http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (3): 355-359, 2006
ISSN 1028-8880

Nutritional Changes of Four Varieties of Mulberry Leaves Infected with Fungus (*Pyllactinia corylea*)

[1]Abul Kashem Tang, [1]M.A. Samad, [1]A.S.M. Aynul Haque Akand,
[1]Bably Sabina Azhar and [2]N. Absar
[1]Department of Applied Nutrition and Food Technology,
Islamic University, Kushtia-7003, Bangladesh
[2]Department of Biochemistry and Molecular Biology,
University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: The nutritional composition such as moisture, ash, lipid protein, carbohydrate, total chlorophyll, crude fibre, phenol, β-carotene, vitamin-B_1, vitamin-B_2, vitamin-C, Calcium, phosphorus and iron content of four varieties of healthy and *Phyllectinia corylea* infected mulberry leaves at mature stage has been analyzed. The mulberry leaves are considered as a good sources of starch, protein, crude fibre, minerals, vitamin-C, β-carotene and were estimated about 6-7, 4.0-4.6, 3.0, 3.0-3.5, 115-132, 100-120 mg%, respectively. Moisture, ash, lipid, crude fibre, carbohydrate, vitamins and minerals content were decreased significantly but protein and phenol content were increased after infected of mulberry leaves with fungus, *Phyllectinia corylea*.

Key words: Mulberry leaves, nutritional changes, *Phyllectinia corylea*

INTRODUCTION

Mulberry (*M. alba* Linn.) is the sole host plants of the silkworm *Bombyx mori* Linn which produces silk. It belongs to the family *Moraceae* under genus *Morus*. is a deep rooted perennial plant widely distributed in Asia, Europe, Africa and Latin America on a wide range of climatic conditions varied from temperate to topical. Mulberry leaves are the sole sources of nutrients e.g., protein, carbohydrate, vitamins, minerals etc. for silkworm's growth. Protein provides the chief structural elements of the muscle, glands and other tissues (Zoadur and Absar, 2001; Hossain *et al*., 1999). It was known that various microorganisms seriously infected the mulberry leaves. Of these microorganisms, fungus is most important because they cause many diseases; as a result the mulberry leaves are unsuitable to feed silkworm. In Bangladesh, mainly *Cercospora moricola* and *Phyllactinia corylea* are infected mulberry leaves widely and the diseases are called leaf spot and powdery mildew respectively, as a result decreased the production of quality leaves by 50% which greatly effect on silkworm rearing as well as the Sericulture Industry (Nomani *et al*., 1970; Sullia and Padma, 1987; Sikdern *et al*., 1979; Gavindaiah *et al*., 1990). Jeyarajan reported that these pathogens infect mulberry leaves and reduces not only yield but also nutritional values, thus making the leaves unsuitable for silkworm feeding (Bakshi *et al*., 1972). *Phyllactinia corylea* parasite infected mulberry leaves that causes powdery mildew in most part of India and Bangladesh during dry season from late September to February and serious outbreak in November to January when the weather is cold and relative humidity is comparatively low (Bakshi *et al*., 1972). As a result production of poor quality of leaves which can not produce good quality cocoons and thus causing a serious set back to sericulture industry. The objective has been ascertained the changes of nutrient content of four varieties mulberry leaves at mature stage.

MATERIALS AND METHODS

The freshly and fungus *Phyllactinia corylea* infected mulberry leaves were collected from Bangladesh Sericulture Research and Training Institute, Rashahi, Bangladesh. The experiment was performed in the month of November-March at the protein and Research laboratory in the year of 2001. Ash and crude fibre content were determined following the methods (AOAC, 1980). Chlorophyll content of the healthy and disease infected mulberry leaves tissues were estimated following the method described by Methods in Physiological Plant Pathology (Mahadevan and Sridar, 1982). Total protein

Corresponding Author: Abul Kashem Tang, Department of Applied Nutrition and Food Technology, Islamic University, Kushtia-7003, Bangladesh

and water soluble protein content of mulberry leaves were determined by the method of Micro-Kjeldahl as described in Laboratory Manual in Biochemistry and Lowry method (Jayaraman, 1981; Lowry *et al*., 1951), respectively. Lipid content of mulberry leaf was determined by the method of Bligh and Dyer (1959). Total sugar and starch content of mulberry leaves were determined by the method as described in the Laboratory Manual in Biochemistry (Jayaraman, 1981). Reducing sugar content was determined by dinitrosalicylic acid method.

