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Surveillance & Molecular Studies of Powdery Mildew Disease on *Platanus orientalis*: An Emerging Threat

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Powdery mildew is a significant foliar disease that affects a wide range of plant species globally, including *Platanus orientalis* (oriental plane tree). This study was undertaken to identify the fungal pathogen responsible for causing powdery mildew on *P. orientalis* in the Murree Hills, Pakistan. A comprehensive survey was conducted across various altitudinal zones, focusing on disease incidence. Symptomatic leaves were collected and analyzed on a morphological and molecular basis to characterize the causal agent, and pathogenicity tests were conducted to confirm their role in disease development. Microscopic and molecular examination revealed characteristic morphological features of *Erysiphe platani*, which was identified as the pathogen responsible. Pathogenicity was confirmed through the successful development of symptoms on healthy *P. orientalis* leaves, fulfilling Koch's postulates. This study marks the first confirmed report of *Erysiphe platani* as a pathogen of *P. orientalis* in Pakistan, contributing to the existing knowledge on plant pathogens in the region. The findings provide a basis for future research on disease management strategies to minimize the impact of powdery mildew on *P. orientalis* and similar hosts.

Keywords: Murree Hills, Microscopic Identification, Powdery Mildew, *Platanus Orientalis*, Pathogenicity

Introduction:

Platanus orientalis, also referred to as the oriental plane tree, is a deciduous tree with exceptional ecological, aesthetic, and environmental value [1]. This species is known for its capacity to adapt to a wide range of habitats and is extensively dispersed in temperate climates [2]. *P. orientalis* thrives in the Murree Hills and Azad Jammu & Kashmir, where it is valued not only for its shade-providing canopy but also for its significant contribution to biodiversity and also responsible for enhancing the aesthetic appeal of landscapes [3]. As a prominent component of urban and natural ecosystems, *P. orientalis* serves as a critical habitat for various organisms and contributes to the ecological stability of the region [4]. The oriental plane tree is a key species in temperate regions, often planted along streets, in parks, and urban green spaces due to its large size, broad canopy, and resilience to environmental stresses [5]. Its extensive root system helps stabilize soil, reduce erosion, and improve water retention, making it a vital species in riparian

zones and degraded landscapes [6][7]. Besides its significant importance, the *P. orientalis* is affected by both biotic and abiotic factors [8]. Among these, powdery mildew, a fungal disease poses a significant threat to this species [9]. Powdery mildew is a common fungal disease affecting a wide range of plant species, including crops, ornamentals, and forest trees. This fungal growth consists of mycelia and spores that proliferate under favorable conditions, particularly in humid environments with poor air circulation. The disease affects the aesthetic value as well as provides an adverse physiological impact on plants [10].

Moreover, Powdery mildew reduces the photosynthetic efficiency of plants by covering leaf surfaces and obstructing light penetration. This leads to a decrease in the tree's energy production [11]. In severe cases, powdery mildew can be responsible to retard the tree's growth, and survival which poses a significant threat to its ecological and ornamental values [12].

In Pakistan, powdery mildew has been reported on various crops and ornamental plants, including wheat, cucurbits, roses, etc. These studies have primarily focused on crops, with limited attention given to forest and urban trees [13]. Globally, powdery mildew on *P. orientalis* has been attributed to *Erysiphe platani*, a species-specific pathogen that infects plane trees [14].

However, in Pakistan, particularly in the Murree Hills, no studies have been conducted to confirm the identity of the pathogen responsible for powdery mildew on *P. orientalis*. By addressing the knowledge gap surrounding powdery mildew on *P. orientalis*, this research will pave the way for future studies on the management of fungal diseases in forest ecosystems, ultimately contributing to the conservation of biodiversity and the maintenance of ecosystem services.

Keeping in view the above-mentioned facts, the Present study is aimed to:

- Conduct a systematic survey of powdery mildew on *P. orientalis* in the Murree Hills
- Characterize the pathogen on morphological and molecular basis to confirm its identity
- Establish the pathogenicity of the identified pathogen to confirm its role in the disease etiology.

