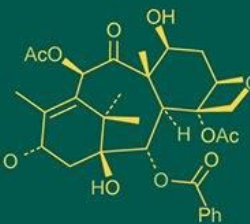
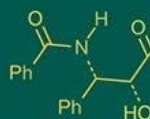
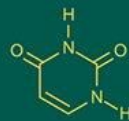
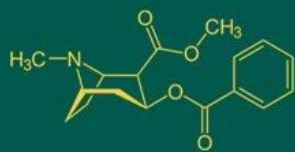


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Elucidation of different Mulberry (*Morus* spp.) genotypes for leaf quality under temperate conditions

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Abstract

Five mulberry genotypes viz., Goshorami, KNG, Mukey, Kokuso-20 and Botatul were analysed for their leaf quality through phytochemical tests during autumn season under temperate climatic conditions of Kashmir. The leaf nutrient analysis of different mulberry genotypes revealed that, KNG had the maximum moisture retention capacity after 06hour (86.25%) and 12 hours (78.81%), total carbohydrates (20.109%), total proteins (20.963%), total flavonoids (58.090 mg QE/g), total phenols (4.855 mg GAE/g), total chlorophyll (3.886 mg/g), total nitrogen (3.351%) and total phosphorous (0.249%). Hence, KNG can be exploited for second commercial rearing (autumn rearing) as an alternative to Goshorami, which is prone to powdery mildew during the autumn season.

Keywords: Carbohydrates, Flavonoids. Protein, Mulberry genotypes

Introduction

Sericulture as an enterprise has been playing a vital role in sustaining the economy of UT (Jammu & Kashmir). It is one of the most eco-friendly cottage-based industry which provides employment to a large number of farmers in its various sectors right from mulberry cultivation, rearing to post cocoon sector. The industry supplements income and employment to large number of semi-literate and semi-skilled workers of the weaker section of the society (Sharma *et al.*, 2018) [35].

In sericulture, more than 60 per cent of total cost of cocoon production goes towards mulberry production alone. Hence, maximum attention needs to be given for the improvement of mulberry both in terms of quality and quantity. Mulberry leaves are the exclusive source of nutrition for growth and development of silkworms (Tang *et al.*, 2005) [38]. The quality of leaves fed to silkworm is considered to be the prime factor for good cocoon production (Ravikumar, 1998) [33]. Mulberry leaves contain a rich source of protein and amino-acids which influence the shell weight of cocoon (Mechii and Katagiri, 1991) [21]. Protein is the main constituent of mulberry leaf which plays a vital role for development of silk gland during the larval period (Quader, 1987) [29]. About 70 per cent of the silk protein produced by the silkworm is directly derived from the mulberry leaves (Quader *et al.*, 1992) [30]. However, the nutritional composition of mulberry leaves varies with different cultivars and seasons which influences growth and cocoon production (Mahmood *et al.*, 1987) [19]. The performance of any mulberry variety with respect to leaf quality and cocoon production varies with agro-climatic conditions and cultivation practices (Ghosh *et al.*, 2000) [11]. Therefore, major contributing factor for seasonal silkworm rearing depends on the quality of leaves. The major steps in increasing the productivity of silk are to increase the leaf yield per unit area coupled with improved leaf quality. The present study is proposed to be taken to generate further information about the mulberry leaf which could be fed to the silkworms for increased and sustainable cocoon production which would ultimately benefit the stakeholders associated with the industry.

Materials and Methods

Five mulberry genotypes viz.; Goshorami, KNG (Kairynezamigaeshi), Mukey, Kokuso-20 and Botatul were analysed for their leaf quality through phytochemical tests. The plantation was maintained in the germplasm bank of College of Temperate Sericulture, Mirgund, Sher-

e-Kashmir University of Agricultural Sciences and Technology of Kashmir, during Autumn, 2021 as per the package of practices (Anonymous, 2003) ^[1].