Estimation of phenol with Folin-Ciocalteu reagent is based on the reaction between phenol and an oxidizing agent phosphomolybdate, which results in the formation of a blue complex (Bray and Thrope, 1954). Calcium and vitamin-C content were determined by titrimetric method (Bernard, 1965; Vogal, 1961). Phosphorus and iron contents of mulberry leaves were determined by the method of Vogel (Bessey and King, 1933). β-carotene, vitamin B_1 and vitamin B_2 contents were determined following the procedures as described in Methods of Vitamin Analysis (Anonymous, 1960).

RESULTS AND DISCUSSION

Moisture plays an important role in the growth activities of plants, herbs etc. Water is indispensable to the absorption and transportation of food to carry on photosynthesis, metabolism of materials and the regulation of temperature. It is also essential for most of the physiological reactions in plant tissue and in its absence life does not exist (Rangaaswami *et al*., 1976). The moisture content was decreased slightly (about 6-7%) after infection with *P. corylea* of four varieties mulberry leaves (Table 1).

Most of the inorganic constituent or minerals are present in ash. The result clearly indicated that the mineral contents of mulberry leave were decreased significantly after infection with *P. corylea*. The ash content of healthy and disease infected mulberry leaves varied between 3.05-3.40 and 2.56-2.83%, respectively (Table 1).

The chlorophyll is most important component of plant. The healthy leaves contained about 255-322 mg%

Table 1: Moisture, dry matter and ash content of healthy and diseased mulberry leaves at mature stage

Variety of leaves	Types of leaves	Moisture (%)	Ash (%)
BM-1	Healthy	71.96±0.14	3.18±0.10
BM-1	Leaf spot	68.44±0.12	2.83±0.12
BM-2	Healthy	72.84±0.10	3.08±0.08
BM-2	Leaf spot	66.23±0.22	2.72±0.06
BM-3	Healthy	74.86±0.18	3.05±0.09
BM-3	Leaf spot	69.43±0.18	2.83±0.06
BM-4	Healthy	70.98±0.09	3.40±0.04
BM-4	Leaf spot	67.31±0.12	2.56±0.10

whereas *P. corylea* infected leaves contained 110-202 mg% of total chlorophyll (Table 2). The result indicated that the total chlorophyll content of mulberry leaves was decreased drastically after infection with *P. corylea*. The result indicated that both chlorophyll-a and chlorophyll-b content were decreased and also indicated that chlorophyll-b content was more pronounced decreased than chlorophyll-a. The similar result indicated by (Tang *et al*., 2005; Hossain *et al*., 1999; Subba *et al*., 1979).

Disease development causes impairment in the photosynthetic pigments that affects the utilization of the light energy by the plants. Disease developments affect not only the total chlorophyll-a and chlorophyll-b (Mahadevan and Sridhar, 1982).

Protein as essential nutrient for growth and development is found to be present in good amount in mulberry leaves as compared to that in other fruits and vegetables. The total protein content of healthy leaves were 4.08-4.62% while diseased leaves contained 5.02-5.28% protein, but the healthy and disease infected mulberry leaves contained 3.12-3.32 and 3.72-3.97% water soluble protein (Table 3), respectively.

