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STUDIES ON BIOCHEMICAL CONSTITUENTS OF DIFFERENT GENOTYPES OF *MORUS ALBA* L.

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ABSTRACT

Growth and development of silk worm *Bombyx mori L*. and cocoon crop are greatly influenced by yield and nutritional quality of mulberry leaf used as feed. The nutritional status of different mulberry varieties is ascertained by its biochemical constituents. Mulberry is rich sources of protein, carbohydrate, carotenoids, lipids, ascorbic acid, anthocyanins etc Hence, five mulberry varieties viz., M_5, RFs-135, V_1, S_{36} and S_{13} were analysed for their leaf quality through phytochemical tests. Results reviled that V_1 is the best one containing highest total soluble protein (111.40mg/g), total free amino acid(9.88µg/g) and total phenol(4.96%) contents compare to other four varieties, so V1 is the highly recommendable feed for silkworm (*Bombyx mori L)* to increase their silk productivity.

KEYWORDS: **Silk worm, Mulberry leaf, proteins, phenols, amino acid**

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INTRODUCTION

Indian Mulberry generally known as *Morus alba* belongs to the family Moraceae of Monochlamydeae of Bentham and Hooker. It is a small genus of trees or shrubs spread in the temperate and subtropical regions of the World. There are about 5 species in India. It is valued for their foliage which constitutes the chief feed for Mulberry silkworms (Bombyx *mori* L.). The common species found in India are, *Morus alba, Morus indica Linn, Morus atropurpurea roxb, Morus nigra, Morus serrata* and *Morus laevigata.* Young leaves which have attained full size are best suited for feeding silkworm larvae[1]. The composition of leaves varies with variety, degree of maturity and the type of soil in which the plants are grown. Silk protein produced by silkworm, *Bombyx mori L*. is directly derived from broad spectrum of proteins and amino acids available in the leaves of mulberry, *Morus alba L.*[2]. Nutritive value of mulberry (*Morus spp*.) leaf is a key factor besides environment and technology adoption for better growth and development of the silkworm larvae and cocoon production[3]. Biochemical constituents of mulberry leaves play an important role for successful cocooning in silkworm, *Bombyx mori* L.[4]. The quality of feed is determined by its major components such as water, carbohydrates, proteins, mineral, elements, fats, amino acids and vitamins. Improvement of quality foliage seems to be an essential part of sericulture.

Silkworm (*Bombyx mori L*.) is essentially monophagous insect feeds solely on mulberry leaves (morus spp.). Its growth and development as well as cocoon and silk production entirely depends upon the quantity and quality of mulberry leaves[5]. Leaf quality is an important parameter used for evaluation of varieties aimed at selection of superior varieties for rearing performance[6]. In spite of this, the qualities of mulberry leaf also vary with age, leaf position and variety of same species. Narayanan etal.[7] also recorded some different varietal superiority of different varieties as food for silkworm greatly affects the economy of sericulture industry. The present work has been amid to determine the total soluble protein, free amino acid and total phenol content of five mulberry (M_5, RFs-135, V_1, S_{36} and S_{13}) varieties grown in Anantapuram agro climatic conditions.

MATERIALS AND METHODS

Mulberry leaf samples preparation

Five popularly cultivated indigenous mulberry (M_5, RFs-135, V_1, S_{36} and S_{13}) varieties were chosen. The healthy leaves were collected from middle portion of mulberry plants situated in Botanical garden, Department of Botany, Sri Krishnadevaraya University, Anantapuramu (District), Andhra Pradesh (State), India.

(i) Estimation of total soluble proteins

Protein content of leaves was estimated by Lowry's method (Lowry *et al.,* 1951)[8].The blue colour developed by the reduction of the phosphomolybdic phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method. Extraction is usually carried out with buffers used for the enzyme assay. 500 mg of the sample was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer. This solution was centrifuged and the supernatant was used for protein estimation. 0.2,0.4,0.6,0.8 and 1 ml of the working standards were pipetted into a series of test tubes 0.1 ml and 0.2 ml of the sample extract was pipetted in two other test tubes. The volume was made to 1 ml in all the test tubes. The tube with 1 ml of water served as the blank. 5 ml of reagent was added to each tube including the blank. This was mixed well and allowed to stand for 10 min. Then 0.5 ml of reagent D was added, mixed and incubated at room temperature in the dark for 30 min. Blue color was developed. The readings were recorded at 660 nm. Standard graph was drawn and the amount of protein in the sample was calculated. Protein percentage was calculated in relation to fresh weight and dry weight basis. The amount of protein mg/g or 100 g sample was expressed.

(ii) Estimation of total free amino acids

Total free amino acids were extracted and determined following the method of Sugano et al.(1975)[9] with slight modifications. The leaf (0.5 g) was homogenized in 70% ethanol in a pestle and mortar. The homogenate was centrifuged at 5,000 g for 10 min and the supernatant was taken. The extraction was repeated four to five times and the supernatants were combined. An appropriate volume (5–10 ml) of this ethanolic extract was evaporated to dryness on a boiling water bath and the residue was dissolved in 5 ml of 0.2 M citrate buffer (pH 5.0). The above sample (2 ml) was taken in a test tube and 1 ml of ninhydrin reagent (4% ninhydrin in methyl cellosolve and 0.2 M acetate buffer in the ratio of 1:1) was added to it. The samples were boiled for 20 min and cooled; the volume was made up to 10 ml with distilled water. Absorbance was noted at 570 nm. Total free amino acids were calculated from a standard curve prepared against glycine(0–100 lg).