Materials and Methods:

Survey and Disease Sample Collection:

To find out the disease incidence (no. of infected trees/ total no. of trees examined x 100) of the powdery mildew, a random survey was conducted from July to September 2024 from different locations of Murree Hills (Figure. 1) Furthermore, the infected leaves were collected from the examined trees, stored in open head paper bags and brought to Fungal Plant Pathology lab of Department of Plant Pathology, PMAS- Arid Agriculture University Rawalpindi for further morphological identification and pathogenicity test.

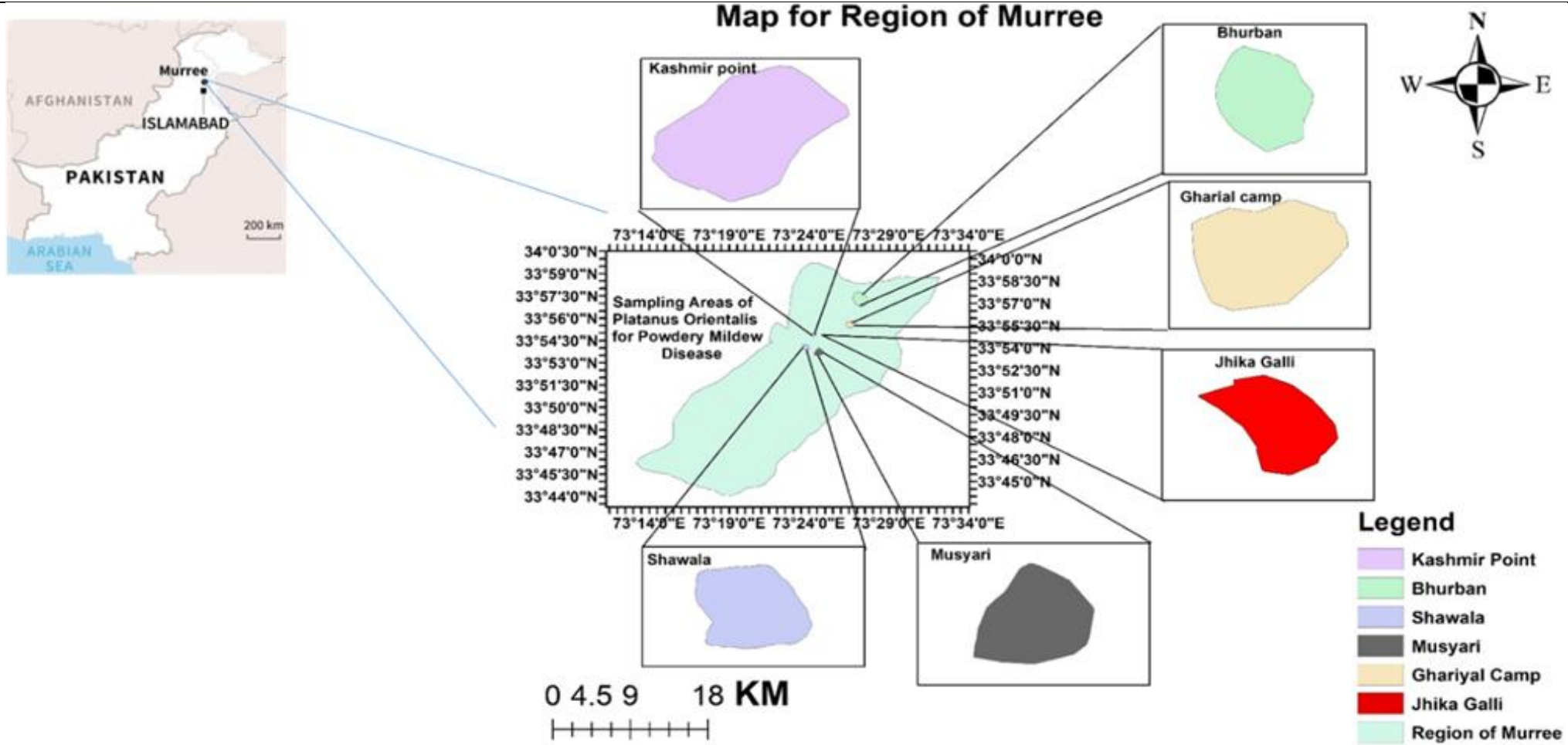

Figure 1: Disease collection sampling sites of *Planatus orientalis* in Murree Hills.

