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# Post Infection Physiobiochemical Alteration at Various Intensities of Leaf spot (*Myrothecium roridum*) in Mulberry

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**Changes in biochemical constituents and physiological alteration were studied in various intensities (1 - 5%, 6 - 15%, 16 - 30%, 31 - 50% and > 50%) of leaf spot (*Myrothecium roridum*) on mulberry leaves and compared with healthy leaves. Chlorophyll, total soluble sugar and total protein were decreased ($P < 0.01$), but total phenol increased due to pathogen infection. Changes in biochemical constituents showed significant correlation with intensity of disease. Chlorophyll ($r^2 = 0.92$), and protein ($r^2 = 0.83$) possessed negative while phenol ($r^2 = 0.61$) possessed positive correlation. Photosynthetic rate, transpiration rate, stomatal conductance, moisture content (%) and physiological water use efficiency (pWUE) were decreased, but stomatal resistance increased in the infected leaves. Physiological parameters also possessed significant ($P < 0.01$) correlation with disease intensity. Photosynthetic rate ($r^2 = 0.96$), transpiration rate ($r^2 = 0.88$), stomatal conductance ($r^2 = 0.65$), physiological water use efficiency ($r^2 = 0.88$) and moisture content ($r^2 = 0.85$) were negatively but stomatal resistance ($r^2 = 0.75$) was positively correlated to disease intensities.**

**Key words**: Mulberry, *Myrothecium roridum*, Leaf spot, Disease intensities, Physiobiochemical alteration

## Introduction

Diseases cause physiological and biochemical alterations in plants. Effect on physiobiochemical characters due to foliar diseases is vital in mulberry since the leaves are directly used for silkworm rearing and the quality of leaves is the key determinant of quality and quantity of the silk produced. Feeding diseased leaves to silkworm results prolonged larval period and deterioration of cocoon characters (Noamani *et al.*, 1970; Sullia and Padma, 1987; Qadri *et al.*, 1998). Similarly, fungal infections cause various physiological and biochemical changes in mulberry foliage. Reduction of photosynthetic pigments, soluble sugar, protein and phenol was observed on infection of various foliar diseases in mulberry (Madhavarao *et al.*, 1981; Umesh Kumar, 1991; Teotia *et al.*, 1997) affecting the nutritive value of the foliage. Likewise, impact of pathogen infection on physiological characters of mulberry foliage was also documented (Madhavarao *et al.*, 1981; Sundareswaran *et al.*, 1988; Shree and Nataraja, 1993; Teotia *et al.*, 1997).

Diseased leaves have cells both directly invaded or killed by the pathogens and cells which are not invaded but are affected by presence of pathogen in the tissues (Scholes, 1992). Therefore, measurement of physiological variables are dependent upon the density of infection and must be compared with measurement taken on healthy control leaves to allow the quantification of impact of disease on leaf physiology (Lopes and Berger, 2001). In addition, photosynthetic competitiveness of diseased leaves with healthy leaves allows quantification of impact of a disease on the remaining green area of the diseased leaves and is the most appropriate way to integrate physiological information in crop loss studies (Rabbinge and Rijisdik, 1981; Boote *et al.*, 1983; Rabbinge *et al.*, 1985; van Oijen, 1990; Bastiaans, 1991). Attempts were made to establish quantitative relation between disease intensities and rate of photosynthesis or of respiration in various pathosystems (Habeshaw, 1979; McGrath and Penny-

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packer, 1990). This study attempts to quantify physiobiochemical characters of mulberry leaf in relation to various intensities of the disease.