The observations on various parameters were recorded during Autumn season and are detailed here under:

Collection of leaf samples

The composite leaf samples comprising of almost equal proportion of tender, medium and coarse leaves were collected early in the morning and fresh weight recorded immediately. Then, the leaves were kept at room temperature and weighed again after 06 and 12 hours. These were then oven dried at 60 °C for 48 hours (Ninge Gowda & Sudhakar, 2002) ^[12] till constant weight was attained. Completely dried leaf samples were pulverized separately and used for analysis.

Leaf moisture

The composite leaf samples comprising of almost equal proportion of tender, medium and coarse leaves were collected early in the morning and fresh weight recorded immediately. Then, the leaves were kept at room temperature and weighed again after 06 and 12 hours. These were then oven dried at 60 °C for 48 hours (Ninge Gowda & Sudhakar, 2002) ^[12]. The dry weight was recorded and the moisture content and moisture retention capacity calculated as per the following formulae:

Moisture content (%)

Moisture content (%) was calculated as follows:

$$\text{Moisture content(\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Moisture retention capacity (%) after 06 hours

Moisture retention capacity (%) after 06 hrs was calculated as follows:

$$\text{Moisture content(\%)} = \frac{\text{Weight after 06 hrs} - \text{Dry weight}}{\text{Fresh weight} - \text{Dry weight}} \times 100$$

Moisture retention capacity (%) after 12 hours

Moisture retention capacity (%) after 12 hours was calculated as follows:

$$\text{Moisture content(\%)} = \frac{\text{Weight after 12 hrs} - \text{Dry weight}}{\text{Fresh weight} - \text{Dry weight}} \times 100$$

Total Nitrogen

The nitrogen content was determined by Microkjaldal's method as described by (Jackson, 1973) ^[16]. 0.5gm of mulberry leaf sample was taken in a digestion tube to which 4-5 gm of digestion mixture (10 parts of potassium sulphate and 0.5 parts of copper sulphate) was added. Then, 10 ml of sulphuric acid was added to it and digestion unit was allowed to run for 3 hours. The tube was then left undisturbed overnight and next day tube was fitted in the condensation unit. The contents were collected in a receiving flask which was then titrated against 0.1N hydrochloric acid and nitrogen content was calculated using the following formula:

$$\text{Nitrogen(\%)} = \frac{(\text{TV} - \text{Blank}) \times \text{Normality of acid} \times \text{DF}}{\text{Weight of sample}} \times 100$$

Total Phosphorous

The phosphorous content was determined by Vandomolybdate phosphoric acid yellow colour method (Jackson, 1973) ^[16]. 0.5gm of mulberry leaf sample was taken in a flask and then 10ml of di-acid (mixture of nitric acid and perchloric acid) in the ratio of 9:4 was added to it. The flask was then kept undisturbed overnight and next day, it was placed on a hot plate at 115-120°C for digestion till a watery transparent aliquot was obtained. The digested samples were then filtered and diluted with double distilled water to make up final volume to 100ml, which was then ultimately used for the estimation of phosphorous content in leaf samples.

Total Potassium

The potassium content was determined by Flame photometry method using corning flame photometer, U.K. (Jackson, 1973) ^[16]. Di-acid digestion of mulberry leaf samples was carried out in the same manner as described for estimation of phosphorous content of leaf. The digest was then used directly for determination of potassium content.

Total Proteins

Protein concentration was determined by the Microkjaldal's method as described by (Jackson, 1973) ^[16].

$$\text{Total proteins} = \text{Nitrogen content} \times 6.25$$

6.25= Most proteins contain 16% of nitrogen, thus the protein nitrogen conversion factor is 6.25

Total Carbohydrates

Carbohydrate content was estimated by (Thimmaiah, 1999) ^[39] using the Anthrone reagent with slight modification. 0.1g of dry leaf samples were taken in test tubes and 5ml of 0.5N HCL added to it. Tubes were kept for 3 hours in hot boiling water bath and cooled at room temperature. Dry sodium carbonate was added to neutralize samples till effervescence ended. Volume of tubes was made 10ml with distilled water. 0.05ml of the resulting solution from each sample tube was taken for analysis. Volume of sample tubes was made up to 1ml with distilled water followed by addition of 4 ml anthrone reagent. Tubes were kept in boiling water for 8 minutes, cooled rapidly and absorbance of green to dark green colour was read at 630nm.

