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INDIAN MULBERRY LEAF SPOT: EPIDEMIOLOGICAL STUDY, DIVERSITY OF FUNGAL PATHOGENS, PRESENT CHALLENGES AND FUTURE PROSPECTS

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ABSTRACT

Leaf spot disease, caused by Cercospora moricola, poses a significant threat to mulberry cultivation, a perennial crop essential for silk production. This study, conducted at the College of Temperate Sericulture, Mirgund, SKUAST-Kashmir, investigates the influence of environmental factors, including temperature, relative humidity, balanced fertilization, and plant spacing, on disease development. Results indicate a high disease incidence during August and September, with optimal conditions being temperatures between 25–30 ºC and relative humidity exceeding 80%. Balanced application of NPK fertilizers significantly reduced disease severity to 6–8%, with plants treated with balanced fertilizers exhibiting lower infection rates compared to those grown with imbalanced fertilizers. Wider plant spacing (90 × 90 cm) further minimized disease progression compared to closely spaced plants (60 × 60 cm). Additionally, mulberry's continuous harvesting makes it susceptible to other fungal pathogens, including Paramyrothecium roridum, Setosphaeria rostrata, and Nigrospora sphaerica, which collectively cause up to 20% yield loss and compromise leaf suitability for silkworms. The study underscores the importance of integrating cultural, chemical, and biological management practices to effectively control fungal diseases and enhance the productivity of the sericulture industry.

***KEYWORDS:* Cercospora moricola, Environment, Leaf spot, Mulberry**

INTRODUCTION

Mulberry (Family: Moraceae) is a perennial, well suited to grow in different climatic zone, woody plant with a short Growth period to use in sericulture*(P. Shukla et al.,)*. Most common Indian Mulberry species are *Morus alba*,*M. indica*, *M. serrata*, and *M. laevigata*. Other exotic species used in breeding programme are M. multicaulis, *M. nigra*, *M. sinensis* and *M. phillipinensis*(*A. Tikader et al*.,). Mulberry (M.*alba*), the primary food source for silkworms (*Bombyx mori*), holds a crucial position in sericulture. Beyond its role in silk production, it is also a valuable resource for food, fodder, fuel, and fiber. However, mulberry is highly vulnerable to various pathogens, among which fungal leaf spot is a major concern (Maqbool et al., 2021). This disease is responsible for a 5–30% reduction in leaf yield (*Sarkar et al., 2020*) and negatively impacts the biochemical composition of the leaves, ultimately diminishing the quality of silk production (*Shree & Nataraja, 1993).*Leaf spot disease in mulberry plants in the Kashmir Valley, India. Common diseases affecting mulberry include leaf spot, bacterial blight, leaf mosaic, powdery mildew, leaf rust, stem canker, violet root rot, white root rot, root knot, and dwarfing *(Reddy et al., 2009; Shree and Nataraj, 1993*). These diseases present major challenges, causing substantial reductions in both the yield and nutritional value of mulberry foliage. Feeding silkworms with diseased leaves negatively impacts their health and reduces cocoon yield in both quality and quantity *(Datta, 2010*). A lack of consistent and systematic studies on leaf spot disease and its epidemics has contributed to significant losses in leaf yield (*Ghoes et al., 2010).*

