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QUANTITATIVE DETERMINATION OF PROTEIN AND CARBOHYDRATE CONTENT IN DIFFERENT VARIETIES AT DIFFERENT STAGES OF MULBERRY LEAF

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Abstract
Mulberry is found in wide variety of climatic, geographic and soil conditions. Mulberry leaf is mainly used to feed the silkworm. As silkworm being the monophagous and solely depends on mulberry leaf. It is distributed in temperate, subtropical and tropical regions with varying varieties to each region. Regional distribution affects the biochemical constituents of leaf. Present study was undertaken to determine the carbohydrate and protein content from three regional varieties of mulberry leaf viz., Botatul (Morus indica), Shahtul (Morus alba) and Chatatul (Morus alba) at three different stages (tender, medium and coarse). In general, decreasing trend in terms of total protein and carbohydrate content was recorded from tender to coarse textured leaves of all the three varieties. The leaf extract of Botatul (tender) and Chatatul (tender) recorded the highest protein and carbohydrate content to the tone of 24.67% and 15.76% respectively. Among the varieties, Botatul recorded highest protein content of 22.42%, whereas highest carbohydrate content (14.98%) was found in Chatatul mulberry variety.
Keywords: Mulberry leaves, Monophagous, Silkworm, Protein and Carbohydrate.

1. Introduction

Mulberry plant has been used for various purposes at various regions around the world. This plant thrives in wide range of climatic, topological and soil conditions. Mulberry as a whole plant has long been used as a functional food due to the presence of rich phytochemical elements. It is also valued for its fruits (consumed fresh, in juice, or as preserves), as a tasty vegetable (young leaf and stem), for its medicinal value (mulberry leaf tea), for landscaping, and as animal feed, depending on where it is grown (Kumar and Chauhan, 2011). Mulberry leaves are most commonly used as feed for silkworm. Silkworm is monophagous and depends only on mulberry leaf. So nutritious and disease free mulberry leaf is prime requirement for silkworm rearing industry. An imbalance in the nutrients of leaf changes the metabolic activity of the larval body (Ito, 1972). The larvae's growth and development, as well as the formation of cocoons, are mainly influenced by the nutritional quality of the mulberry leaves (Krishnaswami, 1978). Mulberry leaf quality varies with age, leaf location, and variety of the same species (Narayanan et al., 1996). Mulberry leaves are rich source of nutrients, but protein and carbohydrates are the main nutrients, which greatly influence the silkworm growth. Present study assess the protein and carbohydrate content of mulberry leaves at various stages (tender, medium, and coarse) from three different mulberry cultivars (Botatul, Chatatul and Shahtul) grown in temperate climate.

2. Materials and methods:

The research was conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir's College of Temperate Sericulture-Mirgund. Leaf samples (tender, medium, and coarse) in almost equal proportion of three mulberry varieties viz., Botatul, Chatatul, and Shahtul were taken.

2.1. Processing of leaf samples:

Collected leaf samples were rinsed under tap water followed by distilled water to remove all impurities from them. Samples were first dried on filter sheets in shade then in oven at 60-65°C (Chapman, 1964) till consistent weight reached. Dry samples were crushed with a mortar pestle, sieved through 2 mm mesh sieve, and stored in labeled tubes for further use.

2.2. Experimental Material

Ethanol (analytical grade), Trichloroacetic acid (TCA), Alkaline copper reagent (Sodium carbonate, Copper sulphate, Sodium potassium tartarate), Hydrochloric acid (HCL), Anthrone reagent, Sulphuric acid (H2SO4), Standard glucose, Bovine Serum Albumin (BSA), Sodium hydroxide (NaOH), Folin- Ciocalteu reagent (FCR), Distilled water and Mulberry leaf (tender, medium & coarse) of three popular varieties viz., Botatul, Chatatul and Shahtul.

2.3. Estimation of total protein content:

Lowry et al., 1951 colorimetric method was used for total protein content estimation. Mortar pestle was used to homogenize 50mg of dried leaves from each sample in 80 percent ethanol. For 20 minutes, homogenates were centrifuged at 5000rpm. The supernatants were discarded, and the residues were suspended for 30 minutes in 10ml of 10% TCA (trichloroacetic acid). The supernatants were discarded after centrifuging the mixtures at 5000rpm for 10 minutes. Protein precipitates (pellets) were dissolved in 1N NaOH and maintained in a hot water bath for 30 minutes after being washed with 5% TCA. With distilled water, the samples were diluted 10 times. 1ml was taken from it and 5mL alkaline copper reagent was added and left for 10 minutes at room temperature. 0.5ml of 50% FCR reagent was quickly added and stirred. After allowing the mixture to rest at room temperature for 30 minutes, the absorbance was measured at 750nm.