Total Phenols

The total phenol content was determined as per the method of (Meda, 2005) ^[22]. Each sample (5g) was diluted to 50ml with distilled water and filtered through Whatman No. 1 paper. The extract of the sample was mixed with 0.5ml of Folin-Ciocalteu reagent. After 3 min, 2 ml of 20% sodium carbonate solution was added. The mixture was mixed thoroughly and incubated for 2 hours at ambient temperature. The absorbance was measured at 760 nm against a methanol blank using a UV-visible spectrophotometer. The total phenolic content was expressed as mg of gallic acid equivalents (mg GAE/g).

Total Flavonoids

The total flavonoid content was determined according to the method of (Meda, 2005) ^[22]. A mixture of the sample in distilled water and 0.3 ml of 5% NaNO₂ were mixed together. After 5 minutes, 0.3 mL of 10% AlCl₃ was added. After 6 min, 2 mL of 1 M NaOH was added and the total volume was

made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The total flavonoid content was expressed as mg of quercetin equivalents (mg QE/g).

Total Chlorophyll

The total chlorophyll content was estimated by the method of (Witham *et al.* 1971) [40]. One gram of finely cut and well mixed representative sample of fresh leaf weighed and ground in mortar and pestle with 20 ml of 80 per cent acetone. Samples were then centrifuged at 5000 rpm for 5 min and supernatant was transferred to a 100 ml volumetric flask after filtering through Whatman No. 1 filter paper. Residues were again ground with 20 ml of 80 per cent acetone, centrifuged and supernatant was again transferred to the same volumetric flask. This procedure was repeated till the residue was colourless. The final volume was made up to 100 ml with 80 per cent acetone in the volumetric flask. The absorbance of the extract was measured at 645 nm and 663 nm wave length filters using UV-visible spectrophotometer for the determination of total chlorophyll content with 80 per cent acetone as blank.

The total chlorophyll content in leaf was calculated by using the following formula

$$\text{Total chlorophyll (mg/g tissue)} = \frac{20.2 \times (\text{O.D.645 nm}) + 8.02 \times (\text{O.D.663 nm})}{1000 \times W}$$

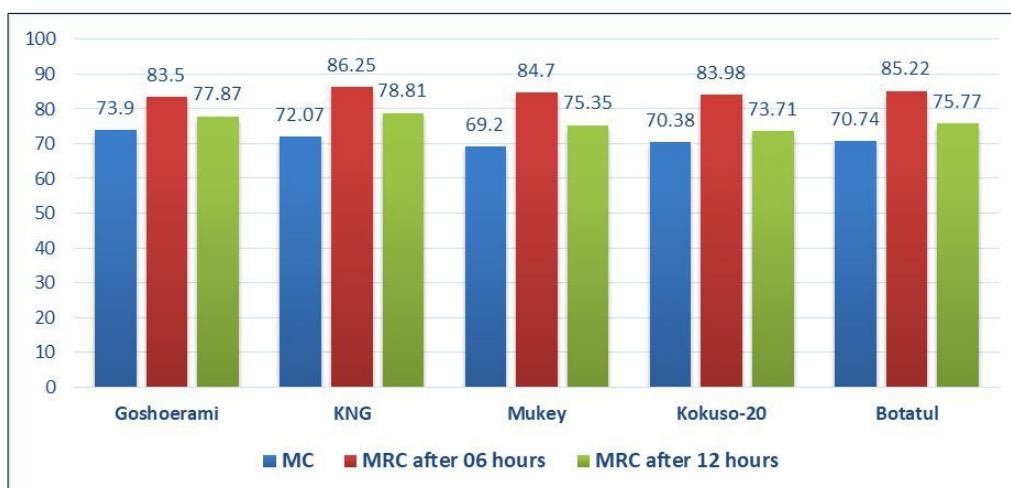


Fig 1: Leaf moisture status of different mulberry (*Morus*) genotypes during autumn season