Microscopic Studies:

The collected leaves were further examined under a compound microscope for detailed morphological characterization based on the color, shape, and size of conidia and conidiophores [10].

Molecular characterization:

Two fungal isolates were randomly selected for further molecular characterization.

DNA Extraction:

A small number of fungal mycelia was collected from a fungal culture plate for DNA extraction. The collected mycelial mass was suspended in 50 µL of PrepMan kit solution inside a 2.0 mL microcentrifuge tube. The sample was then vortexed thoroughly for 10-30 seconds to ensure proper mixing. Next, the tube was placed in a heat block and incubated at 100°C for 10 minutes to break the cell walls and release DNA. Following heat treatment, the sample was centrifuged at 16,000 × g for 2 minutes to separate the cellular debris from the DNA-containing supernatant. The supernatant, containing the extracted DNA, was carefully transferred into a new sterile tube, avoiding any disturbance of the pellet. The DNA in the supernatant was now ready for PCR or other molecular applications. Finally, the extracted DNA was stored at 4°C for up to 1 month or frozen at -20°C for long-term storage.

PCR Amplification:

The extracted DNA was subject to PCR implication using internal transcribed spacer (ITS) region primers viz. ITS1 (5` TCCGTAGGTGAACCTGCCG 3`); ITS4 (5` TCCTCCGCTTATTGATATGC 3`). The PCR test was done in 50 µL reactions (1x PCR buffer, 0.2nM dNTPs, 5U Taq. Polymerase, 20 ng fungal DNA, 10 Pm of each of forward and reverse primers (ITS1 & ITS4), and 1.5 mM of $MgCl_2$. For the amplification of ITS, initial denaturation was carried out at 95 °C for 2 mins, followed by 35 cycles of denaturing at 95° C each for 30 sec. Annealing at 56 °C and extension at 72 °C for 1 min with final extension at 72 °C for 10 mins. For visualization of the reaction, 2 % (w/v) agarose gel was used in gel electrophoresis.

Phylogenetic analysis:

After confirmation of isolates, sequences were aligned by the MUSCLE (Multiple Sequence Comparison by Log-Expectation) method [15], and the neighbor-joining method was performed in MEGA v. 7 for phylogenetic analysis [16].

Pathogenicity Test:

To confirm the pathogenicity of the isolated fungus, an inoculum was prepared by suspending conidia of *Erysiphe platani* collected from infected leaves in sterile water, creating a spore suspension with a concentration of 10^5 spores/ml. During the inoculation process, healthy *Platanus orientalis* plants were sprayed with the spore suspension, while control plants received sterile water. The inoculated plants were then covered with polyethylene bags to maintain high humidity. Symptoms were observed for 10–14 days, and the experiment was repeated three times.

Statistical analysis:

The data were analyzed statistically using Statistix version 8.1. Mean values were compared using Tukey's HSD test at $P \leq 0.05$ following analysis of variance (ANOVA). Significant and non-significant interactions were interpreted based on the ANOVA results.

Results:

Disease Incidence (%):

The research was carried out in the Murree Hills, a temperate region in northern Pakistan known for its moderate temperatures, high humidity, and dense vegetation. From July to September, a survey was conducted across different altitudinal zones ranging from 1,200 to 2,400 meters above sea level. Leaves of *Platanus orientalis* showing symptoms of powdery mildew, such as powdery growth on the leaf surface and ultimately curling of leaves were collected from

various locations Figure 2. The incidence of powdery mildew was calculated as the percentage of infected trees out of the total number of trees examined at each site of Jhika Gali Campus (Kohsar University Murree), Kashmir Point, Misyari, Shawala, Bhurban, Ghariyal. The results indicated that the maximum disease incidence (50%) was observed at Kohsar University Murree, Misyari, and Ghariyal, while the minimum disease incidence (30%) was recorded at Kashmir Point and Bhurban as seen in Figure 3.