(iii) Estimation of total phenol

The total phenol content in the leaf samples was estimated by the method described by Malick and Singh (1980)[10]. Weigh exactly 0.5g of leaf samples of different genotypes and grind it with a pestle and mortar in 10-time volume of 80% ethanol. Centrifuge the homogenate at 10,000rpm for 20min. save the supernatant. Re-extract the residue with five times the volumes of 80% ethanol, centrifuge and pool the supernatants. Evaporate the supernatant to dryness. Pipette out aliquots (0.2ml to 1.0ml) into test tubes. Make up the volumes in each tube to 3ml with double distilled water. Add 0.5ml of folin-ciocalteau reagent. After 3min, add 2ml of 20% Na_2CO_3, solution to each tube. Mix thoroughly, place the tubes in a boiling water bath for exactly 1min, cool and measure the absorbance at 650nm against a reagent blank. Prepare a standard curve using different concentration of gallic acid. From the standard curve the concentration of phenols in the test samples were estimated.

RESULTS

Deficiency of plant nutrients in mulberry leaves causes changes in the metabolic activity of silkworm larva. Nutritional contents of mulberry leaf greatly manipulate the growth and development of silkworm larvae, which in turn affects the quality and quantity of silk production.[12], [13] Leaf quality is a vital parameter used for evaluation of mulberry varieties while selecting preeminent varieties for silkworm rearing and biochemical composition of mulberry leaves varies depending on variety, season, soil, water and cultural practices.[11-12]. The quantitative determinations of various nutritional parameters in leaves of *Mors alba L.* genotypes(M_5, RFs-135, V_1, S_{36} and S_{13}) like total soluble protein, total free amino acids and total phenol contents were estimated and recorded in table 1.

Table I

Biochemical analysis offive different mulberries (Morus alba L)

S.No	Mulberry Varieties	Total soluble protein (mg/g)	Free amino acids (µg/g)	Total phenol%
1	M_5	109.85	7.48	2.88
2	RFs-135	79.14	7.22	3.72
3	V_1	111.40	9.88	4.96
4	S_{13}	82.23	8.02	3.62
5	S_{36}	85.15	8.16	3.66

DISCUSSION

Sericulture in India is an important cottage industry based on agro forestry earning foreign exchange worth about Rs.1500 crores per annum. Presently, sericulture is practised in more than 60,000 villages providing employment to 60 lakh people who hail from the weaker section of the society and are in rural areas. Silk production has reached over 15,000 tones and India is the second largest silk producer (18% of world production) after

China (69%). Even so, our present production falls very much short of the domestic demand. Nearly 90% of our silk is mori silk or mulberry silk produced by the silkworm *Bombyx mori* L. Mulberry leaf quality growth under tropical conditions of India, the first available literature was that of Dasgupta[13] who found differences among leaves of free and bush plantations of mulberry. After, Narayanan et al[14] and Sidhu et al[15] have reported about the quality differences in leaves due to varity, irrigation and manuring. Mulberry improvement is also aimed at bringing qualitative improvement of leaves and a survey of the available literature reveals that extensive studies have been carried out on the varietal response, effect of agronomical inputs, seasons and related aspects on biochemical composition of leaves. Matsumara et al[16], Tangamani and vivekanandan[17], Lie and Sano[18], Fotadar et al[19] and, Chaluvachari and Bongale[20] discussed the importance of quality of mulberry leaves used as feed for silkworm. Growth and development of silkworm (*B. mori* L.) and the cocoon crop yield are influenced largely by the varietal difference and nutritional quality of mulberry (*Morus* sps.) leaf used as food**.** Leaf protein is a major determinant of nutrient quality for many Lepidopteron larvae. It is known fact that, nearly 70% of protein content of raw silk namely fibroin and sericin are directly biosynthesized from mulberry leaf protein and remaining 30% is derived from silkworm body tissue and haemolymph protein, emphasizing the importance of leaf protein in silkworm nutrition[21-23]. Protein content present in different mulberry varieties had a direct bearing on larval growth particularly in silk gland development and cocoon characters of silkworm[24]. Biochemical composition of five different mulberry varieties (M_5, RFs-135, V_1, S_{36} and $S_{13)}$ leaves indicated variation in total protein content. Similar studies were conducted and observed a significant varietal difference in protein content[25, 26].

Mulberry leaves are quite rich in amino acid content (18.6%), forms an important constituent for silkworm nutrition[27, 28]. Considerable amounts of amino acids utilized for the formation of haemolymph, development of silk glands and cocoon production. Amino acids are important for phytophagous insects help them in food selection[29]. Phenolic compounds are responsible for disease resistance in plants and function as hydrogen donors or acceptors in oxidation/reduction reactions. A small change in the host phenol metabolism severely disrupts many processes, which are essential for normal growth and development of plants. Increase of phenolic compounds may result from either synthesis of new aromatic compounds and/or the acceleration of accumulative phenols from neighboring cells[30]. Several workers have reported that, disease resistant varieties possess higher phenol content compared to that of susceptible ones[31]. Presence of higher concentrations of phenolic compounds is considered to be one of the major factors for an incompatible host pathogen interaction[32]. Phenols possess a wide spectrum of biological activities and results show that mulberry extracts could be good sources of these natural constituents[33]. Biochemical analysis of five different varieties of mulberry leaves were analyzed among the five varieties V1 contains highest total soluble protein, free amino acid and phenols. So, V1 mulberry leaves are highly recommendable for mulberry silkworm for their increased silk productivity.

CONCLUSION

The above result and discussion confers V1 has high nutritive value compared with M_5, RFs-135, S_{36} and S_{13} genotypes and also its growth and yield parameter is more convenient compared with other types. So, V1 variety gives good yield and also recommended as a best genotypic feed with high nutritive value for the Silkworm.

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