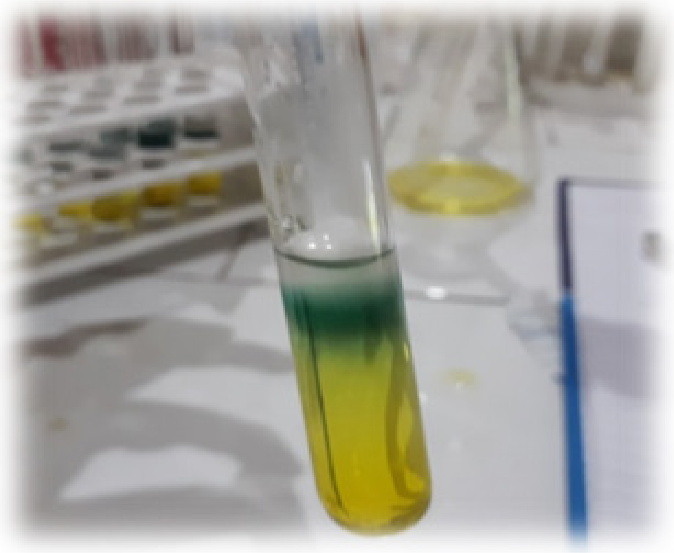
2.4. Estimation of total carbohydrate content:

Total carbohydrate estimation was carried out by Thimmaiah (1999) method using Anthrone reagent with slight modification. Dry leaf samples weighing 0.1g were taken in test tubes. 5ml of 0.5N HCL was added in each tube and left in boiling water bath for hydrolysis for 3 hours. Sample tubes were taken from water bath and kept at room temperature till cooled. Dry sodium carbonate was used to neutralize the samples until the effervescence stopped and volume was made 10ml with distilled water. For analysis, 0.05ml of each sample was taken and the final volume was made to 1ml with distilled water. 4ml Anthrone reagent was added, and samples were boiled for 8 minutes, quickly cooled, and absorbance of green to dark green color was measured at 630nm.

3. RESULTS & DISCUSSION:

3.1. Total Protein:

Table 01 and Figure 01 present the results in terms of overall protein content in mulberry leaves. In general, Botatul tender leaves had the highest total protein content (24.67%), followed by Chatatul tender leaves (22.36%), and Shahtul tender leaves (22.15%). The current findings are consistent with those of Kalaivani, et al., 2013; Murthy et al., 2013; Jyothi et al., (2016), who found that tender mulberry leaves have higher protein content than medium and coarse textured leaves. Because of high protein content in tender leaves, they are best feed during the larvae's early stages as they can acquire more nourishment by eating fewer leaves. The principal nutritional components in leaves are protein and carbohydrates, which have a direct impact on larval growth. The role of soluble and crude proteins in silkworm nutrition has been emphasized by Fukuda et al., (1959) and Takeuchi (1960). Higher values of protein and nitrogen in the feed are known to favour better performance of silkworm larval growth and their cocoon crop (Kalaivani, et al., 2013). Mulberry leaves having water, protein, total sugars, soluble carbohydrates, minerals, and crude fibre are best appreciated and consumed by silkworm larvae, according to Krishnaswami et al., (1978). Deficiency of certain nutrients or an imbalance of nutrients in leaves cause changes in the composition or metabolic activity of silkworm larval body (Ito, 1972).



3.2. Total carbohydrate:

Table 02 and Figure 02 show the carbohydrate content of different mulberry leaves. Chatatul tender (15.76%) had the highest carbohydrate content; followed by Shahtul tender (14.68%) and Botatul tender (12.38%), which gradually reduced with increasing growth periods. The current findings are consistent with earlier findings of Lokesh et al., 2012 and Murthy et al., 2013, who found that tender mulberry leaves have higher carbohydrate content than medium and coarse textured leaves. Carbohydrate content is calculated based on the amount of sugar and starch present in the leaves (Bose and Bindroo, 2001). Silkworms receive more energy and, as a result, silk protein production may be enhanced if the leaves have high carbohydrate content. Carbohydrates are utilized by the silkworm for energy source and for synthesis of both lipid and amino acids (Horie, 1978). The underlying growth and development involving biochemical process of insects are greatly influenced by the proteins, lipids and carbohydrates (Ito and Horie, 1959).