Environmental factors such as air temperature and humidity, rainfall levels, soil temperature, moisture, and fertility significantly influence the initiation and progression of infectious plant diseases. These factors affect disease development by impacting the growth and susceptibility of the host plant, the activity and multiplication of pathogens, and the host-pathogen interaction, which ultimately determines symptom severity *(Agrios, 2005*). Mulberry plants are susceptible to numerous diseases (*Reddy et al., 2009; Sharma et al., 1993*). Among these, leaf spot caused by *C.moricola*(Cooke) is particularly serious during July to September in Kashmir. Severe defoliation is commonly observed, and most commercial mulberry varieties are reported to be vulnerable to this disease (*Sikder and Krisnaswami, 1980*). *Siddaramaiah and Hegde (1990)* found that increased infection intensity by *C. moricola* raises nitrogen and phosphorus levels but reduces potassium in mulberry leaves. Additionally, Cercospora infection alters biochemical constituents such as amino acids, phenols, and sugars, potentially affecting leaf quality. The highest risk of infection occurs when temperatures range between 20– 28°C, coupled with 36 to 72 hours of continuous environmental wetness (*Nelson, 2008*). High humidity further increases the succulence of host plants, making them more susceptible to pathogens, thereby exacerbating disease severity (*Agrios, 2005).*Key pathogens, including *Cercospora moricola, Paramyrothecium roridum,* and emerging threats like *Setosphaeria rostrata,* cause substantial losses, especially under high humidity and rainfall conditions. Effective disease management strategies, incorporating cultural, chemical, and biological approaches, are essential to mitigate these challenges and sustain sericulture productivity globally.

Given unfavorable environmental conditions, Cercospora leaf spot diseases have become widespread in mulberry plantations. Therefore, cultivating healthy plantations is essential for producing high-quality leaves. In Kashmir, studies on the effects of fertilizer application and plant spacing on the development of mulberry leaf spot disease remain limited. Consequently, this study aimed to evaluate the influence of air temperature, relative humidity, balanced fertilizer application, nitrogen fertilizer use, and plant spacing on the incidence and severity of leaf spot disease in mulberry plants.

MATERIALS AND METHODS

To assess the impact of temperature and relative humidity (RH) on the progression of leaf spot disease in mulberry, the percentage of leaf infection and the disease index were observed at monthly intervals from April to September during 2022 2024 (Table 1). The study was conducted on three low-cut mulberry plots located at different sites within the College of Temperate Sericulture Mirgund, SKUAST- Kashmir. From each plot, 15 plants were selected, and disease incidence was monitored. The plots measured 20 × 20 meters, with a spacing of 120 cm between plants. Disease progression was studied under natural environmental conditions.

To evaluate the effect of a balanced dose of NPK fertilizer on disease development, a three-year-old low-cut mulberry plantation with varieties Goshoerami, Ichinose, Kokuso-21 was used. This plot, measuring 20 × 15 meters, had a spacing of 120 cm between plants and 90 cm between rows. The plot was divided into two sections: one received a balanced NPK fertilizer dose of 300:150:100 kg/ha along with 15,000 kg/ha of organic fertilizer, while the other only received 15,000 kg/ha of organic fertilizer. All plants underwent necessary cultural practices and were pruned simultaneously, after 30 days of pruning, a conidial suspension of *C.moricola* was evenly sprayed on all plants, which were then allowed to grow naturally. Leaf infection percentage and disease index (DI) were recorded 30 days after the conidial spray (Table 2).

To study the effect of plant spacing on disease development, a high-bush mulberry plot was established with three different spacing setups (Table 3). In the first plot, the plant-to-plant and row-to-row spacing was 60 cm. In the second plot, the spacing was 60 cm between plants and 90 cm between rows. In the third plot, the spacing was 90 cm in both dimensions. The crown height of all plants in each spacing arrangement was maintained at 75 cm. Two- and three-year-old plants were selected for this study, and all were pruned simultaneously. The plants were naturally exposed to infection during the rainy months of June, July, and August. Disease progression was recorded 80 days after pruning.

The study measured leaf infection percentage, plant height, disease index (DI), and leaf yield to evaluate the impact of leaf spot disease. For all measurements, the total number of diseased and healthy leaves was recorded on three long branches of each plant. The DI was calculated using the grading method described by *Siddaramaiah et al. (1978).*

Grading method

0 = No infection (Healthy)

I = 1-5%
II = 6-25%
III = 26-50%
IV = 51-75%
V = 76-100%

Disease severity in terms of Disease index (DI) was calculated by the formula:

$$DI = \frac{\text{Sum of numerical value x 100}}{\text{Total no. of leaves graded x Maximum grading in 5}}$$

Where numerical values were obtained by multiplying the number of leaves with their respective grading. All the studied were made in the three replications and average results were calculated. The DI values were transferred into angular values and statistical analysis was completed using Microsoft Excel software. LSD was determined, whenever; the calculated 'F' values were significant at 5% level (*Sendecor and Cochran, 1980*).

