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STUDIES ON CHLOROPHYLL CONTENT, SOLUBLE PROTEIN, CARBOHYDRATES AND MOISTURE CONTENT OF *Morus alba Linn.*

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ABSTRACT
Proper cultivation methods, proper irrigation of mulberry field, proper construction of the rearing houses, proper rearing space for the larvae in the tray, proper sanitation and hygienic methods are some of the subsidiary factors in increasing the cocoon production and yarn production. Vellore district on the North part of Tamil Nadu stand as the second largest producer of mulberry silk in the State. Hence an attempt is made in this study the impact of rearing space leaf quality, diet rationing and sanitation measures on silkworm rearing in Vellore district. From the data obtained in rearing space experiments, it is concluded that 1.39 m^2 is required for 100 DFLs for the I instar larvae of cross breed proportionately the space and larvae is multiplied into 3 times into second, third, fourth and fifth instar stages of larva. The spacing is increased for hybrid larvae because of their large size. From the present study which has shown light on the rearing space, leaf quality, diet rationing and sanitation measures the following suggestions are made for the future mulberry cultivation, rearing methods, management and future prospects. The most farmers in Sericulture Industry in this district are using Mysore local variety of mulberry which has less content of moisture, chlorophyll, protein and carbohydrate when compared to MR_2 variety. Hence this research suggests the farmers to cultivate only MR_2 variety of mulberry leaf.

KEYWORDS: *Morus alba,* chlorophyll content, soluble protein, carbohydrates, moisture contents, experimental methods etc.

INTRODUCTION

India is the second largest producer of silk after China. Hence the scope for increasing the silk production in our country is immense. In India the major states concentrating on silk production are Karnataka, Andhra Pradesh, Tamil Nadu, West Bengal and Jammu & Kashmir. Nearly 2,146.905 acres are found under the cultivation of mulberry in this district and annual yield of 13,418 tonnes of mulberry is harvested every year. Commonly Mysore local, kanva-2, Mr2, S36 and S54 are cultivated in this district Natrampalli, Parnampet and Alangayam range consist maximum number of rearing houses. The cross breed (MV x B) Lx NB_4D_2 variety of *Bombyx mori* is used largely for the cocoon production. Hybrids like NB_7X NB_4D_2 (BVx BV) are also used in some places. From the temperature, humidity and rainfall obtained throughout the year October to January are more humid and rainy months which are not favorable for cocoon yield. In this district from January to June high temperature, less humidity and less rain fall are also not favorable for the harvest of cocoons. Hence from the data it is concluded that from June month to October, average rainfall, average humidity and average temperature favors the growth of mulberry rearing and reeling processes in this district.

Indian Mulberry commonly known as *Morus alba* belongs to the family Moraceae of Monochlamydeae of Bentham and Hooker. It is a small genus of trees or shrubs distributed in the temperate and sub tropical regions of the World. There are about 5 species in India. It is valued for their foliage which constitute 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 (Koul *et al.,* 1994). The composition of leaves varies with variety, degree of maturity and the type of soil in which the plants are grown. The protein content of leaves decreases and the carbohydrate content increases with the maturity of leaves, fiber, fat and ash constituents also increase. Young leaves are more acidic than older ones. Analysis of leaves collected from different localities in India gave the following ranges of values (dry basis). Crude protein: 16.0 – 39.0, soluble sugars, 7.6 – 26.0, ash 8.0 – 17.0, calcium (CaO) 0.7 – 2.7, and iron (Fe_2O_3), 0.05 – 0.12%.The relation between the composition of Mulberry leaves fed to larvae and the resultant silk production has been extensively investigated. It has been found that accumulation of protein in larvae depends largely on the concentration of carbohydrates in the leaves (Ohnuma *et al.,* 1997). The maximum growth of larvae occurs when they are fed on leaves containing 3-4% sugars at the first instar period and 4 - 5% at the second instar period. When the leaf is low in soluble carbohydrates, addition of sucrose produces favorable results both as regards yield and quality of silk (Singh and Ninaergi, 1995). The professional food value of mulberry leaf for silkworm larvae is attributed to the presence of 3 stimulant factors, in it *viz*. an attractant, a biting factor and a swallowing factor (David *et al.,* 1970). The substances which attract the larvae to the leaves have been identified as citral,

