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Changes in Some Biochemical Parameters of Mulberry (*Morus sp.*) Leaves after Infected with Leaf Spot Disease

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Abstract: The biochemical parameters such as moisture, ash, protein, fat, carbohydrate, Vitamin-C, phenol, pectin and mineral contents of healthy and leaf spot disease infected mulberry (*Morus sp.*) leaves were analyzed. Most of these nutrient contents in mulberry leaves were affected greatly with the infection of fungi. Total sugar, reducing sugar, non-reducing sugar, starch, moisture, ash, protein and lipid contents were found to be decreased in infected mulberry leaves due to leaf spot disease caused by *Cercospora moricola* Cooke and these contents were decreased by 8.58, 1.6, 60.09, 20.22, 24.52, 38.16, 33.84 and 40.26%, respectively. Among the contents, only Vitamin-C, phenol, pectin and mineral contents were found to be increased in infected samples over healthy samples and these contents were increased by 90, 84.21, 35 and 39.97%, respectively. The changes of total sugar, reducing sugar, non-reducing sugar, starch, protein, ash and lipid contents decreased remarkably but those of Vitamin-C, phenol, pectin and mineral contents increased significantly in leaves of disease affected mulberry as compared to those in healthy mulberry.

Key words: Biochemical parameters, mulberry, leaf spot disease, *Cercospora moricola*

Introduction

Cultivation of mulberry (*Morus sp.*) and rearing of silkworm (*Bombyx mori L.*) are the basis of Sericulture Industry that is dependent primarily on mulberry leaves, the only source of nutrition for silkworm, the growth and development of the larvae and subsequent cocoon production are very much influenced by its nutritive value (Anonymous, 1975 and Krishnaswami, 1978). It is reported that about 70% of the silk protein produced by silkworm are directly derived from the protein of mulberry leaves (Narayanan *et al.*, 1967; Krishnaswami *et al.*, 1970; Petkov and Dona, 1979 and Fukuda *et al.*, 1959). Increased carbohydrate content of mulberry leaves is favorable or healthy growth of silkworm larvae (Anonymous, 1975). The moisture content of mulberry leaf plays an important role in growth and development of silkworm (Sengupta *et al.*, 1971). Krishnaswami (1978) observed that mulberry leaf containing more water, protein, total sugar and soluble carbohydrate and less mineral is best relished and utilized by the silkworm larvae.

Mulberry is affected by various diseases and pests. Among the various diseases, leaf spot is the most devastating for mulberry cultivation. The disease is caused by *Cercospora moricola* Cooke and it decreases the leaf production during the months of April to September. Rural

people of Bangladesh consider sericulture as their pastime profession and earn extra money to help their family. To encourage the farmers it is essential to protect the leaves from the diseases like leaf spot as it hampers cocoon crop during the rainy season.

During the growth period of mulberry plant in rainy season fungal pathogen ***Cercospora moricola*** comes in contact with mulberry leaves under existing environmental condition and causes disease with interfering the normal physiological function of the leaf. The net result is the degradation of quality leaves. Ullal and Narasimahana (1978) stated that the affected leaf of mulberry by ***Cercospora moricola*** are unfit for silkworm consumption. It is reported that ***Cercospora moricola*** infects mulberry leaves, reduce leaf yield, deteriorate the nutritional value of the leaves and make the leaves unsuitable for silkworm larvae (Jeyarajan, 1986). Sharma ***et al.*** (1993) reported leaf spot; powdery mildew; leaf rust and leaf blight are the most serious diseases of mulberry leaf. In Bangladesh, leaf spot disease is found as major important fungal disease and degraded the normal cocoon production (Ali, 1995 and Ghosh, 1996). But not enough information is available on the comparative studies among the nutritional composition of healthy and infected mulberry leaves. The primary objectives of the present study were to quantify the changes of some biochemical parameters in the plant of healthy and disease infected mulberry leaves.

Materials and Methods

Mature mulberry leaves (healthy and diseased) of a selected variety are collected from the Bangladesh Sericulture Research and Training Institute, Rajshahi, Bangladesh. All the reagents used in the study were of analytical grade.

Ash content was determined following the method of AOAC (1980). Protein content was analyzed by the Kjeldahl method (Jayaraman, 1981) while the lipid content was determined following the method of Bligh and Dyer (1959). Total sugar and starch content of mulberry leaves were estimated following the method as described by Jayaraman (1981), reducing sugar content was determined by di-nitrosaly cylic acid method (Miller, 1972), while non-reducing sugar i.e., sucrose content was calculated following the formula as described by Ranganna (1979).