RESULT AND DISCUSSION

It was hypothesized that climatic factors, specifically temperature and humidity, play a significant role in determining the timing and severity of disease infection. The impact of these factors on the development of leaf spot disease in mulberry is presented in Table1.

Table 1. Effect of temperature and relative humidity on the development of leaf spot disease.

Month	Average temperature (°C)			Average relative humidity (%)			Infection (%)			Disease index (%		
Year	2022	2023	2024	2022	2023	2024	2022	2023	2024	2022	2023	2024
April	29.41	30.11	28.39	50.11	46.63	59.03	19.20	17.10	18.70	7.33	6.54	7.00
May	29.71	29.48	28.68	77.48	66.62	73.90	18.90	24.20	24.40	7.10	8.70	9.11
June	29.46	30.44	29.26	83.94	73.79	81.01	29.80	31.70	29.60	8.24	12.60	10.60
July	29.21	28.75	29.36	84.40	85.93	83.54	30.00	36.70	34.50	9.52	15.22	12.73
Aug.	29.54	29.4	29.31	85.38	82.40	86.43	44.50	42.10	41.20	18.30	17.40	16.50
Sept.	28.61	28.78	25.46	89.26	88.26	87.23	50.62	52.70	51.79	21.60	23.50	20.80

Above result indicated that temperatures ranging from 25 to 30 ºC and relative humidity exceeding 80% significantly promote the progression of the disease. The disease exhibited rapid development during June, July, August, and September, when the average temperature and humidity were approximately 25–30 ºC and above 80%, respectively. Although the disease was observed as early as April, its progression was slower compared to the peak period from June to September. Leaf spot disease was notably severe in conditions of abundant moisture and frequent rainfall, followed by warm and humid weather.

Research by Wolf and Verreet (2005) highlighted that relative air humidity above 95% or leaf wetness is essential for Cercospora infection and subsequent lesion formation in sugar beet. Similarly, according to Wu et al.(1999) studied the impact of temperature and Humidity duration on Cercospora arachidicola infection in three peanut cultivars. Another research by Alderman and Beute (1986) reported that Cercospora conidia require a saturated atmosphere to germinate optimally at temperatures of 16 to 25 ºC. They also observed that germ tube elongation occurred at R.H levels as low as 94.5%. These studies underscore the critical role of temperature and relative humidity in Cercospora infections on mulberry leaves.

Additionally, the effects of a balanced fertilizer application in our research comprising 300 kg/ha, 150 kg/ha, and 100 kg/ha of NPK along with 15,000 kg/ha of organic fertilizer on the development of leaf spot disease in mulberry are presented in Table 2.

Table 2. Effect of Balanced Fertilizer on the Development of Leaf Spot Disease

Treatment	Goshoerami	Ichinose	Kokuso-21	Kanva-2
Fertilizer Type	**Leaf Infection (%) ± SE**	**Disease Index (%) ± SE**	**Leaf Infection (%) ± SE**	**Disease Index (%) ± SE**
NPK Organic Fertilizer	26.82 ± 0.537	7.69 ± 0.526	28.54 ± 0.687	7.77 ± 0.0366
Nil Organic Fertilizer	37.12 ± 0.515	12.46 ± 0.325	32.6 ± 0.325	10.67 ± 0.32
C.D. at 5% level	2.982	1.903	3.031	2.376

The data highlights the impact of balanced fertilizer application (NPK and organic fertilizer) compared to no organic fertilizer on leaf spot disease in four mulberry varieties: Goshoerami, Ichinose, Kokuso-21, and Kanva-2. Leaf infection (%) was significantly reduced in all varieties when NPK and organic fertilizer were used. Infection rates were lower for Goshoerami (26.82 ± 0.537), Ichinose (28.54 ± 0.687), Kokuso-21 (29.82 ± 0.433), and Kanva-2 (25.36 ± 0.852) compared to higher infection rates without organic fertilizer: Goshoerami (37.12 ± 0.515), Ichinose (32.6 ± 0.325), Kokuso-21 (36.57 ± 0.497), and Kanva-2 (33.12 ± 0.523). The greatest reduction in infection was observed in Goshoerami and Kokuso-21 with fertilizer application.