linalyl acetate, linalol, terpinyl acetate and hexenal, the first three being more effective than the rest. β-sitosterol (C.0.2% in leaves), along with some sterols and a water soluble substance, is the main factor which stimulates the biting action; the amount of food eaten by larvae is controlled by the concentration of β-sitosterol. The third factor which stimulates continues swallowing of leaves by larvae is present in the methanol insoluble, but water soluble fraction. The absence of anyone of these factors inhibits feeding by larvae. A prolamine has been separated from alcoholic (alkaline) extracts of Mulberry leaves; it forms the principal protein of the leaves. The nitrogen distribution in a preparation containing 12.64% N was as follows; HCl insol. N, 0.50; humin N, 0.45; amide N, 0.96; diamino acid N (arginino N, 0.49; lysine N, 0.35; cystein N, 0.01) 1.74; and monoamino acid N, 7.89%. Protein preparations from young Mulberry leaves form an excellent supplement to protein deficient diets. Non protein nitrogen accounts for C.22% of the total nitrogen in young leaves and C.14% in mature leaves. The amino acids identified in the free form are phenyl alanine, leucine, valine, tyrosine, proline, alanine, glutamic acid, glycine, serine, arginine, aspartic acid, cystine, threonine, sarcosine, γ-amino butyric acid, pipecolic acid and 5-hydroxy pipecolic acid.The leaves are a good source of ascorbic acid, 200 – 300 mg/100g, of which over 90% is present in the reduced form (Madhu babu *et al.,* 1992). They contain also carotene, vitamin B1, folic acid, folinic acid and vitamin-D. The presence of glutathione in leaves has been reported (Das and Sikdar, 1970).Mulberry leaves are rich in calcium, the mineral constituents present in the leaves are copper, zinc, boron and manganese occur in traces. Phylate phosphorus accounts for 18.2% of total phosphorus. Mulberry leaves are sometimes eaten as vegetable. They are also useful as cattle fodder. They are nutritious and palatable, and are stated to improve milk yield when fed to dairy animals. Analysis of leaves (from Uttar Pradesh) gave the following values (dry weight basis); protein 14.0; ether extract 6.8; N-free extract 49.7) total ash 13.8%, calcium (CaO), 2.74; and phosphorus (P_2O_5) 0.45%. Feeding experiments have shown that up to 6 kg of leaves per day can be fed to milk cows without adversely affecting the health of animals or the yield and butter content of milk. Since silkworm has been domesticated for many centuries, they are by nature quite delicate and are very sensitive to various factors. Among the various factors which influence the cocoon crops, the most important one are the quality and quantity of leaf supply and the techniques of rearing adopted such as feeding, cleaning, spacing etc. The behaviour of silkworms in relation to these above factors shows that the response varies with the different stages in the growth of worms to all these factors. Based on these responses suitable rearing techniques have been evolved for obtaining best results in silkworm rearing. Hence in order to analyze some of the factors which influence the silkworm rearing in respect to Vellore district the present study has been undertaken to assess some objectives i.e. to assess the quality of mulberry varieties which are cultivated in Vellore district, analyze the feeding methods and the quantity of feeding, understand the spacing schedule and study the various disinfection measures that are followed by the rearers of this district.

MATERIALS AND METHODS

Estimation of chlorophyll

The chlorophyll content in leaves was estimated by the method of Arnon (1949), chlorophyll is extracted in 80% acetone and the absorption at 663 nm and 645 nm are read in a spectrophotometer. Using the absorption coefficients the amount of chlorophyll is calculated.