## Materials and Methods

The experiment was conducted at Central Sericultural Research & Training Institute, Berhampore, West Bengal using popular mulberry cv S1. Plants were grown in earthen pots (12" diameter and 20" height) following recommended package of practices (Subbarao, 1989). The fungi *Myrothecium roridum* was grown in potato dextrose agar. After 10 days of growth, the conidia were harvested using a camel hairbrush and the conidial suspension ($10^4$ spores/ml) was prepared in sterilized distilled water. The suspension was sprayed on 30 days old shoots of mulberry plants with a hand held sprayer and allowed for development of the disease. A control group was kept without inoculation. After 15 days, when the disease was developed well, the leaves were collected from 5 – 7 position randomly from ten plants and graded into five groups as 1 (1 – 5% leaf area infected), 2 (6 – 15% leaf area infected), 3 (16 – 30% leaf area infected), 4 (31 – 50% leaf area infected) and 5 (> 50% leaf area infected). The leaves without infection collected from uninoculated plants grown in similar condition were taken as control for comparison. Three replications were kept for each grade and control.

Total chlorophyll content, total soluble protein, total soluble sugar, and phenol of these leaves were estimated following the methods of Arnon (1949), Lowry *et al.* (1951), Morris (1949) and Bray and Thorpe (1954), respectively.

The leaves of same disease intensities in 0 – 5 scale were assessed for gas exchange parameters. Gas exchange parameters were measured using a portable photosynthetic system (Model LI-6200, LI COR Inc. Lincoln, NE) with 250 ml leaf chamber. All measurements were taken around noon (10.00 – 12.00 a.m.) to minimize diurnal variation in chlorophyll titre and photosynthetic ratio. At the time of measurement, $CO_2$ was near ambient (300 – 400 $\mu^{-1}$). Exchange of $CO_2$ between the atmosphere and leaf was measured by monitoring the rate at which the $CO_2$ concentration changed at short interval of time. The net photosynthetic rate was then calculated from the rate of change. Transpiration, stomatal conductance, stomatal resistance and physiological water use efficiency were (pWUE) also measured. Water content of the leaves was calculated from fresh and dry weight of the leaves.

Data were analysed statistically for variance and *F* test (Snedecor and Cochran, 1967) was carried out. Correlation between the disease intensities and physio-biochemical alteration was done and regression equations were made.

## Results

### Biochemical changes

The disease influenced on all the biochemical activities of the leaves. Chlorophyll content reduced in infected leaves significantly ($P < 0.05$). Total soluble sugar content increased in the infected leaves, compared to control with maximum in grade 5 (38.59%). Total soluble protein content significantly decreased in the infected leaves. Maximum reduction of protein (85.81%) was observed in grade 5. However, there was no significant decrease between grade 2 and grade 3. Phenol content increased during the course of pathogenesis. Increase was high in disease intensity grade 4 followed by in grade 3 and grade 5. There was no significant variation in phenol content among leaves of intensity grade 1 and grade 2 (Table 1).

### Physiological alterations

Photosynthetic rate declined sharply in the infected leaves and significantly ($P < 0.05$) lowered from disease intensity grade 2 onwards. Reduction was higher in intensity grade 5 (6.08), which was 69.45% less compared with healthy

**Table 1.** Some biochemical changes at various intensities of *Myrothecium* leaf spot in mulberry leaves

| Disease severity (%) | Total chlorophyll (mg/g. fr.wt.) | Total soluble sugars (mg/g. fr.wt.) | Total soluble proteins (mg/g. fr.wt.) | Total phenols (mg/g. fr.wt.) |
|---|---|---|---|---|
| 0 (No disease) | 2.63 (0.00) | 45.17 (0.00) | 28.60 (0.00) | 19.73 (0.00) |
| 1 (1 – 5%) | 2.50 (4.56) | 47.70 (5.60) | 16.73 (41.49) | 27.96 (41.72) |
| 2 (6 – 15%) | 2.51 (4.45) | 52.13 (15.42) | 14.26 (50.11) | 27.23 (38.00) |
| 3 (16 – 30%) | 2.47 (5.86) | 58.90 (30.40) | 12.53 (56.17) | 38.03 (92.03) |
| 4 (31 – 50%) | 2.45 (6.62) | 54.46 (20.59) | 9.83 (65.60) | 48.56 (146.12) |
| 5 (> 50%) | 2.34 (10.92) | 62.60 (38.59) | 1.06 (85.81) | 32.56 (65.03) |
| CD $P < 0.05$ | 0.081 | 2.91 | 2.36 | 4.56 |

Figures are average of three replications. Figures in parenthesis are percent increase or decrease compared with control.