Nitrogen, Phosphorous and Potassium

Nitrogen content was significantly highest (3.351%) in the genotype KNG which was followed by Mukey (3.213%). Lowest nitrogen content (2.680%) was recorded in Kokuso-20 respectively. Phosphorous content in leaf varied among the different mulberry genotypes and was significantly higher (0.249%) in KNG. Goshorami had 0.180 per cent phosphorous content whereas the lowest (0.138%) was recorded in Mukey. Significant differences were also recorded with regard to potassium content and the highest potassium content (1.650%) was registered in Botatul followed by Kokuso-20 (1.278%). However, least potassium content (0.939%) was present in the mulberry genotype KNG (Table-2) (Fig.2).

Results

Moisture content and moisture retention capacity after 06 and 12 hours

Significant differences were observed with regard to moisture content and moisture retention capacity after 06 and 12 hours. Mulberry genotype Goshorami was higher (73.90%) with regard to moisture content followed by KNG (72.07%) genotype. However, lowest moisture content (69.20%) was present in Mukey. Moisture retention capacity (%) after 06 hours was higher (86.25%) in the genotype KNG followed by Botatul (85.22%) and the least moisture content (83.50%) after 06 hours was registered in Goshorami. Similarly, KNG recorded the highest moisture content (78.81%) after 12 hours and the genotype Goshorami had 77.87 per cent MRC after 12 hours. Least moisture retention capacity (73.71%) after 12 hours was recorded in Kokuso-20 (Table-1) (Fig.1).

Table 1: Leaf moisture status of different mulberry (*Morus*) genotypes during autumn season

Treatment	Moisture content (%)	Moisture retention capacity (%) after 06 hrs	Moisture retention capacity (%) after 12hrs
Goshorami	73.90	83.50	77.87
KNG	72.07	86.25	78.81
Mukey	69.20	84.70	75.35
Kokuso-20	70.38	83.98	73.71
Botatul	70.74	85.22	75.77
C.D (p ≤ 0.05)	0.44	0.68	0.13

Table 2: Leaf macronutrient (NPK) status of different mulberry (*Morus*) genotypes during autumn season

Treatment	Nitrogen (%)	Phosphorous (%)	Potassium (%)
Goshorami	3.072	0.180	1.086
KNG	3.351	0.249	0.939
Mukey	3.213	0.138	0.964
Kokuso-20	2.686	0.159	1.278
Botatul	2.938	0.140	1.650
C.D (p ≤ 0.05)	0.007	0.007	0.012

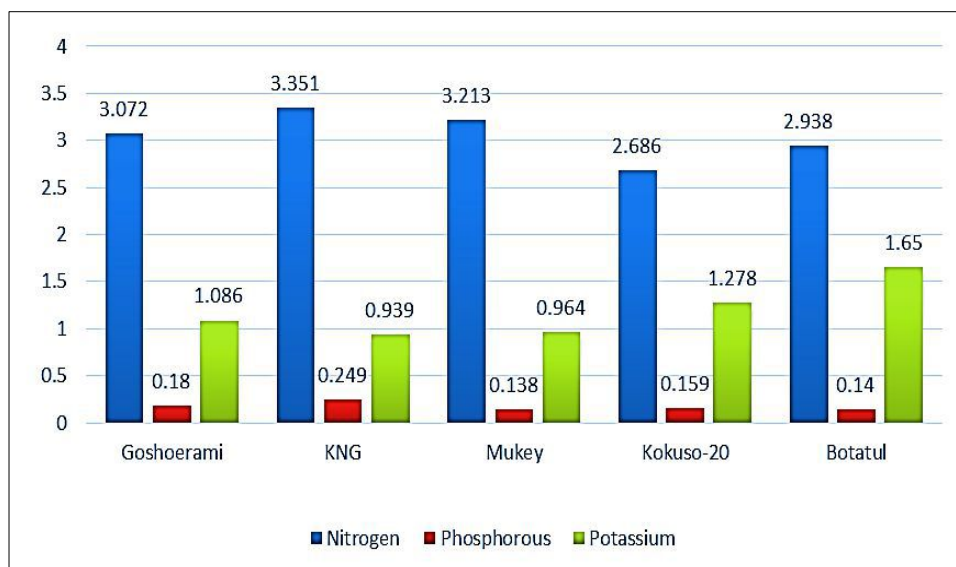


Fig 2: Leaf macronutrient (NPK) status of different mulberry (*Morus*) genotypes during autumn season

