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FIRST REPORT OF CLADOSPORIUM CLADOSPORIOIDES ON MULBERRY IN PAKISTAN: EXPANDING ITS HOST RANGE

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Abstract

Cladosporium, commonly occurring fungal pathogen, results in the losses of commercially significant plants. Different diseased plants were collected during phytopathogenic surveys conducted in Lahore. Morpho-anatomical, culturing and molecular techniques revealed the occurrence of *Cladosporium cladosporioides* on *Morus alba*. The results were supported by sequencing of ITS region of DNA. *Morus alba* is being recorded here as a new host plant for *C. cladosporioides*. This investigation may be helpful for taxonomic study of the genus *Cladosporium*, a cosmopolitan saprophytic and pathogenic fungus. This investigation may be helpful for taxonomic study of the genus *Cladosporium*, a cosmopolitan saprophytic and pathogenic fungus.

Key words: Fungal cultures, ITS, Lahore, Mulberry, Phytopathogenic fungi.

INTRODUCTION

The diseases caused by fungal pathogens are widespread in environment. Fungal spores are used to identify and classify different fungal species. Plants help in the provision of nutrients to fungal pathogens (Vargas *et al.,* 2012). Pathogenic fungi including *Alternaria* Nees, *Aspergillus* Link, *Cladosporium* Link., *Drechslera* (Bainier) Arx, *Fusarium* J. Sheld., *Helminthosporium* (Bainier) Nicot, *Macrophomina* (Tassi) Goid., and *Penicillium* G. Sm., cause different disease symptoms in different plants (Anonymous, 2004).

Among different fungal pathogens, *Cladosporium* Link, is hetergenous, cosmopolitan, saprobic in nature (Abdollahzadeh *et al.,* 2020; Lee *et al.,* 2023). Examination of its molecular and morphological characteristics is necessary for developing the taxonomy of this genus. Molecular markers along with polyphasic approaches can be used for the identification of species. It survives as a secondary invader in fresh or on senescent leaves. This pathogenic fungus causes leaf scab and leaves spot disease in economically significant plants (Nam *et al.,* 2015). About 170 species of *Cladosporium* have been known worldwide and three (03) species from Pakistan (Ahmad *et al.,* 1997; Bensch *et al.,* 2012).

Morphological examination along with phylogenetic study help in differentiating different pathogenic fungi and to solve the evolutionary relationships between different species of same genus. The current study is an attempt to examine the morphological and cultural characteristics of the *C. cladosporioides* and also phylogenetic analysis of this species for authentic identification.

MATERIALS AND METHOD

Current work was done in FBSR Lab, Institute of Botany, University of the Punjab, Lahore, Pakistan.

Sampling of infected plants

Phytopathogenic surveys were conducted in Lahore, Pakistan (Figure 01) and plants with fungal infection were collected. Tagging was done and then they were shade dried. Photography of diseased plants were done both in the field and also under stereomiroscope.

Preparation of MEA medium and culturing

Phyto–pathogenic fungal species were isolated from infected plant samples on 2% Malt Extract Agar (MEA) medium. MEA medium was prepared using 4g of each agar and malt extract in distilled water. Autoclaving of prepared media was done. Streptomycin was also added and cooled at approximately 40˚C. The medium was then poured in the petri plates and allowed to cool for some time (Reyes *et al.,* 2025). Sterilized blades were used for inoculating the small sections of diseased leaves on MEA medium. Parafilm was used for sealing purposes and was labelled properly. Growth of fungal cultures were observed after few days. Fungal mycelium was shifted to new autoclaved petri plates to obtain their pure cultures (Imathiu *et al.,* 2014).

Macroscopic Characterization

Colony morphology, growth rate, dia., color and habit of colony were observed.

Microscopic Characterization

Agar block was placed on clean glass slide. Lactophenol was used as a staining agent to observe different structures present in the culture. Light microscope was used to visualize different anatomical features at different magnification powers. Line drawings were also done.

Molecular Analysis

Modified CTAB protocol was used for the extraction of DNA. PCR amplification of extracted DNA was done using ITS primers. Visualization of PCR product was done using agarose gel electrophoresis. Then the sequencing of amplified product was completed commercially.