The sample of the leaf was finely cut and mixed well; 1g of tissue was weighed and ground to a fine pulp with the addition of 20 ml of 80% acetone. This solution was centrifuged at 5000 rpm for 5 minutes and the supernatant was transferred to 100-ml volumetric flask. This procedure was repeated until the residue is colorless. The mortar and pestle were washed thoroughly with 80% acetone and clear washings were collected in the volumetric flask. This was made to 100 ml with 80% acetone. This absorbance was recorded at 645,663 and 652 nm against the solvent (80% acetone) blank. The amount of chlorophyll present in the extract mg chlorophyll per g tissue was calculated using the following equations.

$$\text{Mg chlorophyll a/g tissue} = 12.7\ (A_{663}) - 2.69\ (A645) \times \frac{V}{1000XW}$$

$$\text{Mg chlorophyll b/g tissue} = 22.9\ (A_{645}) - 4.68\ (A663) \times \frac{V}{1000XW}$$

$$\text{Mg total chlorophyll /g tissue} = 20.2\ (A_{645}) + 8.02\ (A663) \times \frac{V}{1000XW}$$

where
A = Absorbance at specific wavelength
V = Final volume of chlorophyll extract 80% acetone
W = Fresh weight of tissue extracted

Determination of total carbohydrate

The total carbohydrate was estimated by anthrone method (Hedge and Hofreiter, 1962), carbohydrates are first hydrolyzed in to simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630 nm. 100 mg of the sample was weighed into a boiling tube. This was hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N – HCl and cool to room temperature. Then this solution was neutralized with solid sodium carbonate until the

effervescence ceases and made to 100 ml and centrifuged. The supernatant was collected and 0.5, 1ml aliquots were taken for analysis. Standard solution was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard 'O' served as blank. The volume is made to 1 ml in all the tubes including the sample tubes by adding distilled water. Than 4 ml of anthrone reagent was added and this was heated for eight minutes in a boiling water bath and made to cool. Green to dark green colour was recorded at 630 nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. The amount of carbohydrate present in the tube was calculated from the graph. Carbohydrate percentage was calculated relation to fresh weight and dry weight basis. The Calculation is done by using this formula.

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \frac{\text{Mg of glucose}}{\text{Volume of test sample}} \times 100$$

Estimation of protein

Protein content of leaves was estimated by Lowry's method (Lowry *et al.,* 1951).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 tartrate 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 colour 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.

Moisture content (%)

Fresh leaves were collected from each plant at 9-11 am and weighed in gms. They were dried in hot air oven at 60^oC for 72 hrs. and dry weight was recorded. Moisture % was calculated by using the following formula.

Moisture content (%) ={(Fresh wt-Dry wt)/Fresh wt} x 100

Moisture content (%) after 6 hrs.

Fresh leaves were kept at room temperature for 6 hrs after plucking. During the period temperature and room humidity were recorded. After 6 hrs, sample were weighed again and moisture content was calculated using the formula.

Moisture content after 6 hrs = {(Fresh wt - 6 hr. wt)/Fresh wt} x 100

Moisture Retention capacity (%)

It is the capacity of leaves to retain moisture after 6 hrs. from harvest which varies from accession to accession. The moisture retention capacity was calculated using the formula.

Moisture retention capacity % = {(6 hrs. wt – Dry wt)/Fresh wt – Dry wt} x 100

Fresh leaf weight

30 leaves were harvested from a sapling as 10 leaves top, middle 10 leaves and bottom 10 leaves and recorded in gms for 6 saplings.

Dry weight of leaves

After recording the fresh weight, the leaves were oven dried at 60^oC for 72 hrs. to record dry weight in gms.

Statistical Procedures

The mean and the standard deviations were calculated from the determined values by using the standard procedures (Bailey, 1984). The standard deviations were calculated by using the formula.

$$S = \frac{(X_1 - \bar{X})^2}{n - 1}$$

where X_1 = value of individuals

$\bar{X}$ = mean value of the sample

n = number of samples

In order to examine whether the difference in results obtained was significance or not the following formula (Student's `t' test) was employed.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}$$

Where $\bar{X}_1$ = mean value of one sample

$\bar{X}_2$ = mean value of other sample

S_1 and S_2 = are corresponding standard deviations

n_1 and n_2 are the number of tests for each sample

The level of significance (P-value) between X_1 and X_2 was determined by using the students `t' distribution table of fractiles and critical values