The mulberry leaves are considered as good source of starch, protein, crude fiber, minerals, vitamin-C and β-carotin and their contents were foumd to be about 6-7, 4-4.6, 3, 3.0-3.5, 115-132 and 100-120 mg%, respectively (Kashem *et al*., 2003). Nutrients content of Moringa fruit such as moisture, total sugar, starch, total soluble solid, vitamin-C are decreased why ash, total protein, total lipid and reducing sugar contents were decreased after infection with Rhizopus Stolonifer infected disease (Zoadur and Absar, 2001). Nutrients contents of mango leaves such as lipid, total sugar and reducing contents decreased significantly while protein, calcium and iron content increased moderately after infection (Hossain *et al*., 1999).

The total protein content of healthy leaves were 4.12~4.62 g% while diseased leaves contained 5.02~5.28 g% protein while the water soluble protein content of healthy leaves was 3.12~3.32 g% and diseased leaves was 3.89~3.96 g% (Table 3). The result indicates that in both cases the protein content of pyllactinia corylea infected leaves increased significantly. Similar result was also reported by Nuhu *et al*. (1960) in purple vein virus infected tomato leaves.

According to Hayashi (1962) the accumulation of nitrogen is due to increase in the activity of amino acid activating enzymes, suggesting that an increase in protein content in virus infected leaves is due to synthesis of viral protein by the causative organism.

Table 2: Chlorophyll content of healthy and diseased mulberry leaves at mature stage

Variety of leaves	Types of leaves	Total chlorophyll (mg%)	Chlorophyll-a (mg%)	Chlorophyll-b (mg%)
BM-1	Healthy	275.25±0.03	185.20±0.02	90.52±0.01
BM-1	Leaf spot	110.75±0.02	79.95±0.05	30.81±0.03
BM-2	Healthy	255.45±0.04	175.25±0.02	80.20±0.05
BM-2	Leaf spot	130.86±0.02	88.74±0.03	42.06±0.02
BM-3	Healthy	322.25±0.04	194.30±0.04	127.95±0.02
BM-3	Leaf spot	202.25±0.04	132.56±0.03	69.69±0.02
BM-4	Healthy	265.20±0.04	179.83±0.04	83.22±0.01
BM-4	Leaf spot	150.28±0.04	106.56±0.03	43.72±0.05

Table 3: Protein, lipid and crude fibre content of healthy and diseased mulberry leaves at mature stage

Variety of leaves	Types of leaves	Protein (%)	Water soluble protein (%)	Lipid (%)	Crude fibre (%)
BM-1	Healthy	4.12±0.08	3.12±0.12	1.97±0.09	6.12±0.12
BM-1	Leaf spot	5.02±0.06	3.89±0.16	1.30±0.02	3.73±0.10
BM-2	Healthy	4.08±0.09	3.23±0.10	2.00±0.05	6.08±0.14
BM-2	Leaf spot	5.09±0.06	3.97±0.09	1.32±0.08	4.08±0.08
BM-3	Healthy	4.12±0.08	3.22±0.08	2.18±0.05	7.04±0.10
BM-3	Leaf spot	5.02±0.10	3.72±0.04	1.42±0.04	4.28±0.09
BM-4	Healthy	4.62±0.12	3.32±0.06	2.10±0.08	6.78±0.12
BM-4	Leaf spot	5.28±0.08	3.96±0.08	1.38±0.04	4.28±0.09

Table 4: Carbohydrates content of healthy and diseased mulberry leaves at mature stage

Variety of leaves	Types of leaves	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Starch (%)
BM-1	Healthy	2.82±0.04	0.62±0.04	2.20±0.06	6.46±0.11
BM-1	Leaf spot	1.42±0.08	0.34±0.05	1.08±0.05	3.94±0.09
BM-2	Healthy	3.02±0.03	0.71±0.05	2.31±0.10	6.82±0.07
BM-2	Leaf spot	1.46±0.03	0.38±0.01	1.08±0.06	4.11±0.08
BM-3	Healthy	2.82±0.06	0.59±0.02	2.23±0.04	7.22±0.04
BM-3	Leaf spot	1.44±0.06	0.37±0.03	1.07±0.10	4.14±0.08
BM-4	Healthy	2.74±0.03	0.68±0.03	2.06±0.03	6.38±0.05
BM-4	Leaf spot				

