = Concentration of metal in ash solution of sample (µg/ml)
CB = Concentration of metal in blank solution (µg/ml)
V = Volume of ash solution made up (ml)
D = Dilution volume (ml)/aliquot taken for dilution (ml), if original solution is diluted
W = Sample weight (g).

3. RESULTS AND DISCUSSION

3.1. Composition of Mulberry Leaves

Mulberry leaves are highly digestible and contain the following high concentrations of crude protein and minerals. The leaves consist of moisture 75–82%; crude protein 24–56%; ash (minerals) 7–8%; and carbohydrate 12–20%. Mineral content details are summarised in Tables 1-5.

3.2. Macronutrients

It is a constituent of protein. It is the basic nutrient in the synthesis of proteins, amino acids, chlorophyll, and alkaloids. Nitrogen increases the vegetative growth, no, size, and weight of leaves and ultimately yield. The leaves become succulent improving their feed value to the silkworms.

The mulberry cultivar V1 was recorded significantly higher nitrogen content in 9th leaves (3.34%), 7th leaf (3.29%), 5th leaves (3.07%), and 3rd leaves (2.83%), followed by S36 9th–3rd leaves (3.49%, 3.22%, 3.17%, and 2.76%); M5 (3.13%, 3.08%, 2.99%, and 2.56%); and S13 9th–3rd leaf (3.11%, 3.02%, 2.82%, and 2.85%).

The mulberry cultivar V1 was recorded significantly higher phosphorus content in 9th leaves (0.29%), 7th leaf (0.14%), 5th leaves (0.19%), and 3rd leaves (0.16%), followed by S36 9th–3rd leaves (0.24%, 0.19%, 0.18%, and 0.16%); M5 (0.22%, 0.20%, 0.17%, and 0.18%); and S13 9th–3rd leaf (0.10%, 0.14%, 0.15%, and 0.13%) and lowest in Anantha (0.20%, 0.17%, 0.19%, and 0.15%).

Table 1: Mineral constituents of V1 mulberry cultivar different stages.

Varieties	Macronutrients percentage of dry matter					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
3rd leaf	2.83	0.16	1.93	1.85	0.58	0.14	0.09	0.12	0.8
5th leaf	3.07	0.19	1.98	2.06	0.59	0.15	0.11	0.14	0.10
7th leaf	3.29	0.14	2.24	2.17	0.61	0.18	0.13	0.15	0.10
9th leaf	3.34	0.21	2.45	2.32	0.67	0.21	0.13	0.17	0.12

Table 2: Mineral constituents of S13 Mulberry cultivar different stages.

Varieties	Macronutrients percentage					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
3rd leaf	2.85	0.13	1.87	1.64	0.44	0.16	0.08	0.14	0.07
5th leaf	2.82	0.15	1.90	1.77	0.52	0.18	0.10	0.15	0.09
7th leaf	3.02	0.14	2.14	1.90	0.55	0.20	0.11	0.17	0.10
9th leaf	3.11	0.19	2.23	2.16	0.59	0.22	0.13	0.19	0.13

Table 3: Mineral constituents of S36 Mulberry cultivar different stages.

Varieties	Macronutrients percentage					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
3rd leaf	2.76	0.16	1.87	1.73	0.55	0.15	0.07	0.11	0.06
5th leaf	3.17	0.18	1.99	1.92	0.58	0.18	0.10	0.13	0.09
7th leaf	3.22	0.19	2.25	2.17	0.58	0.19	0.12	0.15	0.11
9th leaf	3.49	0.24	2.39	2.34	0.63	0.21	0.13	0.16	0.12

The mulberry cultivar V1 was recorded significantly higher potassium content in 9th leaves (2.45%), 7th leaf (2.24%), 5th leaves (1.98%), and 3rd leaves (1.93%), followed by S36 9th–3rd leaves (2.39%, 2.25%, 1.99%, and 1.87%); M5 (2.15%, 1.99%, 1.92%, and 1.89%); and S13 9th–3rd leaf (2.23%, 2.14%, 1.90%, and 1.87%) and lowest in Anantha (2.07%, 1.82%, 1.73%, and 1.56%).

3.3. Micronutrients

The supply of micronutrient in adequate amount and in proper proportion is one of the main factors which govern the growth, development, and yield of mulberry. In the absence of micronutrients, the plants are known to suffer from physiological disorders which eventually lead to low yield and loss of quality. Some of the important factors which directly or indirectly affect the availability of micronutrients to mulberry are soil conditions such as the soil reaction, organic matter content, nature and amount of clay minerals, microbiological activity, physical and chemical conditions of the soil as well as water aeration and climatic conditions of the locality.