Proteins and Carbohydrates

Protein content in leaf was highest (20.963%) in KNG being significantly higher than the rest of the genotypes. Mukey had 20.156 per cent protein content in leaf whereas Kokuso-20 had 16.783 per cent leaf protein content being the least among the genotypes. Similarly, carbohydrate content in leaf too was the highest (20.109%) in KNG which was statistically significant over the values recorded in other genotypes. Goshierami the most popular genotype in the region registered 19.142 per cent whereas Mukey had the least value (16.130%) (Table-3) (Fig-3).

Phenols and Flavonoids

Mulberry genotypes depicted significant differences among themselves as far as the phenol content in leaf was concerned. It was the highest (4.855mg GAE/g) in KNG followed by Goshierami (3.553 mg GAE/g) and the least (2.780mg GAE/g) in Mukey. Differences among the mulberry varieties in terms of flavonoid content in leaves were significant being the highest (58.090mg QE/g) in KNG. Goshierami had 56.163 mg QE/g flavonoid content whereas it was the lowest (35.853 mg QE/g) in Mukey (Table-3) (Fig.3).

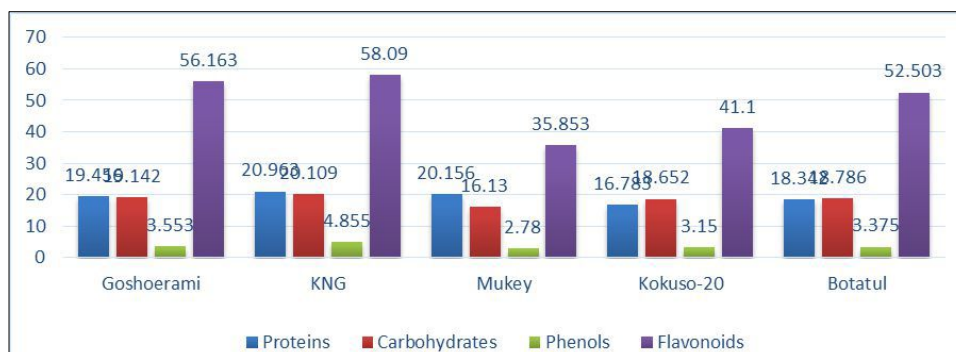


Fig 3: Phytochemical status of leaf of different mulberry (*Morus*) genotypes during autumn season

Table 3: Phytochemical status of leaf of different mulberry (*Morus*) genotypes during autumn season

Treatment	Total Proteins (%)	Total Carbohydrates (%)	Total Phenols (mg GAE/g)	Total Flavonoids (mg QE/g)
Goshierami	19.456	19.142	3.553	56.163
KNG	20.963	20.109	4.855	58.090
Mukey	20.156	16.130	2.780	35.853
Kokuso-20	16.783	18.652	3.150	41.100
Botatul	18.342	18.786	3.375	52.503
C.D (p ≤ 0.05)	0.133	0.051	0.041	0.952

Chlorophyll a, Chlorophyll b and total Chlorophyll

Chlorophyll "a" content differed significantly in various mulberry genotypes being highest (2.486 mg/g) in KNG. Mukey registered 2.282 mg/g chlorophyll "a" content and the least (1.670 mg/g) was present in Kokuso-20. KNG registered highest chlorophyll "b" content which was significantly higher than the other genotypes. Goshierami had 1.257 mg/g chlorophyll "b" content whereas Kokuso-20 had the least value (1.208 mg/g) for chlorophyll "b". The mulberry genotypes exhibited significant differences in terms of total chlorophyll content. Total chlorophyll was highest (3.886 mg/g) in KNG which was followed by Goshierami (3.372mg/g). However, least total chlorophyll (2.878 mg/g) was registered in Kokuso-20 respectively (Table-4) (Fig.4).