Phylogeny

After analyzing the nucleotide peaks, consensus sequence was made using Clustral W Alignment in BioEdit software. Final sequence was then subjected to NCBI BLAST search to compare the final sequence with sequences already present in the GenBank. MEGA software was used for the reconstruction of phylogenetic tree. Maximum likelihood phylogram was constructed using the Kimura model. Rapid bootstrap analysis was performed with 1000 replicates.

RESULTS

Taxonomy

Cladosporium cladosporioides (Fresen.) G.A. de Vries, Contrib. Knowledge of the Genus Cladosporium Link ex Fries: 57 (1952)

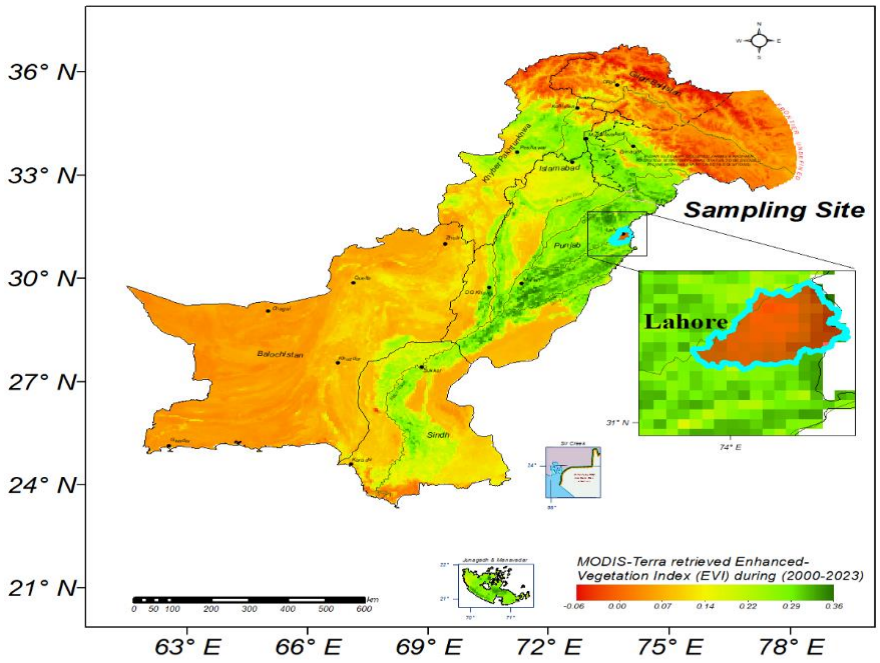

Figure 1: Location map showing sampling site Lahore, Punjab, Pakistan (By the software ArcGIS, version 10.3).