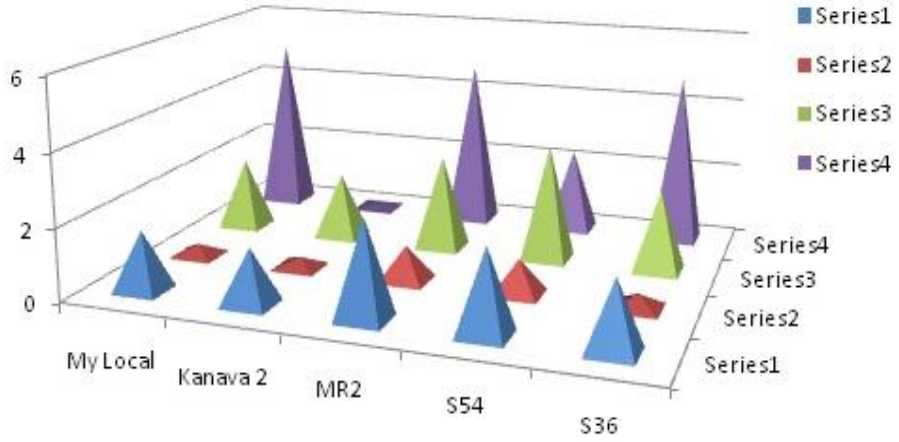
RESULTS

Table.1. describes the amount of primary metabolites of Mulberry leaf. Considering the chlorophyll content, MR_2 has the maximum (2.68) chlorophyll a content and Kanva-2 has the minimum (1.54) chlorophyll a content. Chlorophyll b is maximum MR_2 and minimum (0.98) in Kanva-2. Total chlorophyll content is more in MR_2 (3.66) less in Kanva-2 (1.84).The ratio between chlorophyll a and chlorophyll b is maximum in Mysore local (4.98) and minimum (0.08) in Kanva-2. Regarding the protein soluble content, the fresh percentage is maximum (6.79) in MR_2 and minimum (4.09) in S_{54}. The dry percentage is maximum (28.90) in MR_2 and minimum (13.74) in S_{54}. The soluble carbohydrate fresh percentage is maximum (4.81) in MR_2 and minimum (3.37) in S_{54}. The dry percentage is maximum (15.70) in MR_2 and minimum (11.32) in S_{54}. The moisture content of mulberry leaf of different varieties is shown in Table 2, MR_2 variety has the highest moisture (72%) and the lowest moisture content is seen in Mysore local (60%). The MR_2 variety retains highest moisture content (61.71%) after 6 hours and the S_{54} variety retains lowest moisture content (44.10%) after 6 hours. The moisture retention capacity is maximum in MR_2 (78.45) variety and minimum (44.57%) in S_{54} variety.

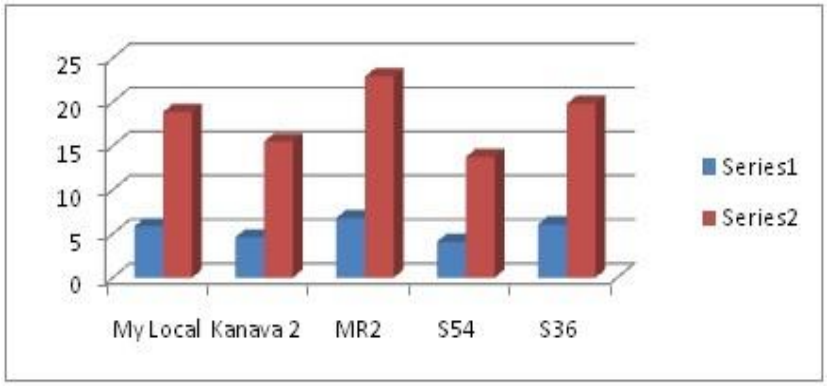
TABLE 1. Primary metabolites of Mulberry leaf