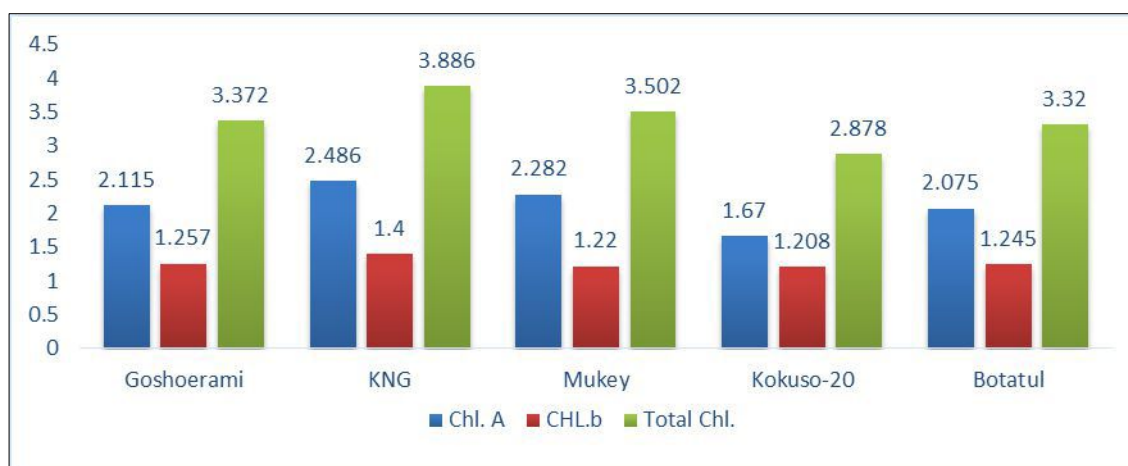


Fig 4: Chlorophyll content of different mulberry (*Morus*) genotypes during autumn season

Discussion

Leaf moisture content and moisture retention capacity

In mulberry leaves, moisture content plays a vital role in improving nutrition levels which in turn improve the palatability and digestibility of leaves as well as normal growth and development of silkworms and cocoon quality. Availability of moisture content in leaves enhances feeding efficiency of silkworm larvae which in turn increases growth rate. Moisture retention capacity plays an important role because leaves with high moisture content remain fresh and acceptable to silkworms for longer time (Murthy *et al.* 2013) [26]. The results of current study revealed that all the mulberry genotypes exhibited significant differences in leaf moisture content, moisture retention capacity after 06 hours and 12 hours. Goshoerami had highest moisture content (73.90%) which was followed by KNG (72.07%) and it was lowest (69.20%) in Mukey. With respect to moisture retention capacity after 06 hours, highest value (86.25%) was recorded in KNG followed by Botatul (85.22%) genotype. However, lowest moisture retention capacity after 06 hours (83.50%) was observed in Goshoerami. Further, KNG registered the highest moisture retention capacity after 12 hours (78.81%) and the genotype Goshoerami had 77.87 per cent MRC after 12 hours. Least moisture retention capacity after 12 hours (73.71%) was recorded in Kokuso-20.

The variation in moisture content and its retention can be attributed to leaf anatomical and morphological features including stomatal frequency, cuticle thickness and mesophyll thickness. The results are also supported by the findings of Nazim (2022) [27], Mir *et al.* (2012) [23], Khan *et al.* (2007) [18] who also observed that moisture retention capacity after 06 and 12 hours are governed by anatomical features including stomatal size and frequency etc.

Major nutrients

Primary macronutrients play a very important role in improving the yield and quality of crops. Three main elements are nitrogen, phosphorous and potassium (N, P and K) and are required in abundance. They must be readily available through soil medium or fertilizer (Israel and Yonas, 2021) [15].