Vitamin-C content was estimated by the titramatric method (Bessey and King, 1993), while phenol content was calorimetrically by Folin-Cicocalten method (Bray and Thrope, 1954). Pectin content was determined by the king's gravimetric method of estimation of pectin as calcium pectate (Pearson, 1984). Moisture and mineral contents were determined by AOAC methods. (AOAC, 1980). Statistical analysis of data given as percentage was carried out from angular transformed values and performed using Microsoft Excel software.

Results and Discussion

The disease infected mulberry leaves can be easily distinguished from healthy mature mulberry leaves by physical observation only. By comparing the photographs of healthy and leaf spot diseased infected leaf as shown in Fig. 1 it can be concluded that the light green color of healthy mature leaf changed completely to yellowish color after infection.

The biochemical parameters such as moisture, ash, carbohydrate, protein, lipid, vitamin-C, phenol, pectin and mineral contents of healthy and infected mulberry leaves were analyzed and the results were summarized in Tables 1-3.

Table 1: Moisture, ash, protein and lipid contents of healthy and disease affected mulberry leaves

Mulberry leaves	Moisture (g %)	Ash (g %)	Protein (g %)	Lipid (g %)
Healthy	74.41±0.69	1.25±0.23	17.59±0.91	0.89±0.44
Diseased	68.02±2.54	1.23±0.31	7.02±1.83	0.71±0.38
% decreased after infection	8.58	1.60	60.09	20.22

Table 2: Total sugar, reducing sugar, non-reducing sugar, starch contents of healthy and disease affected mulberry leaves

Mulberry leaves	Total sugar (g %)	Reducing sugar(g %)	Non-reducing sugar (g %)	Starch(g %)
Healthy	5.83±0.00	1.31±0.38	3.25±0.20	16.79±0.07
Diseased	4.40±0.81	0.81±0.25	2.15±0.12	10.03±2.61
% decreased after infection	24.52	38.16	33.84	40.26

Table 3: Vitamin-C, phenol, pectin and mineral contents of healthy and disease affected mulberry leaves

Mulberry leaves	Vitamin-C (g %)	Phenol (g %)	Pectin (g %)	Mineral (g %)
Healthy	0.20±0.02	0.19±0.32	4.60±0.79	6.83±0.01
Diseased	0.38±0.07	0.35±0.19	6.21±0.69	9.56±0.03
% Increased after infection	90.0	84.21	35.00	39.97

Moisture, ash, protein and lipid contents of.. healthy and diseased mulberry leaves are presented in Table 1. The results clearly indicated that protein, lipid and moisture contents of mulberry leaves decreased significantly, while ash content decreased slightly after infection with disease. The decrease in above mentioned nutrient contents might be due to the accumulation of insoluble solid materials on the diseased area by the organism. Of the nutrients, the protein content decreased by 60.09% followed in decreasing order lipid by 20.22%, moisture by 8.58% and ash by 1.6%. As in the present study Saud ***et al.*** (2000) also reported the protein, lipid and moisture contents were decreased in guava by 56.09%, 24.24% and 20.73% after infected with ***Aspergillus niger*** respectively. Mogle and Mayee (1981) observed considerable reduction of amino acids in pearlmillet infected with downy mildew. Anwar (1986) also reported the reduction of amino acid contents of the infected tissue of sorghum and maize. Singh and Sinha (1983) found that the protein content was decreased in guava by 65% and 33% after infected with ***Aspergillus flavus*** and ***Aspergillus parasiticus***, respectively.

As given in Table 2 total sugar, reducing sugar, non-reducing sugar and starch contents in mulberry leaves were found to be also decreased remarkably after infection with disease and these contents were decreased by 24.52%, 38.16%, 33.84% and 40.26% respectively. Siddaramaiah and Hegde (1990) studied on change in biochemical constituents of ***Cercospora*** infected mulberry leaves and found that infection by the pathogen induced changes in the chemical constituents like total amino acids, phenol and sugars. Madhava Rao ***et al.*** (1981) and Sundares Waren ***et al.*** (1988) reported that diseased leaves are biochemically poor in nutritive value and indicated the reduction of moisture, protein and sugar contents. It is reported that total sugar, reducing sugar, non-reducing sugar and starch contents of mulberry leaves were found to be decreased with the increase of disease intensity (Ali, 1995 and Ghosh 1996). Similar results have been reported in fruit rot disease infected guava by Saud ***et al.*** (2000). Naik ***et al.*** (1988) reported that the total sugar content decreased in ***Colletotrichum gloeosporiodies*** infected betelvine leaf.