SNo.	Mulberry varieties	Chlorophyll				Protein soluble		Carbohydrate soluble	
		a	b	Total	a/b	Fresh %	Dry %	Fresh %	Dry %
1	Mysore Local	1.66 ± 0.12	0.33± 0.13	1.99 ± 0.08	4.98 ± 0.34	5.85 ±0.39	18.86± 0.65	3.75± 0.52	12.09± 0.12
2	Kanva-2	1.54 ± 0.53	0.30 ± 0.28	1.84 ± 0.18	0.08 ± 0.05	4.58 ± 0.33	15.50± 0.67	4.64± 0.36	15.70± 0.50
3	MR_2	2.68 ± 0.50	0.98 ± 0.33	2.66 ± 0.52	4.71 ± 0.50	6.79 ± 0.25	22.90± 0.88	4.81± 0.47	15.90± 0.36
4	S_{54}	2.30 ± 0.63	0.97 ± 0.12	3.27 ± 0.49	2.37 ± 0.29	4.09± 0.41	13.74± 0.23	3.37± 0.48	11.32± 0.22
5	S_{36}	1.90 ± 0.62	0.40 ± 0.25	2.29 ± 0.25	4.78 ± 0.45	6.05± 0.18	19.79± 0.43	4.71±0.67	15.40± 0.62

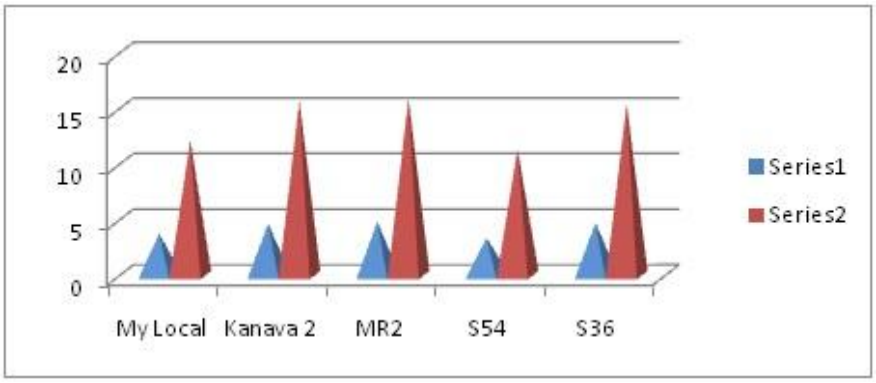
FIGURE 1. Chlorophyll content in different mulberry varieties



Series-1. Chlorophyll a, Series-2. Chlorophyll b, Series-3. Total chlorophyll, Series-4. a/b



Series1.Chlorophyll a, Series-2.Chlorophyll b, Series-3.Total chlorophyll, Series-4.a/b



Series-1. Chlorophyll a, Series-2. Chlorophyll b, Series-3. Total chlorophyll, Series-4. a/b

TABLE 2. Moisture content of Mulberry leaf

S.No.	Mulberry varieties	Moisture content (%)	Moisture content after 6 hours (%)	Moisture retention capacity (%)
1	Mysore Local	60.00±0.43	51.52±1.02	59.48±0.54
2	Kanva-2	65.00±0.60	59.52±0.67	71.31±0.53
3	MR_2	71.00±0.70	61.71±0.69	78.45±0.57
4	S_{54}	63.00±0.94	44.10±0.63	44.57±0.49
5	S_{36}	64.00±0.38	61.71±0.86	78.45±0.50

DISCUSSION

Sericulture is a cottage industry catching up in almost all the villages in Vellore district due to the subsidies encouragement and also training and advice given by Government technical service centres. Villagers start their own sericulture unit to compensate the loss of revenue yield from the agriculture during the lean period. But the available data indicated that the status of sericulture industry is not catching up as per the expectation. The cocoon yield directly related to the technology involved in silkworm rearing and the quality of mulberry leaves. Mulberry being practically the sole food of the silkworms, it is at once obvious that the quality of mulberry leaf has a predominating influence on the development of the worm and the quality of cocoon. If silkworm rearing and cocoon production are to be a success, it is very necessary that mulberry leaf to be fed to the silkworms is very nutritive and fresh. Mulberry is a very quick growing plant and its leaves can be harvested several times in a year, especially in tropical countries like India.