The mulberry cultivar V1 was recorded significantly higher calcium content in 9th leaves (2.32%), 7th leaf (2.17%), 5th leaves (2.06%), and 3rd leaves (1.85%), followed by S36 9th–3rd leaves (2.24%, 2.17%, 1.92%, and 1.73%); M5 (2.26%, 2.15%, 1.86%, and 1.77%); and S13 9th–3rd leaf (2.16%, 1.90%, 1.77%, and 1.64%) and lowest in Anantha (2.13%, 1.73%, 1.94%, and 1.60%).

The mulberry cultivar V1 was recorded significantly higher magnesium content in 9th leaves (0.67%), 7th leaf (0.61%), 5th leaves (0.59%), and 3rd leaves (0.58%), followed by S36 9th–3rd leaves (0.63%, 0.58%, 0.58%, and 0.55%); M5 (0.61%, 0.63%, 0.56%, and 0.55%); and S13 9th–3rd leaf (0.59%, 0.55%, 0.52%, and 0.44%) and lowest in Anantha (0.61%, 0.58%, 0.53%, and 0.39%).

The mulberry cultivar V1 was recorded significantly higher iron content in 9th leaves (0.21%), 7th leaf (0.18%), 5th leaves (0.15%), and 3rd leaves (0.14%), followed by S36 9th–3rd leaves (0.21%, 0.19%, 0.18%, and 0.15%); M5 (0.19%, 0.15%, 0.18%, and 0.11%); and S13 9th–3rd leaf (0.22%, 0.20%, 0.18%, and 0.16%) and lowest in Anantha (0.19%, 0.16%, 0.15%, and 0.13%).

Table 4: Mineral constituents of M5 Mulberry cultivar different stages.

Varieties	Macronutrients percentage					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
3rd leaf	2.56	0.18	1.89	1.77	0.55	0.11	0.09	0.13	0.06
5th leaf	2.99	0.17	1.92	1.86	0.56	0.18	0.10	0.15	0.09
7th leaf	3.08	0.20	1.99	2.15	0.63	0.15	0.13	0.16	0.11
9th leaf	3.13	0.22	2.15	2.26	0.61	0.19	0.12	0.18	0.11

Table 5: Mineral constituents of Anantha mulberry cultivar different stages.

Varieties	Macronutrients percentage					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
3rd leaf	2.72	0.15	1.56	1.60	0.39	0.13	0.05	0.11	0.06
5th leaf	2.97	0.19	1.73	1.94	0.53	0.15	0.07	0.12	0.07
7th leaf	3.15	0.17	1.82	1.73	0.58	0.16	0.08	0.14	0.09
9th leaf	3.07	0.20	2.07	2.13	0.61	0.19	0.11	0.16	0.11

The mulberry cultivar V1 was recorded significantly higher copper content in 9th leaves (0.13%), 7th leaf (0.13%), 5th leaves (0.11%), and 3rd leaves (0.09%), followed by S36 9th–3rd leaves (0.13%, 0.12%, 0.10%, and 0.07%); M5 (0.12%, 0.13%, 0.10%, and 0.09%); and S13 9th–3rd leaf (0.13%, 0.11%, 0.10%, and 0.08%) and lowest in Anantha (0.11%, 0.08%, 0.07%, and 0.05%).

The mulberry cultivar V1 was recorded significantly higher manganese content in 9th leaves (0.17%), 7th leaf (0.15%), 5th leaves (0.14%), and 3rd leaves (0.12%), followed by S36 9th–3rd leaves (0.16%, 0.15%, 0.13%, and 0.11%); M5 (0.18%, 0.16%, 0.15%, and 0.13%); and S13 9th–3rd leaf (0.19%, 0.17%, 0.15%, and 0.14%) and lowest in Anantha (0.16%, 0.14%, 0.12%, and 0.11%).

The mulberry cultivar V1 was recorded significantly higher zinc content in 9th leaves (0.12%), 7th leaf (0.10%), 5th leaves (0.10%), and 3rd leaves (0.8%), followed by S36 9th–3rd leaves (0.12%, 0.11%, 0.09%, and 0.06%); M5 (0.11%, 0.11%, 0.09%, and 0.06%); and S13 9th–3rd leaf (0.13%, 0.10%, 0.09%, and 0.07%) and lowest in Anantha (0.11%, 0.09%, 0.07%, and 0.06%).

4. CONCLUSION

In the present study, authors estimated the micronutrients in mulberry leaves, the content of the nutritional management in mulberry leaves varied according to the mulberry cultivars leaf taken apical, medium, and coarse leaves. The leaves consist of moisture 75–82%, crude protein 24–56%, ash (minerals) 7–8%, and carbohydrate 12–20%. The mulberry variety V1 showed the highest nutritional content followed by S36, M5, S13, and Anantha, comparatively among the five mulberry cultivar.

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