Table 01:
Total protein percentage in leaf at different stages

Leaf Stage	Mulberry varieties		
	Botatul	Chatatul	Shahtul
Tender	24.67	22.36	22.15
Medium	22.39	22.05	19.48
Coarse	20.42	19.56	18.65
CD	0.030	0.020	0.015
SE (d)	0.012	0.008	0.006
SE (m)	0.008	0.006	0.004
CV	0.065	0.047	0.037

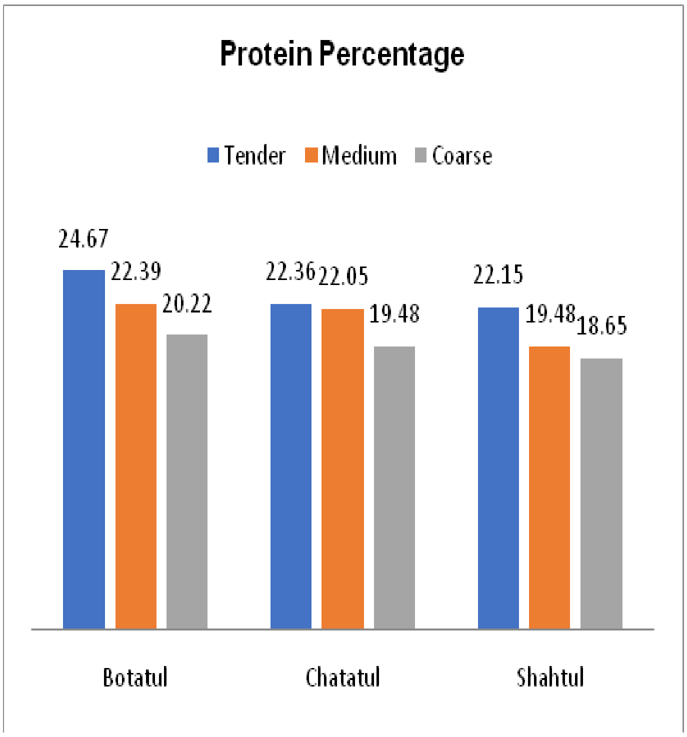


Figure 01: Graphical representation of total protein percentage in leaf at different stages.

Table 02:
Total carbohydrate percentage in leaf at different stages

Leaf Stage	Mulberry varieties		
	Botatul	Chatatul	Shahtul
Tender	12.38	15.76	14.68
Medium	12.06	15.59	12.89
Coarse	10.38	13.60	13.56
CD	0.024	0.028	0.031
SE (d)	0.010	0.011	0.012
SE (m)	0.010	0.008	0.009
CV	0.104	0.092	0.111

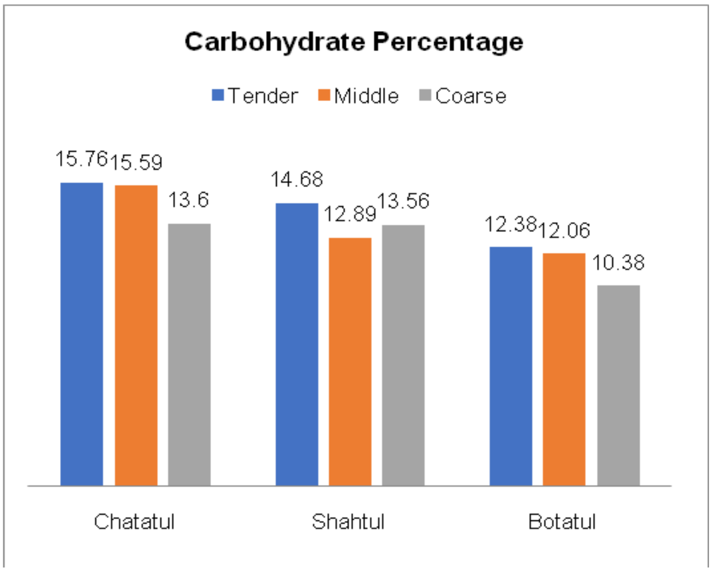


Figure 02: Graphical representation of total carbohydrate percentage in leaf at different stages.

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