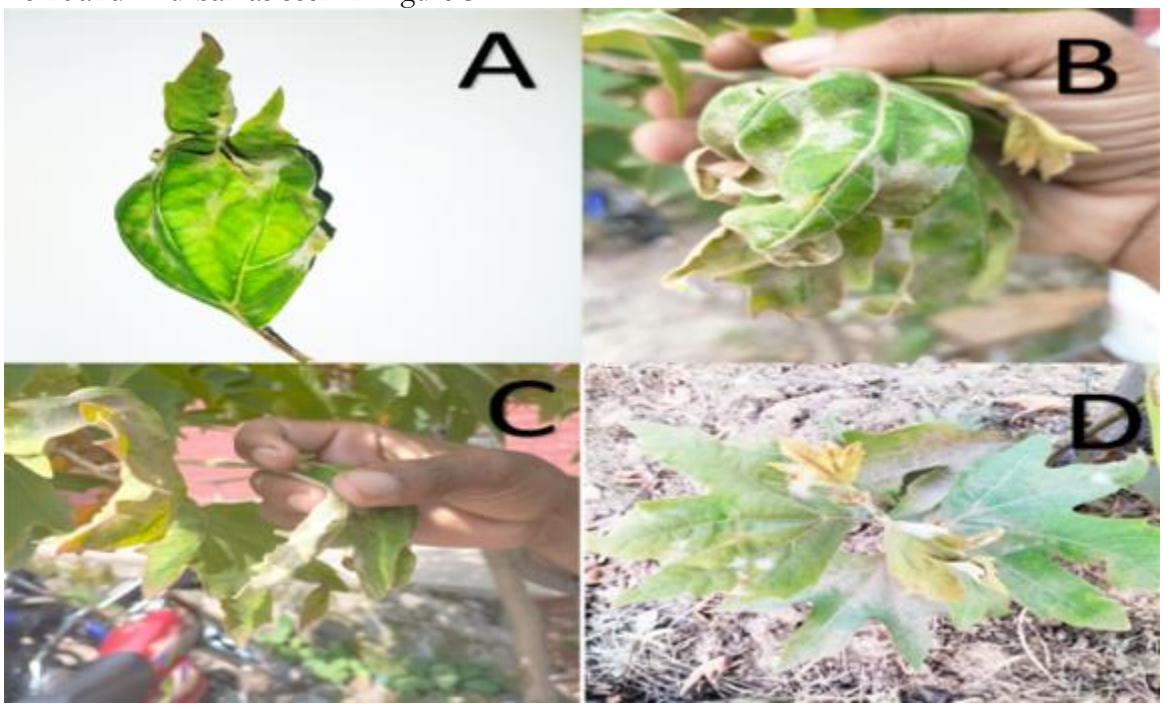

Figure 2 (A-D) Disease specimen of powdery mildew on Planatus orientalis

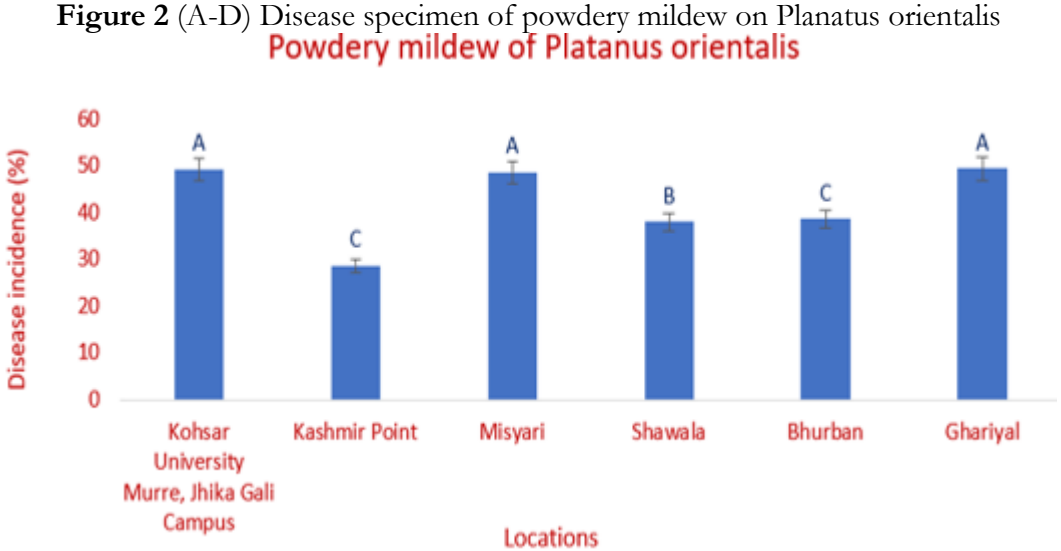

Figure 3: Disease incidence % of powdery mildew on Planatus orientalis

Microscopic Characterization of Pathogen Causing Powdery Mildew of Planatus Orientalis:

The study analyzed the microscopic characteristics of 50 isolates of *Erysiphe platani* causing powdery mildew of *Planatus orientalis*. The Conidia of *E. platani* observed (Color) Hyaline, Shape (Elliposoid to ovoid), Size measuring 30-45 µm in length and 10-15 µm in width, produced in chains of 2-4. Similarly, the conidiophores were erect, cylindrical, and measured 80.6–247 × 4.6 -7 µm. Conidiophores were also found unbranched, hyaline, and smooth in texture Figure 4.

Molecular Identification:

From 50 morphological identified isolates of *Erysiphe platani*, 02 representative isolates viz. P6EPKP7 and P20EPJG13 were selected for molecular characterization. The Inter transcribed spacer region (ITS) gene regions were amplified by using primer pairs ITS1/ITS4 respectively. The gel electrophoresis resulted in the production of clear single bands of amplified product that was further used for nucleotide sequencing. The amplified PCR product had fragments size of about 600-650 (ITS) and aligned sequences were submitted to Genbank to obtain accession numbers viz. PVO35243 and PVO35262 with ITS gene regions respectively. The genetic similarity of each of the above-mentioned isolates was analyzed with a BLAST search for ITS gene regions. The results showed that all 02 isolates of *Erysiphe platani* with ITS gene region exhibit 99% genetic similarity with previously reported isolates of Erysiphe platani (accession nos. OM049212, OM049216, OM049210, AB926022, KX611158 and OM049208) respectively shown in Figure 5.