Similarly, the disease index (%) was lower when NPK and organic fertilizer were applied, with values for Goshoerami (7.69 ± 0.526), Ichinose (7.77 ± 0.366), Kokuso-21 (6.94 ± 0.39), and Kanva-2 (5.72 ± 0.473) compared to higher values without organic fertilizer: Goshoerami (12.46 ± 0.325), Ichinose (10.67 ± 0.32), Kokuso-21 (9.97 ± 0.412), and Kanva-2 (9.57 ± 0.08). Among the varieties, Kanva-2 exhibited the lowest disease index with NPK and organic fertilizer, suggesting its potential resistance to leaf spot. These findings emphasize the importance of balanced fertilizer application in reducing the severity of leaf spot disease in mulberry.

The present study suggested that farmers can be understand about the possible development and spread of the leaf spot disease of mulberry so that proper management of the disease can be done. To increase the productivity of silk, information regarding importance of NPK and proper dose of organic fertilizer along with proper plant spacing are also important to manage the severity of the disease.

The study demonstrated that applying balanced fertilizers significantly reduced disease infection and severity across all four mulberry varieties— Goshoerami, Ichinose, Kokuso-21, Kanva-2.when compared to imbalanced fertilizer application. The role of proper fertilizer application in enhancing both productivity and the quality of mulberry leaves is well-established. Previous research *(Sharma et al., 1993*) reported that nitrogen and phosphate fertilizers increased disease incidence, while applying a complete NPK fertilizer in the soil reduced disease progression.

Balanced fertilizer use improves the efficiency of photosynthesis in chlorophyll molecules, surpassing that of plants not provided with adequate nutrients. NPK fertilizers are crucial for the production of high-quality mulberry leaves and play a key role in minimizing chlorophyll degradation caused by infections. The influence of plant spacing on leaf spot disease progression is detailed in Table 3.

Table 3. Effect of plant spacing on the development of leaf spot disease due to *Cercospora moricola*

Plant spacing (cm)	Leaf infection (%)	Disease index (%)	Leaf yield/plant (g)
60 □ 60	37.89 □ 0.242	11.03 □ 0.191	265.43 □ 0.654
0 □ 90	33.9 □ 1.017	9.03 □ 0.22	290.3 □ 1.616
90 □ 90	28.94 □ 0.691	7.99 □ 0.098	302.37 0.092
C.D. at 50% level	1.387	0.274	14.789

This study revealed that wider plant spacing reduced the development of leaf spot disease compared to narrower spacing. Narrow spacing of 60 × 60 cm resulted in an increased disease development rate of 37.89%, along with a lower leaf yield. In contrast, wider spacing of 60 × 90 cm and 90 × 90 cm reduced disease development to 33.90% and 28.98%, respectively, while yielding higher leaf production.

Plants grown with narrow spacing showed greater disease severity, which could be attributed to factors such as reduced temperature, limited air circulation, and inadequate light penetration within the densely planted population. Closer spacing also raised humidity levels and blocked light, further hindering air circulation. According to*Sharma et al. (1993*), closer plant spacing (60 × 60 cm) led to significantly higher disease incidence across various diseases they examined, including leaf spot, powdery mildew, and leaf rust. Agronomic practices such as plant spacing, crown height, and harvesting methods were found to influence disease incidence. Additionally, broader agricultural practices like fertilization, irrigation, and spacing play a key role in determining disease prevalence in different crops.