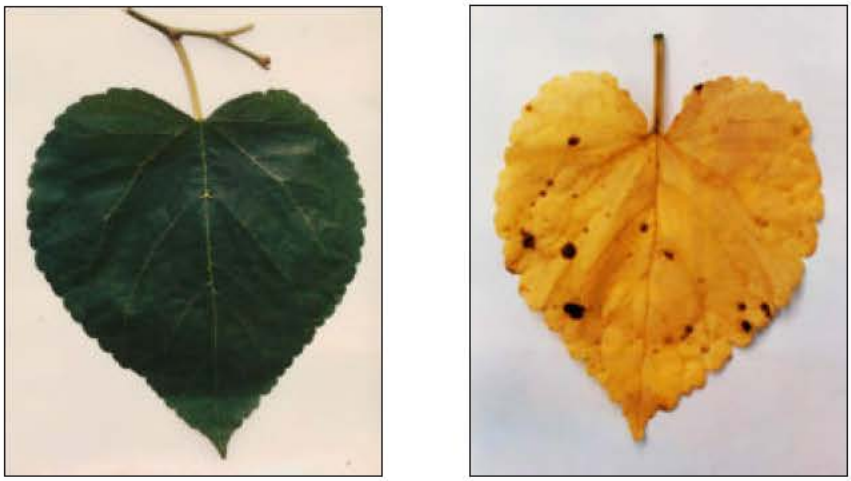

Fig.1: Photograph showing the healthy (left) & infected (right) mulberry leaves with leaf spot disease

The decrease in starch, total sugar, reducing sugar and non-reducing sugar is expected due to deficiency in photosynthetic pigments, the magnitude of which has been reported to be directly proportional to the rate of photosynthesis (Livne 1964, Ghosh 1996).

Vitamin-C, phenol, pectin and mineral contents of healthy and infected leaves of mulberry plants have been shown in Table 3. Results indicated that only these four contents were increased in leaf spot infected leaves as compared to the healthy ones. From the findings it can be suggested that the insoluble materials produced by the causative organism might be contained vitamin-C, phenol, pectin, mineral etc. The content of Vitamin-C and pectin was found to be increased 90.0% and 35% after infected with leaf spot disease of mulberry leaves. Ravindranath *et al*. (1965) reported that susceptibility is directly related to increase in ascorbic acid content of the leaf. Chahal *et al*. (1972) and Reddy *et al*. (1977) indicated the changes of ascorbic acid level in plants infected with pathogen.

Ascorbic acid content of leaf appears to play some role in susceptibility and resistance to disease. The disease susceptibility is directly related to increase in ascorbic acid content of the leaf (Singh, 1978). Ali *et al*. (1992) indicated that vitamin-C content is increased after infected of leaf spot disease in mulberry leaves which in turn increased susceptibility leading to premature defoliation. Siddaramaiah *et al*. (1980) reported production of toxins due to disease as the cause of premature defoliation.

It is observed that as the pathogenesis progressed there was a gradual increase of phenol and mineral contents in comparison to the healthy leaves. As in the present study Thind *et al*., (1977), Ghosh (1979), Singh and Sinha (1983), Saud *et al*. (2000) also reported higher content of phenol in infected apple fruit, orange, lemon and guava. Siddaramaiah and Hedge (1990) reported that with the increase in disease intensity phenols increased considerable in maize. It is reported that phenol content was found to be increased after infection of betelvine leaf and mango leaf (Naik *et al*., 1988 and Tofazzol *et al*., 1999).

Minerals were present to a lower level in both healthy and diseased mulberry leaves. The content was found to increase moderately in disease affected mulberry leaves than that of healthy ones. It is probably due to the increased activity of certain enzymes, which are very crucial for the metabolism of causative organism. Salim Uddin ***et al.*** (2000) reported that minerals (calcium and iron) were found to be increased after infection of sugarcane fleshes.

Considering the results obtained it may be concluded that due to changes of moisture, ash, protein, lipid, sugar, reducing sugar, non-reducing sugar, total sugar, vitamin-C, phenol, pectin and mineral contents in infected leaves, the metabolic process of infected plant is altered as compared to healthy ones which leads to cause yield reduction of mulberry leaves.

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