Table 5: Minerals and Phenol content of healthy and diseased mulberry leaves at mature stage

Variety of leaves	Types of leaves	Phenol (mg%)	Calcium (mg%)	Phosphorus (mg%)	Iron (mg%)
BM-1	Healthy	145.24±0.18	240±2	75.81±0.12	11.82±0.04
BM-1	Leaf spot	208.38±0.14	152±1	44.25±0.10	6.44±0.05
BM-2	Healthy	150.84±0.16	225±3	81.89±0.11	12.34±0.06
BM-2	Leaf spot	208.28±0.18	130±4	52.08±0.10	6.82±0.05
BM-3	Healthy	148.25±0.14	245±1	78.04±0.09	13.04±0.08
BM-3	Leaf spot	216.34±0.11	145±1	52.23±0.08	6.44±0.02
BM-4	Healthy	160.86±0.18	238±2	76.78±0.06	14.00±0.05
BM-4	Leaf spot	218.65±0.20	152±2	50.42±0.06	6.75±0.04

Crude fibre has pronounced effect on the digestion and absorption processes of nutrients. The mulberry leaves contained about 7% crude fibre that was decreased about 30-40% after infection with disease. Tang found that crude fibre content was decreased of mulberry leaves after infection with *Cercospora moricola* (Tang *et al*., 2005). The lipid content of mulberry leaves decreased remarkably after infection with disease. The healthy mulberry leaves contained about 2% lipid. While the fungus infected leaves contained between 1.30-1.42% lipid (Table 3).

Table 4 represents the total sugar, reducing sugar, non-reducing sugar and starch contents of healthy and disease infected mulberry leaves. The amount of total sugar, reducing sugar and non-reducing sugar were found to vary between 2.74-3.02, 0.59-0.71 and 2.06-2.31% in healthy leaves and 1.42-1.46, 0.34-0.38 and 1.07-1.08% in diseased leaves, respectively. The total sugar content of mulberry leaves decreased drastically (about 50%) after infection with disease. Starch is the storage carbohydrate of chlorophyll containing plants and the starch content of healthy and diseased mulberry leaves are between 6.38-7.22 and 3.94-4.14%, respectively (Table 4). The present data indicated that the starch content of mulberry leaves also decreased remarkably after infection with disease and the reduction in starch content may be due to the hydrolysis of starch by microorganism for their carbon source and synthesis of lower amount of carbohydrate because chlorophyll pigment decreases drastically. From the present data it can be concluded that the contents of all types of sugar in mulberry leaves decreased significantly after infection with fungus, *P. corelia*. The results showed good agreement with that reported for infected pods (Hedge and Manjol, 1967) and for infected betelvine leaves (Naik *et al*., 1988). Mehta also found that sugar content decreased due to fruit-rot disease of tomato (Mehta *et al*., 1995).

The four varieties of healthy mulberry leaves contained 145.24-160.86 mg% whereas disease affected leaves contained 208.38-218.65 mg% phenol (Table 5)

Table 6: Vitamin content of healthy and diseased mulberry leaves at mature stage

Variety of leaves	Types of leaves	Vitamin-C (mg%)	β-carotene (mg%)	Vitamin-B_1 (mg%)	Vitamin-B_2 (mg%)
BM-1	Healthy	124.95±0.14	102.46±0.10	44.23±0.05	0.48±0.01
BM-1	Leaf spot	91.24±0.15	70.2±0.12	30.25±0.02	0.28±0.01
BM-2	Healthy	126.82±0.14	98.38±0.09	46.32±0.03	0.50±0.01
BM-2	Leaf spot	89.28±0.16	69.86±0.10	32.06±0.02	0.30±0.02
BM-3	Healthy	132.75±0.14	105.32±0.09	60.79±0.01	0.46±0.01
BM-3	Leaf spot	94.22±0.12	83.79±0.09	50.36±0.02	0.26±0.02
BM-4	Healthy	116.00±0.18	120.23±0.10	54.28±0.03	0.52±0.01
BM-4	Leaf spot	80.27±0.12	86.76±0.11	40.06±0.04	0.29±0.01