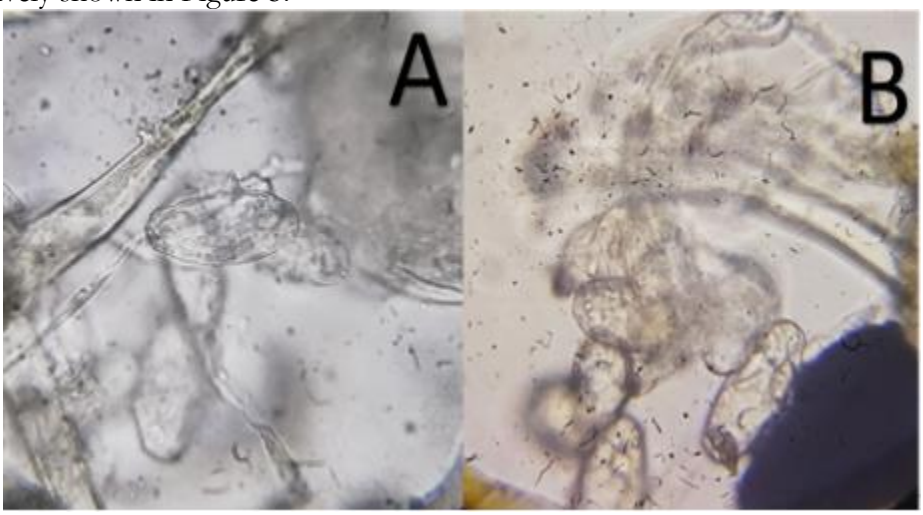

Figure 4: (a & b) Microscopic features of *Erysiphe platani*

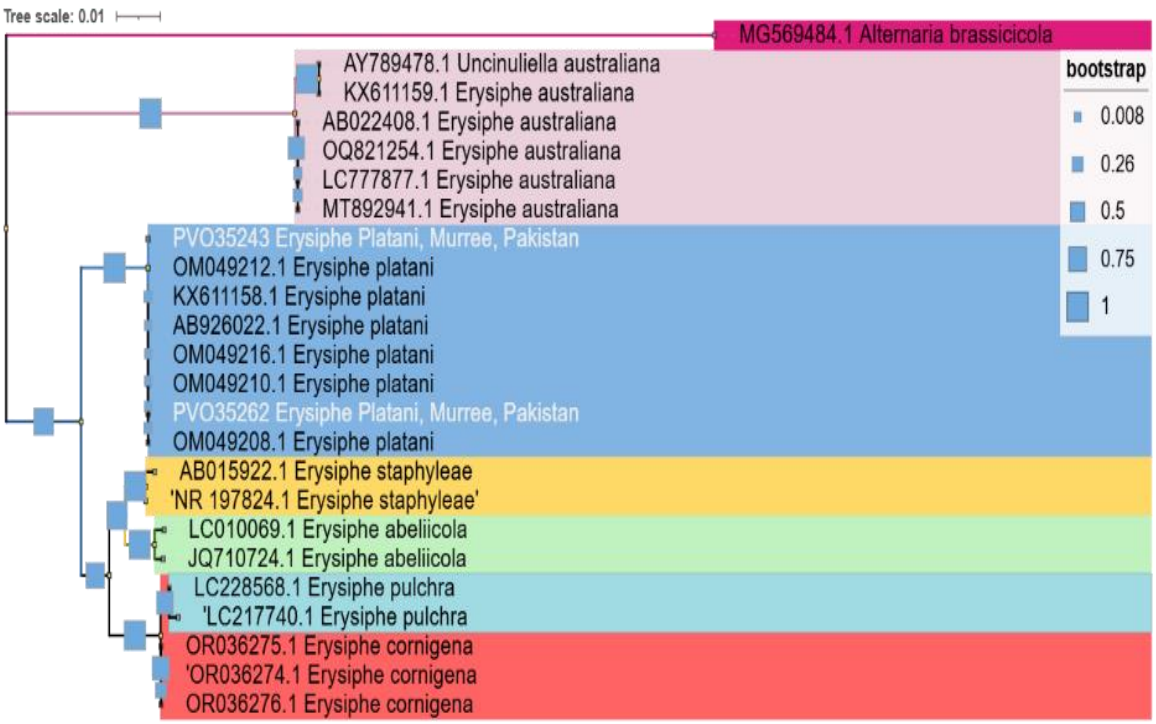

Figure 5: Phylogenetic analysis of *Erysiphe platani*

Pathogenicity Test:

The results revealed that inoculated plants with pathogens developed characteristic powdery mildew symptoms within 10 days, confirming the pathogenicity of the isolated fungus. No symptoms were observed on the control plants shown in Figure 6 (a & b).