Research conducted on breeding of mulberry has resulted in evolving over 200 varieties of mulberry. Studies on the ecological factors and adaptability of these varieties have helped in selecting varieties suitable to different agro-climatic tracts. Most of the Indian varieties of mulberry belong to *Morus indica*. Among them, Mysore local Kanva-2, MR2, S54 and S36 varieties are cultivated in Vellore district. The cultivation of mulberry for raising silkworm cocoon crops should aim not only at increased production of leaves per unit area but also leaves of suitable quality for the maximum utilization of the leaf crop produced. For it has been fairly well recognized that both chemical composition and nutritive value of the leaves as reflected in the silkworm cocoon crop differ considerable from variety to variety, season to season or according to growth and maturity of the leaves, manurial application, irrigation and so on. Despite considerable amount of researches carried out by various workers on this problem of quality of leaves, there seem to considerable contradictions and it has not been possible to make any positive recommendations as to the mulberry cultivation practice to be followed for the production of suitable type of leaves. As regards mulberry leaf quality growth under tropical conditions of India, the first available literature was that of Dasgupta (1961) who found differences among leaves of free and bush plantations of mulberry. After, Narayanan *et al.,* (1966) and Sidhu *et al.* (1969) have reported about the quality differences in leaves due to Varity, irrigation and manuring. Systematic studies on the qualities of mulberry leaves were initiated at central Sericultural Research Station, Berhampore since 1963 on the various factors influencing the quality of leaf. 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.,* (1955), Tangamani and vivekanandan (1984), Lie and Sano (1984), Fotadar *et al.,* (1989) and chaluvachari and Bongale (1995) discussed the importance of quality of mulberry leaves used as feed for silkworm. Present findings show that MR_2 variety is photosynthetically more efficient in Vellore District. Tangamani and Vevekandan (1984) observed higher quantity of chlorophyll in MR_2 and Japanese genotypes under tropical conditions. High quantity of chlorophyll 'a' and 'b' is advantageous since they are the most important pigment in photosynthesis. The role of soluble and crude proteins in silkworm nutrition has been emphasized by Fukuda *et al.,* (1959) and Takeuchi (1960). Feeding of silkworm is known to be strongly stimulated by sugars. Higher values of protein and nitrogen in the feed are known to favour better performance of silkworm larval growth and their cocoon crop. (Legay 1958, Horie 1980) 14% of soluble sugar and 26.5% of protein in the feed was most effective on larval

growth as reported by Ueda *et al*. (1969), Ito and Arani (1963). Bongale *et al.,* (1994) reported that Kanva-2 recorded higher values of sugar content associated with better moulting ratio. Carbohydrates of the mulberry leaves are synthesized by the photosynthetic action of the leaves. Photosynthesis does not occur at night and since carbohydrates are consumed and transferred to other parts due to respiration. The carbohydrate content of the leaves is lowest in the morning. Carbohydrates particularly the sugar content in mulberry leaves in closely related to the health of the silkworm. Mulberry leaves with high sugar content fields good results of rearing. Moreover by adding sugar artificially to the feed, the occurrence of flacherie was reported to have greatly reduced. (Kichisaburo minamizawa, 1970). Present study reveals that MR_2 recorded more amount of soluble protein content more carbohydrate content.

Importance of the nutritive care for young age silkworms and its influence on cocoon crop performances have been widely accepted (Yokoyma, 1965, Krishnaswami *et al.* 1970; Chaluvachari 1995). Leaf moisture content and moisture retention are reported to positive influence on the growth of silkworm larvae. Paul *et al.*(1992) reported that absolute consumption of feed and growth rate of larvae increased with increasing levels of leaf moisture content. They also recorded high positive correlation between larval weight and leaf moisture content Lie and Sano (1984) reported that lower values of leaf moisture and protein content in the feed recorded lower rates of larval growth body weight and cocoon weight. Chaluvachari and U. D. Bongale (1996) reported that higher values of moisture content and moisture retention in the leaves favoured the moulting ratios and larval weight and a clear positive influence of leaf moisture retention on the young age larvae was also recorded. Paripev (1968) reported that high moisture content favored both palatability and content favoured both palatability and assimability of the nutrients in the silkworm. It could be concluded from the present study that MR_2 recorded higher values of moisture content and moisture retention capacity.

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