indicating that the content of phenolic substances increased by (25-35%) after infection with fungus. Changes in phenolic substances of almost all the inoculated plants have been reported. Thind reported that phenol contents increased in date apple fruits infected with smut (Thind *et al*., 1977).

As given in Table 5, it was found that the minerals such as calcium, phosphorus and iron contents of healthy leaves 240~238, 75.81~76.78 and 11.82~14.00 g% while those of the disease leaves were 152, 44.25~50.42 and 6.44~6.75 g%. So it was found to decrease in *P. corylea* infected mulberry leaves about 30-45%, respectively. Hossain *et al*. (1999) found that the calcium and iron contents decreased in the mango leaves after infection with Colleotrichum gloeosphorioides while Prasad *et al*. (1976) reported that phosphorus content decreased in diseased condition of sunflower leaves.

From Table 6 it was found that the mulberry leaves contained good amount of vitamin-C, β-carotene and vitamin-B_1. The amount of vitamin-C, β-carotene, vitamin-B_1 and vitamin-B_2 were found to vary between 116.00-132.75, 98.38-120.23, 44.23-60.79 and 0.468-0.523 mg% in healthy leaves and 74.25-82.44, 70.21-88.29, 33.33-49.21 and 0.272-0.326 mg% in disease infected leaves, respectively. Like minerals, the contents of all the above mentioned vitamins in mulberry leaves decreased after infection with disease and the content of vitamin-C, β-carotene, vitamin-B_1 and vitamin-B_2 were decreased by 40, 25, 30 and 35%, respectively. Agarwal reported that ascorbic acid content decreased in lemon, musambi and orange fruits induced by *Colleotrichum gloeosporioides* (Agarwal and Ghose, 1979). Singh found that *A. flavus* and *A. parasiticus* caused considerable losses in the quantity of ascorbic acid in Musambi fruits (Singh, 1982).

In conclusion, this study gave almost detailed nutrient analysis of four varieties of mulberry leaves were produced widely in Bangladesh. Further the nutritional quality of mulberry leaves was found to be seriously affected after infection with fungus, *P. corylea*.

REFERENCES

Anonymous, 1960. Report on determination of β- carotine, Vitamin-B, Vitamin-B of betel leaves in this study. Bio. Nature, 46: 412.

Agarwal, G.P. and K. Ghose, 1979. Reports on post infectional changes in ascorbic acid content in lemon, musambai and orange fruits induced by *C. gloeosporioides* Penz. and reports on Potassium, Iron and Zinc. Ind. Phytopayhol., 32: 108-109.

AOAC, 1980. Official Methods of Analysis. 13th Edn., Association of Official Analytical Chemists, Washinton DC.

Bessey, O.A. and C.G. King, 1933. The distribution of Vitamin C in plant and animal tissues and its determination. J. Biol. Chem., 103:687-698.

Bray, H.G. and W.V. Thorpe, 1954. Reports on FCR methods for estimation of phenol. Biochem. Invea. J., 17: 134-137.

Bligh, E.G. and W.J. Dyer, 1959. Total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911.

Bernard, L.O., 1965. Hawk"s Physiological Chemistry. 14th Edn., McGraw Hill Book Company, New York, pp: 1264.

Bakshi, B.K., M.A. Reddy Ram, Y.N. Puri and S. Singh, 1972. Survey of the disease of important FRIC, Deharddun, India, pp: 93-98.

Gavindaiah, P.T., K. Girindhar Sengupta and A.N. Suryanarayan, 1990. Incidence of leaf spot disease in relation to epidermal and cuticle thicness in some indigenous varieties of mulberry. Sericologia, 30: 257-260.