During the course of present investigation, the data revealed that all the five mulberry genotypes exhibited significant differences for various macro-nutrients of the leaf viz., nitrogen, phosphorous and potassium. The highest nitrogen content (3.351%) was recorded in KNG which was followed by Mukey (3.213%) and least (2.680%) was recorded in

Kokuso-20. Phosphorous content was significantly higher (0.249%) in KNG. Goshoerami had 0.180 per cent phosphorous content whereas the lowest (0.138%) was recorded in Mukey. Further, highest potassium content (1.650%) was registered in Botatul followed by Kokuso-20 (1.278%) and the least potassium content (0.939%) was present in the mulberry genotype KNG. The findings on the increased uptake of nutrient by the plant are in agreement with the findings of Mandal and Sinha, 2002 [20]. The reason behind the variability of the NPK content could be attributed to the nutrient use efficiency (NUE) of the genotype to capture nutrients from the soil which might have increased nutrient uptake and their accumulation in plant.

Bio-chemical compounds

Protein

Leaf protein is a major constituent of nutrient quality for feed of many Lepidopteron larvae. It is a known fact that, nearly 69 per cent of protein content of raw silk namely fibroin and sericin are directly biosynthesized from mulberry leaf protein and remaining 31 per cent is derived from silkworm body tissue and haemolymph protein, emphasizing the importance of leaf protein in silkworm nutrition (Bose *et al.*, 1991) [9].

In the present investigation, protein content in leaf was the highest (20.963%) in KNG being significantly higher than the rest of the genotypes. Mukey had 20.156 per cent protein content in leaf whereas Kokuso-20 had 16.783 per cent leaf protein content being the least among the genotypes. The present findings are in harmony with Ramachandra *et al.* (2008) [31] and Bose and Bindroo (2001) [10] who also reported existence of varietal differences in protein content of mulberry leaf. The variation in the protein content might be due to the nutrient use efficiency of the plant and the availability of sufficient quantity of nitrogen in the soil which have assimilated/absorbed by the plant. The nutrient use efficiency of the plant is a genetical trait and it varies from genotype to genotype. Besides, it may also be due to the activities of different enzymes including peroxidase and superoxide dismutase (Hadimani *et al.* 2019) [13].

Carbohydrate

Carbohydrate is a major source of energy for most of the insects. For, silkworm, carbohydrate is a very important fuel source. They may be converted to lipids and may contribute to the production of amino-acids. Many carbohydrates, especially sugars, are powerful feeding stimulant. A functional carbohydrate may appear as non-functional if it is

not ingested effectively (Borah and Boro, 2020). In the present study, carbohydrate content in leaf was the highest (20.109%) in KNG which was statistically significant over the values recorded in other genotypes. Goshorami the most popular genotype in the region registered 19.142 per cent whereas Mukey had the least value (16.130%). The results are in line with observations of Srivastava and Elangovan, Ramamoorthy *et al.* (2018) ^[32] who also reported existence of varietal differences in carbohydrate content of mulberry leaf. The reason behind the variation in the carbohydrate content of the leaf might be due to varied photosynthetic efficiency by the plant.

Phenol

Presence of higher concentrations of phenolic compounds is considered to be one of the major factors for an incompatible host pathogen interaction. Phenols possess a wide spectrum of biological activities and results show that mulberry extracts could be a good source of these natural constituents. In the present study, mulberry genotypes depicted significant differences among themselves as far as the phenol content in leaf was concerned. Highest phenol content (4.855 mgGAE/g) was recorded in KNG which was followed by Goshorami (3.553 mgGAE/g). However, lowest phenol content (2.780 mgGAE/g) was recorded in Mukey. Sanchez-Salcedo *et al.* (2015) ^[34] reported that among the studied clones, *Morus alba* had the lowest phenolic content (from 12.81 to 15.50 mg GAE/g) whereas the *Morus nigra* clones displayed slightly higher levels (from 13.48 to 16.13 mg GAE/g) which can be due to the variability of phenolic content among the mulberry leaves due to their genetic diversity. Furthermore, several workers have reported that, disease resistant varieties possess higher phenolic content compared to that of susceptible ones (Sharma *et al.* 1993) ^[36]. The variation among the genotypes might have also occurred due to developmental stages and environmental variations.