**Table 2.** Some physiological alterations at various intensities of *Myrothecium* leaf spot in mulberry leaves

| Disease severity (%) | Photosynthetic rate ($\mu$ mol$^{-1}$S$^{-1}$) | Transpiration rate (mol m$^{-2}$S$^{-1}$) | Stomatal conductance (Cm s$^{-1}$) | Stomatal resistance (S cm$^{-2}$) | pWUE | Moisture (%) |
|---|---|---|---|---|---|---|
| 0 (No disease) | 19.90 (0.00) | 0.021 (0.00) | 1.76 (0.00) | 0.58 (0.000) | 930.03 (0.00) | 75.80 (0.00) |
| 1 (1 – 5%) | 19.07 (4.17) | 0.019 (0.11) | 1.15 (34.86) | 0.88 (55.80) | 886.77 (46.51) | 68.37 (9.80) |
| 2 (6 – 15%) | 16.55 (16.83) | 0.021 (0.99) | 1.211 (31.34) | 0.85 (50.35) | 579.10 (37.73) | 67.33 (11.11) |
| 3 (16 – 30%) | 10.877 (45.34) | 0.021 (0.99) | 1.08 (38.83) | 0.99 (75.17) | 616.17 (33.74) | 66.57 (12.17) |
| 4 (31 – 50%) | 9.44 (52.55) | 0.021 (0.99) | 1.037 (41.21) | 0.99 (74.47) | 560.60 (30.04) | 64.40 (12.17) |
| 5 (> 50%) | 6.080 (69.45) | 0.021 (0.99) | 0.997 (43.48) | 1.03 (81.33) | 381.90 (58.93) | 62.50 (17.05) |
| CD P < 0.05 | 3.40 | NS | 0.40 | 0.26 | 121.81 | 1.73 |

Figures are average of three replications. Figures in parenthesis are percent increase or decrease compared with control. NS- not significant.