DIVERSITY OF FUNGAL PATHOGENS

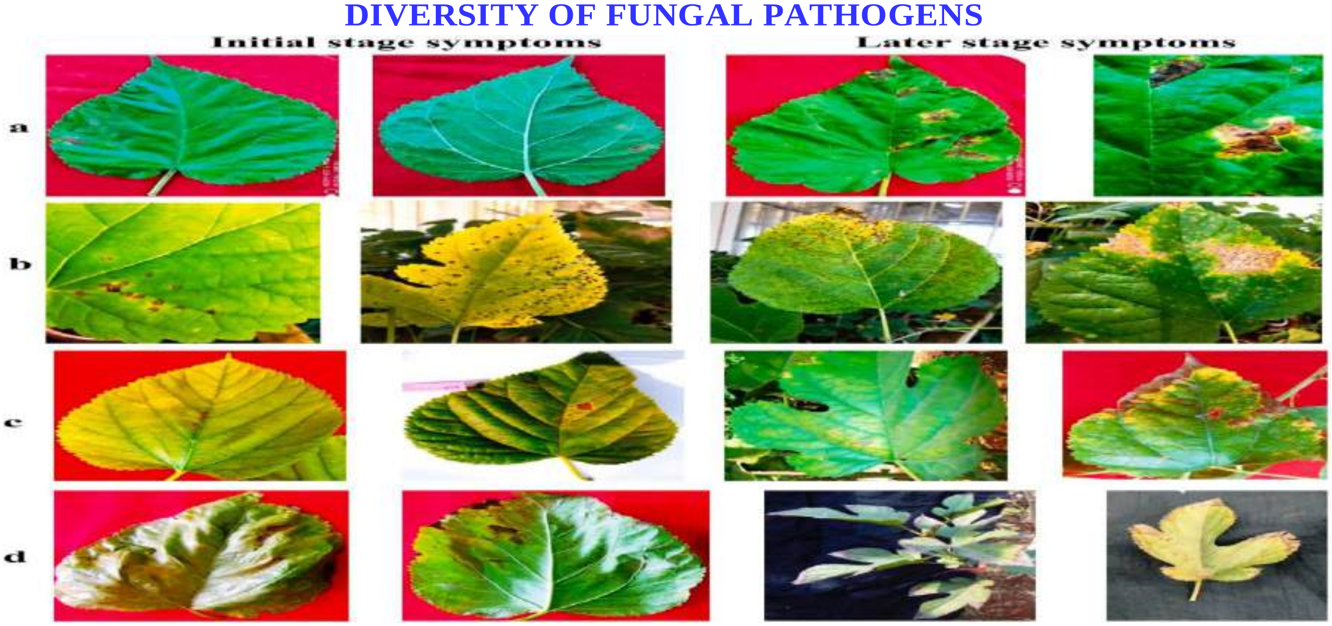

Fig. 1. Symptoms of mulberry leaf spot diseases at initial and later stage caused by a) Bipolaris sorokiniana b) Curvularia lunata c) Cladosporium sphaerospermum and d) Epicoccum sorghinum.

Mulberry, being a perennial crop, is harvested continuously for silk production. Its perennial nature and vulnerability to various fungal diseases, such as leaf spot, necessitate effective and precise disease management strategies (*G.S. Arunakumar et al.,)*. Studies show that infected leaves lose their suitability for silk production more significantly than those affected by nutritional deficiencies. Consequently, there is an urgent need for a cost-efficient, widely applicable, and effective disease management approach to combat the increasing pathogen-induced infections.