Flavonoid

Mulberry leaves have shown antioxidant and anti-inflammatory properties owing to their rich flavonoid contents (Chon SU *et al.*, 2009) ^[10]. The most promising activities of flavonoids in addition to antioxidants are their potential to modulate different metabolic pathways especially those linked with energy homeostasis in the body (Berger *et al.*, 2015) ^[3].

In the present investigation, the differences among the mulberry varieties in terms of flavonoid content in leaves were significant being the highest (58.090mg QE/g) in KNG. Goshorami had 56.163 mg QE/g flavonoid content whereas it was the lowest (35.853 mg QE/g) in Mukey. The findings are in harmony with Sanchez-Salcedo *et al.* (2015) ^[34] who also reported the variation of polyphenols in different species of mulberry viz., *Morus alba*, *Morus nigra*. Hu *et al.* were of the opinion that flavonoid accumulation differs throughout the mulberry growth period. Like phenols, the variation in the flavonoid content might also be due to the genetic variation and developmental stages.

Chlorophyll

Chlorophyll content is very important for quantifying the photosynthetic efficacy of plant and is an essential constituent in assessing quality of foliage (Murthy *et al.*, 2013) ^[26].

Based on the results obtained, significant differences were observed amongst the five mulberry genotypes as far as

chlorophyll-a (Chl.a), chlorophyll-b (Chl.b) and total chlorophyll are concerned. With respect to chlorophyll-a, highest value (2.486 mg/g) was recorded in KNG which was followed by Mukey (2.282 mg/g) and lowest (1.670 mg/g) was noticed in Kokuso-20. KNG registered highest chlorophyll "b" content (1.400 mg/g) which was significantly higher than the other genotypes. Goshorami had 1.257 mg/g chlorophyll "b" content whereas Kokuso-20 had the least value (1.208 mg/g) for chlorophyll "b". Total chlorophyll was highest (3.886 mg/g) in KNG which was followed by Goshorami (3.372 mg/g). However, least total chlorophyll (2.878 mg/g) was registered by Kokuso-20. Similar results have been reported by Kalaivani *et al.* (2013) who also reported varied total chlorophyll content in mulberry leaf. Total chlorophyll content of genotype KNG might be associated with better photosynthetic efficiency that leads to better quality of the leaf. Higher level of leaf chlorophyll is indicative of photosynthetic efficiency of the plant system.

The leaf of KNG variety of mulberry turned out to be superior with respect to qualitative parameters viz., moisture retention capacity after 06 hours and 12 hours, total nitrogen, total phosphorous, total proteins, total carbohydrates, total phenols, total flavonoids, total chlorophyll and thus the economic traits of silkworm *Bombyx mori* L. viz., growth index, cocoon yield by number, cocoon yield by weight, single cocoon weight, single shell weight, shell percentage, filament length and raw silk percentage.

Hence, for popularization of autumn crop in the Temperate zone of Jammu and Kashmir, KNG can be exploited for second commercial rearing for increased and sustainable cocoon production. In addition to that, KNG genotype will provide an alternative to Goshorami which is prone to powdery mildew during the autumn season.

Conclusion

Thus, it has been concluded that the leaf of KNG variety of mulberry turned out to be superior with respect to qualitative parameters viz., moisture retention capacity after 06 hours and 12 hours, total nitrogen, total phosphorous, total proteins, total carbohydrates, total phenols, total flavonoids, total chlorophyll. Hence, for popularization of autumn crop in the Temperate zone of Jammu and Kashmir, KNG can be exploited for second commercial rearing for increased and sustainable cocoon production. In addition to that, KNG genotype will provide an alternative to Goshorami which is prone to powdery mildew during the autumn season.

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