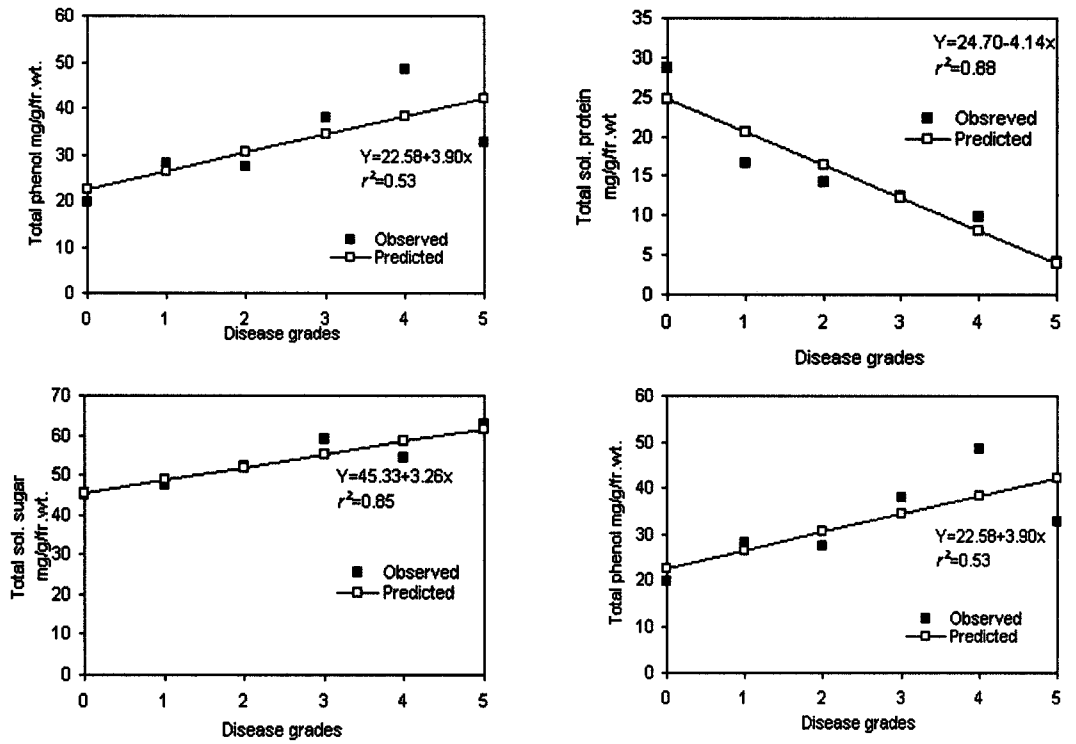


**Fig. 1.** Relation between some biochemical changes and disease intensities in mulberry leaves.

leaves. No statistically significant reduction in photosynthetic rate was observed between intensity grades 1 (19.07) and 2 (16.55), among grade 3 and 4 similarly among grade 4 and 5. No significant variation in transpiration rate observed due to infection. In the infected leaves, stomatal conductance was significantly lower. Minimum conductance was recorded in the leaves under severity grade 5 with maximum reduction 43.48%. No statistically significant difference was noticed among grade 1 (1.15) and 2 (1.21 as well as among grades 3 (1.08), 4 (1.037) and 5 (0.99). Concomitantly, stomatal resistance increased significantly in the infected leaves with maximum in grade 5 (1.03). Nevertheless, no significant increase in stomatal resistance noticed between various disease intensity grades. Physiological water use efficiency (pWUE) consistently lowered in the infected leaves. No significant reduction in pWUE was observed between the healthy leaves and leaves under grade 1 (886.7). Similarly, there was no significant difference among grade 2 (579.1), grade 3 (616.17) and grade 4 (560.6), that were showed reduction of 37.73%, 33.74% and 30.04% respectively. However, pWUE in grade 5 (381.90) was significantly less than that observed in all other grades with a reduction of 58.93% compared with healthy leaves. Least moisture content was

observed in the leaves of intensity grade 5 (62.5) with moisture loss of 17.05%. No significant reduction in moisture content was observed between the leaves of grade 1 (68.37) and 2 (67.33) as well as between grade 2 and grade 3 (Table 2).

Analysis of interrelation between biochemical alterations due to disease intensity revealed, increase or decrease in biochemical constituents significantly ($P < 0.01$) correlated with the disease intensities. Chlorophyll content possessed negative correlation ($r^2 = 0.88$) and changed 0.04 units per intensity grade. Similarly, total soluble sugar content possessed a significant positive correlation ($r^2 = 0.85$) increasing 3.26 unit on disease progress to next grade. Total protein content was negatively correlated ($r^2 = 0.88$) differing 4.14 unit per grade. Phenol content however increased progressively ($r^2 = 0.53$) with disease adding 3.90 unit from one grade to next higher intensity grade (Fig. 1).

Photosynthetic rate showed significant negative correlation ($r^2 = 0.96$) with the disease intensity, 2.96 unit decrease in photosynthetic rate was estimated with increase in disease severity. Similar significant negative correlation was observed in case of stomatal conductance ($r^2 = 0.65$). Stomatal resistance was, however, inversely proportional to stomatal conductance and increased along disease intensity possessing a positive correlation ($r^2 = 0.75$) with the disease severity altering 0.08 units with change in severity grade. pWUE was negatively correlated ($r^2 = 0.88$) with the disease intensity. A change in 105.24 units was estimated with change in disease severity. Likewise, moisture content decreased with increase in the disease intensity level, possessing a significant negative correlation ($r^2 = 0.85$) with 2.26 unit change (Fig. 2).