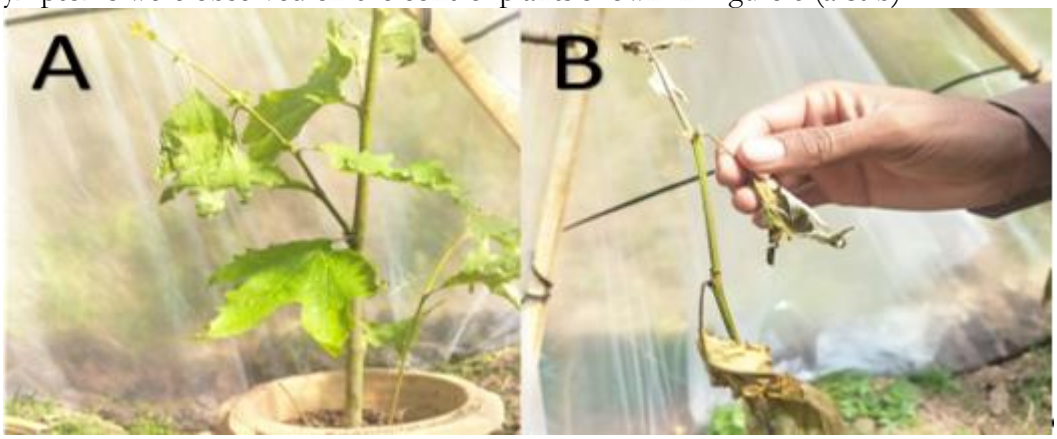

Figure 6: Pathogenicity test (a) Control (b) Inoculated

Discussions:

Powdery mildew is a foliar disease affecting a wide range of plant species globally, including *Platanus orientalis* (oriental plane tree). This disease is caused by obligate biotrophic fungi, which emerge in epidemic form during moderate temperatures and high humidity, leading to significant economic and ecological impacts. *Erysiphe platani*, a well-documented causal agent of powdery mildew on *P. orientalis*, has been reported in various regions worldwide (Ortiqov et al., 2023; Lee et al., 2013). but its presence and impact in Pakistan have not been documented before this study.

The current research aims to identify and confirm the fungal pathogen responsible for powdery mildew on *P. orientalis* in the Murree Hills of Pakistan and to evaluate the disease incidence across different altitudinal zones. This study contributes to the growing body of knowledge on plant pathogens in the region and provides a foundation for future disease management strategies. The study revealed *Erysiphe platani* as the pathogen is responsible for powdery mildew on *P. orientalis* in the Murree Hills. A survey conducted from July to September across altitudes ranging from 1,200 to 2,400 meters recorded varying disease incidences at different sites. The highest disease incidence (50%) was observed at Jhika Gali Campus (Kohsar University Murree), Misyari, and Ghariyal. The variation in disease incidence across sites can be attributed to microclimatic differences. Sites with higher humidity and dense vegetation exhibited greater disease incidence. This aligns with previous studies indicating that powdery mildew becomes more epidemic in environments with high humidity and reduced airflow [17]. Conversely, the other locations with lower humidity and more open landscapes, recorded lower disease incidence, supporting findings from other regions where microclimatic conditions influence disease prevalence [18].

Microscopic examination of 50 isolates confirmed the morphological characteristics of *E. platani*, including hyaline conidia that were ellipsoid to ovoid in shape, measuring 30-45 µm in length and 10-15 µm in width, produced in chains of 2-4. Conidiophores were unbranched, hyaline, smooth, and measured 80.6–247 × 4.6-7µm which is further confirmed through molecular identification. Pathogenicity tests validated the results, with inoculated plants developing characteristic powdery mildew symptoms within 10 days, while control plants remained symptom-free. The findings of this study align with previous reports of *Erysiphe platani* as a significant pathogen of *P. orientalis* in temperate regions globally [19]. Similar morphological characteristics of the conidia and conidiophores have been reported in studies conducted in Europe, North America, and parts of Asia [20]. Studies in Mediterranean countries have also

identified *E. platani* as a dominant pathogen of *P. orientalis*, particularly in areas with high humidity and moderate temperatures [21]. The dimensions of conidia and conidiophores observed in the current study fall within the ranges reported in earlier research, corroborating the identification of the pathogen [22].

The confirmation of E. platani as the causal agent of powdery mildew on *P. orientalis* in Pakistan has significant implications for disease management. The current findings underscore the need for localized strategies in the Murree Hills to mitigate this issue such as targeted fungicidal applications at high-incidence sites during peak disease periods may help mitigate the impact.

Conclusions:

This study identifies *Erysiphe platani* as the pathogen responsible for powdery mildew on *Platanus orientalis* in the Murree Hills, providing the first documented evidence of this pathogen in Pakistan. The findings underscore the need for further research on the genetic diversity of the pathogen and the development of sustainable management strategies.

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