Foliar diseases, including leaf spot, adversely affect the nutritive quality of mulberry leaves. Research conducted across various nurseries identified Cercospora moricola as a major pathogen, leading to a 10–20% reduction in leaf yield. This pathogen not only diminishes the yield but also degrades the nutritional content, rendering the leaves unsuitable for feeding silkworm larvae *(R.A.Baiyewu et al.,)* Biochemical analyses revealed significant differences in the nutritional parameters between healthy and diseased leaves (*S.K. Ghosh et al*.,)

Another significant leaf spot disease, Myrothecium or brown leaf spot, caused by *Paramyrothecium roridum* (formerly Myrothecium roridum Tode ex. Fr.), poses a serious threat to mulberry cultivation, particularly in Eastern and Northern India. This disease has also been reported in Japan and other regions of India, significantly impacting larval survival rate, larval weight, development stage, and the pupa's digestive absorption rate (*X.Jianget al.,)*. Management strategies for mulberry diseases typically involve cultural, mechanical, chemical, and biological methods, used either individually or in combination (*A.Pappachan et al.,)*

In India, Cladosporium cladosporioides was reported in 1995 as the cause of mulberry leaf margin burning (*K.P.Singh et al.,)*. Neophloeospora maculans has been observed affecting Morus nigra as a new host beyond its usual host, Morus alba, and expanding its presence in Brazil (*C.A.daCostaet al*.,) More recently, Setosphaeria rostrata and Nigrospora sphaerica were identified as contributors to leaf spot in India (*G.S.Arunakumar et al.,*). Globally, the leaf spot pathogen Mycosphaerella mori is commonly found on Morus species. In 2017, it was reported on Morus nigra and Morus rubra in Poland (K.Pieczul et al.,) In Morocco, Drechslera australiensis was identified as a new parasite on Morus species (*N.Lamrani et al* .,)

Leaf spot caused by Phloeospora maculans is particularly prevalent during the rainy season, thriving in conditions of frequent rainfall and high humidity, as observed in Korea and Turkey*(S.K.Hong, S.Soylu et al.,)*. Additionally, other fungal pathogens such as Drechslera yamadi, Alternaria tenuissima, Septogloeum mori, Fusarium solani, and Colletotrichum gloeosporioides significantly affect mulberry leaves, causing substantial economic losses to the sericulture industry worldwide.

This is Result obtained after maintaining proper Temperature, Humidity, Spacing and Fertilizer (NPK)

CONCLUSION AND FUTURE PERSPECTIVE.

Fungal diseases, particularly leaf spot, pose a significant threat to mulberry cultivation, directly impacting sericulture and its economic sustainability. Key pathogens such as *Cercospora moricola*, *Paramyrothecium roridum*, and emerging threats like *Setosphaeria rostrata* demand immediate and effective management strategies. Environmental factors, including high humidity and rainfall, exacerbate disease severity, highlighting the urgency for timely interventions. Sustainable mulberry production hinges on integrated disease management strategies that combine cultural, chemical, and biological approaches. Proactive measures, such as balanced fertilization, optimal plant spacing, and timely use of organic amendments, are instrumental in mitigating disease incidence and improving leaf quality, thereby enhancing silk productivity.

However,combating leaf spot disease requires innovative, multidisciplinary approaches that extend beyond traditional methods. In the short term, integrating precision-based Integrated Pest Management (IPM) strategies, including the use of biological control agents, cultural practices, and eco-friendly fungicides, is essential. Strengthening diagnostic tools and phenotyping as well as Genotypic techniques for identifying resistant varieties is critical for disease control.

In the mid-term, advancements in plant breeding technologies, such as, genomic selection, and transcriptomics, Functional Studies and CRISPR-Cas9 offer promising avenues to develop disease-resistant mulberry varieties. Collaborative research initiatives focusing on host-pathogen interactions, novel resistance mechanisms, and adaptability to abiotic stresses will play a crucial role in building a robust mulberry production system.

The long-term vision should emphasize integrating durable resistance into mulberry plants and adopting adaptive IPM frameworks that incorporate biological, botanical, and agronomic practices. Predictive modeling and AI-driven decision-making tools can further enhance the resilience of mulberry cultivation to evolving pathogen populations and climate challenges.

A holistic approach, driven by scientific innovation, ecological stewardship, and active farmer participation, is essential for the sustainable management of leaf spot disease and other challenges in mulberry cultivation. This will ensure the long-term profitability, productivity, and environmental resilience of the sericulture industry.

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