Hayashi, Y., 1962. Amino acid activation in tabacco leaves infected with tobacco mosaic virus. Virology, 18: 140-142.

Hedge, R.K. and R.L. Monjol, 1967. Physiological changes in the bean pods due to infection of *C. lindemuthianum.* Ind. Phyotopathol., 24: 186-188.

Hossain, T., Z. Alam and N. Absar, 1999. Changes in different nutrients and enzyme contents in mango leaves infected with *Colletotrichum glaeosorioides*. Ind. Phytopayhol., 52: 75-76.

Jayaraman, J., 1981. Laboratory Mannual in Biochemistry. Wiley Eastern Ltd., New Delhi Ltd., India, 53: 75-76.

Kashem, M.A., F. Parveen and N. Absar, 2003. Nutritional changes of four varieties of Mulberry leaves at infected with Fungus (*Cercospora moricola).* J. Bio-Sci., 11: 113-119.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mahadevan, A. and R. Sridhar, 1982. Methods in Physiological Plant Pathology. Sivakami Publications. Madras, pp: 5-8, 36.

Mehta, P., M. Vyas and S.B. Suksena, 1995. Metabolic changes during pathogenesis of fruit-rot disease of tomato. Ind. Phyotopathol., 28: 253-255.

Nuhu, A., A.M. Akanda and A. Khair, 1960. Biochemical changes of tomato due to purple vein virus. Bangladesh J. Bot., 25: 321-349.

Nomani, M.K.R., P.K. Mukheerjce and S. Krishnaswami, 1970. Studies on the effect of feeding multivoltine silkworms (*B. mori*) larvae with mildew affected leaves. Ind. J. Seric., 9: 49-52.

Naik, M.K., P.C. Hirmath and S.V. Hiremath, 1988. Post infectional changes in the betel vine leaves infected with *Colletotrichum glaeosorioides.* Ind. Phyotopathol., 41: 370-372.

Prasad, B., O.P. Verma and L.N. Daftan, 1976. Biochemical changes in Safflower leaves, caused by rust infection. Ind. Phytopayhol., 29: 91-93.

Rangaaswami, G., M.N. Narasimhanna, C.R. Sastry and K. Kasiviswanathan, 1976. Sericulture manual-1. Mulberry Cultivation. FAO. Rome, pp: 150.

Sikder, A.K., M.V. Samson, Y.R. Madhava Rao, B. Murtuza and Nataraju, 1979. Effect of feeding leaf spot affected and systemic fungicide sprayed leaves of mulberry (*Morus indica* L.). Ind. J. Ser., 28: 73-77.

Subba, R.B.L., A. Ghosh and V.T. John, 1979. Effect of rice tungro virus on chlorophyll and anthocyanin pigmants in two rice cultivars. Phytopathology, 94: 367-371.

Singh, A., 1982. Total sugar, reducing sugar and non-reducing sugar content in plants. Natl. Bio., 34: 145-162.

Sullia, S.D. and B.D. Padma, 1987. Acceptance of mildew affected leaves by silkworm (*B. mori*) and its effects on cocoon characteristics. Sercologia, 27: 693-696.

Thind, T.S., S.B. Saksena and S.C. Agarwal, 1977. post infectional changes in amino acids, sugars, phenolic substances and organic acids of apple fruits incited by *Clathridium Corticola*. Ind. Phytopayhol., 30: 445-448.

Tang, M.A.K., K.A. Salam, M.A. Samad and N. Absar, 2005 Nutritional Changes of four varieties of Mulberry leaves at infected with Fungus (*Cercospora moricola*). Pak. J. Biol. Sci., 8: 127-131.

Vogal, I.A., 1961. A text book of Quantitative Inorganic Analysis. 3rd Edn., Longman (London), 787b: 806-808.

Zoadur, M. and N. Absar, 2001. Indian Phytopathol., 54: 293-298 .