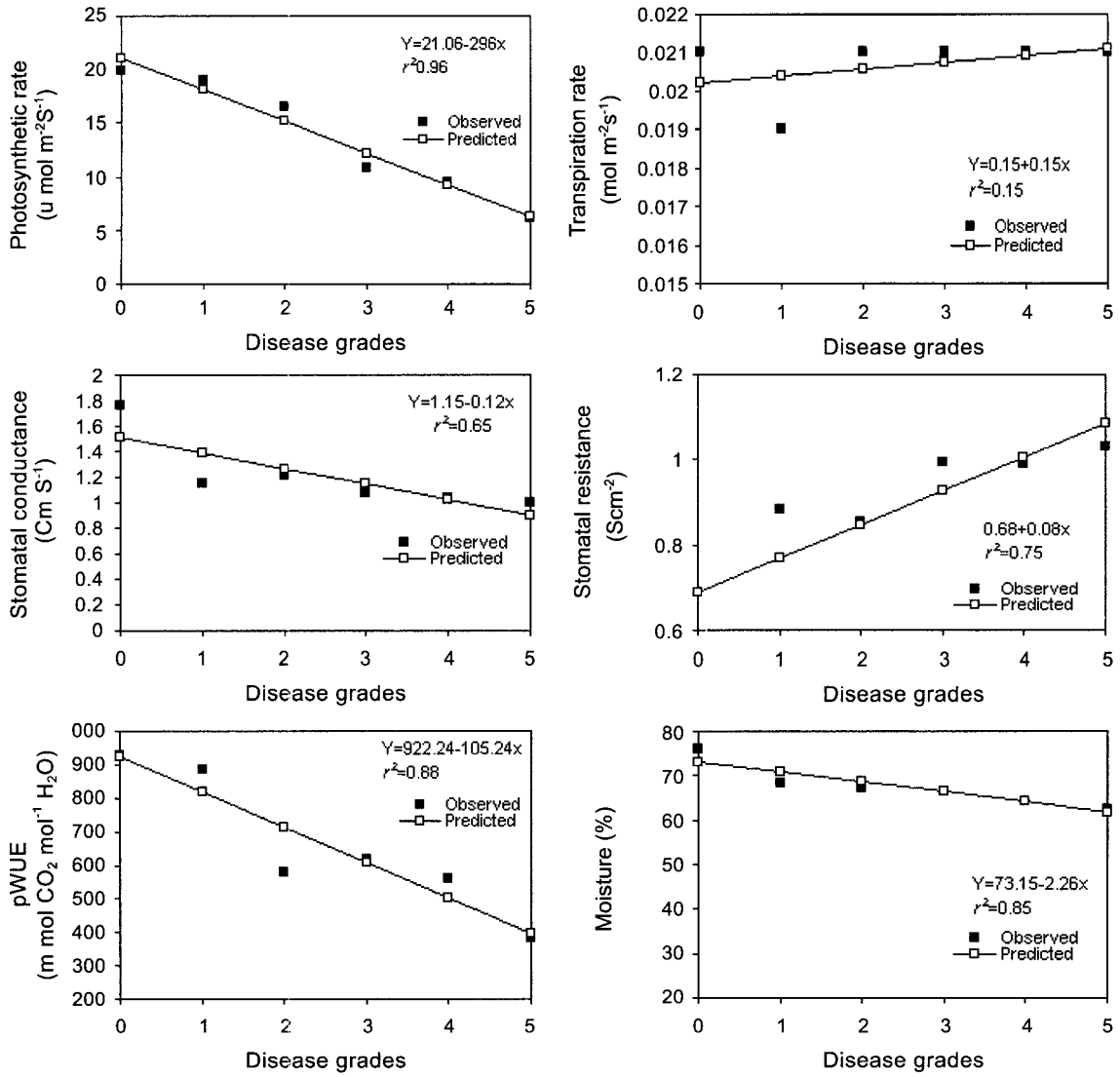


**Fig. 2.** Relation between some physiological alterations and disease intensities in mulberry leaves.

## Discussion

Diseases develop in individual plant sequentially beginning with the arrival of inoculums. After the invasion of pathogen, a complex series of interacting biochemical events are initiated in all the stages of pathogenesis, these alterations are manifested in all affected cells and tissues, the membranes and organelles, the metabolic pathway and the growth regulating process of the host. Present study reveals magnitude of changes in biochemical constituents and alteration in physiological activities at various intensities due to *Myrothecium* leaf spot in mulberry leaves. As elucidated by Gorpade and Joshi (1980), decrease in chlorophyll might be occurred due to its breakdown or the inhibition of its synthesis. Sakari and Misawa (1974) explained gradual decrease in the activity of enzyme chlorophyllase with progress of disease as a plausible reason for decrease in chlorophyll content in diseased leaves. This is supported by the observations of Srikantaswami *et al.* (1996) and Ali *et al.* (1997) respectively in *Cercospora* leaf spot and powdery mildew infected mulberry leaves, increase in sugar content during pathogenesis is reported in many agricultural crops and in mulberry (Nema, 1991; Lodha *et al.*, 1993; Shree and Nataraja, 1993)

Reduction in total protein is attributed to their breakdown by proteolytic enzymes secreted by the pathogens and subsequent utilization by the pathogen at a faster rate (Sambroski *et al.*, 1958; Agarwal *et al.*, 1982). Similar observations were made in mulberry due to leaf spot (Madhavarao *et al.*, 1981) and leaf rust (Sundareswaran *et al.*, 1988). Phenol contents increased gradually with the disease intensity. Metabolic changes that occurred during host pathogen interaction might have triggered the production of more phenols or in other words the tissues offered resistance to the invading pathogens as explained by Kosuge (1969). Similar results on increase in phenol content in various pathosystems have been reported (Nema, 1991; Singh *et al.*, 1993; Srikantaswamy *et al.*, 1996; Teotia *et al.*, 1997)

The disease significantly influence all physiological activities. Cells penetrated by fungal structures or killed by secretion of enzymes loss their chlorophyll and presumably their capacity to photosynthesis. Reduction in net photosynthesis is reported in a range of pathosystems (Shtienberg, 1992). Reduction in photosynthetic activities is caused by decrease in photosynthesizing leaf area and or its reduced efficiency (Yarwood, 1967; Goodman *et al.*, 1986).

Moisture content of the leaves reduced drastically in the diseased leaves. Blockage caused by the growth of pathogen compounded by its secretion of pectiolytic enzymes causes host response by producing gums and mucilage. These gums and mucilage form tylosis in the vessels resulting reduction in water flow and causing severe water stress (Dickinson and Lucas, 1997). This is a plausible explanation for reduction in moisture content in the infected leaves. In the present study, stomatal resistance increased and stomatal conductance decreased. In sugar beets infected with beet yellows virus reduced stomatal aperture was reported to be the most important factor limiting $CO_2$ uptake and photosynthesis (Hall and Loomis, 1972). Barriers to movement of gases to leaf tissue may depict as diffusive resistance since the water vapour and $CO_2$ have a common pathway in plants. Stomatal resistance likewise provides a barrier to $CO_2$ uptake and ultimately photosynthesis (Duniway and Slatyer, 1971). In tomatoes infected with *Fusarium*, rsistance to $CO_2$ diffusion increased because stomates did not open normally (Duniway, 1976).

The quantitative determination of effect of disease on the physiology of individual leaf is the first step towards a broader understanding of crop losses (Bastiaans *et al.*, 1994). Present study correlates the intensity of *Pseudocercospora* leaf spot disease on virtual loss in important biochemical and physiological nature of mulberry leaves which is completely due to the loss of area to the pathogens. Integration of this information in the crop loss studies may be useful for developing effective disease management strategies.

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