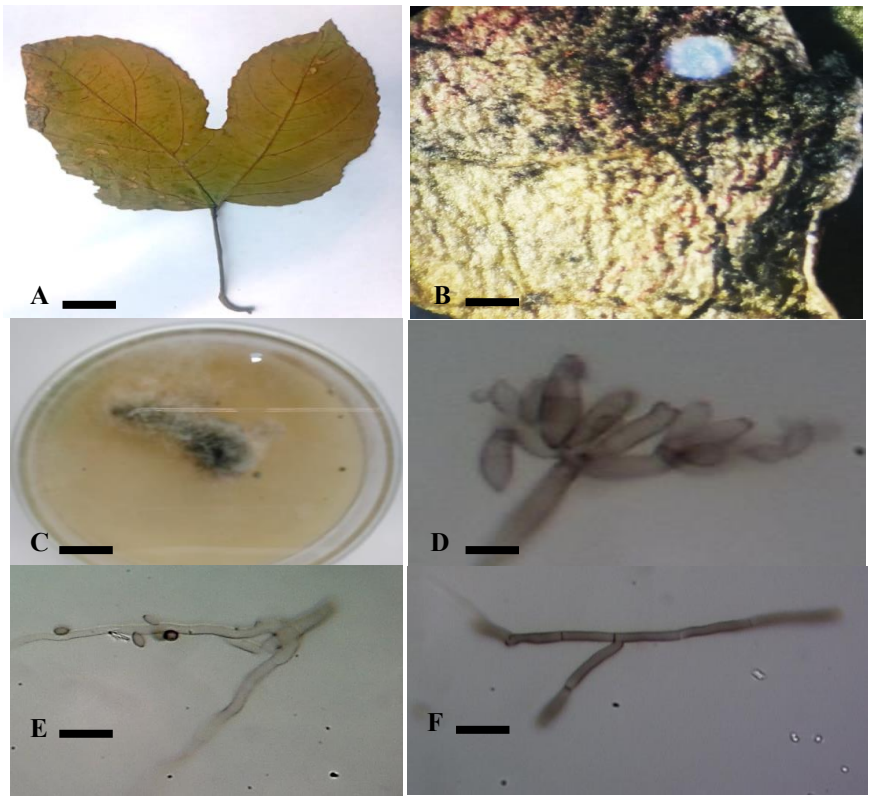

Plate 1(a): Infected host plant *Morus alba*. **A.** Infected leaf; **B.** Infection under stereomicroscope; **C.** Colony on MEA medium; **D.** Conidiophore with conidia; **E.** Conidium**; F.** Hyphae. Scale bars: A=3 cm; C=2 cm; D=9 µm; E=6 µm; F=4 µm.

Anatomical Characterization

Conidiophore were solitary, oblong to cylindrical in shape, most of the conidiophores were unbranched, 12–90 × 4–5µm. **Hyphae** were septate, branched, 3–4 µm wide, sub hyaline. **Conidia** ovoid to limoniform, symmetrical, sometimes subglobose, in a long chain, 7–11 × 2–3 µm, long germ tubes present, aseptate.

Colony Characterization

The appearance of fungal mycelium on MEA medium was observed after 3 days of inoculation. Colony was filamentous in appearance, and the margins of colony are white in color.

Material Examined

On *Morus alba* L., Pakistan, Punjab, Lahore, Jilani Park, 217 m a. s. l., 12 January 2021, Ramsha Liaqat and Dr. Najam ul Sehar Afshan, NW#03.

Molecular analyses of ITS region

C. cladosporioides sequence was amplified and the amplified sequence has a length of 474 base pairs. It exhibited 99 % identity with *C. cladosporioides* (HM148021) in an initial BLAST results. Closely linked sequences were retrieved from GENBANK and were incorporated for the final ITS dataset. MUSCLE alignment was used for the final alignment of the dataset.

Then the sequences were imprted to the Bioedit and trimming of the sequences were done. Then MEGA 11 software was used for construction of phylogenetic tree following Jukes–Cantor nucleotide substituation model. Out of 508 characters, 385 were conserved, 100 were variable, 75 were singleton sites and 22 were Parsimony informative.

DISCUSSION

According to Abdollahzadeh *et al*. (2020), *Cladosporium* Link. lies in the phylum Dothideomycetes and are distinguished from other genera of the order Cladosporiales having septate, pigmented and branched hyphae. Coronate conidiogenous loci are also the characteristic feature of *Cladosporium.* Conidiophores are simple to branched and hyaline to pigmented in appearence. Conidia are present in acropetal manner having verrucose ornamentation. Color of conidia varies from sub–hyaline to pigmented arranged in short or long chains (Crous *et al.,* 2007).

Leaves of *Morus alba* L. infected with fungal disease were collected from Lahore, Pakistan and analyzed both morphologically, anatomically and phylogenetically. From the leaves of *M. alba,* species of *Cladosporium* were isolated. The morphological features of studied taxa match well with the description of *C. cladosporioides*. Molecular confirmation of the studied taxa was done with the help of sequencing of ITS region. Our collection grouped with the *C. cladosporioides* sequences from areas of world in the phylogenetic tree. Morpho–anatomical and phylogenetic characterization confirmed it as *C. cladosporioides* and *Morus alba* has been reported here as a new record for this fungus from Pakistan. This fungus has been formerly reported on *Paspalum distichum* L. and *Zea mays* L. (Lenne**,** 1990; Ahmad, 1969; Ahmad *et al.,* 1997).

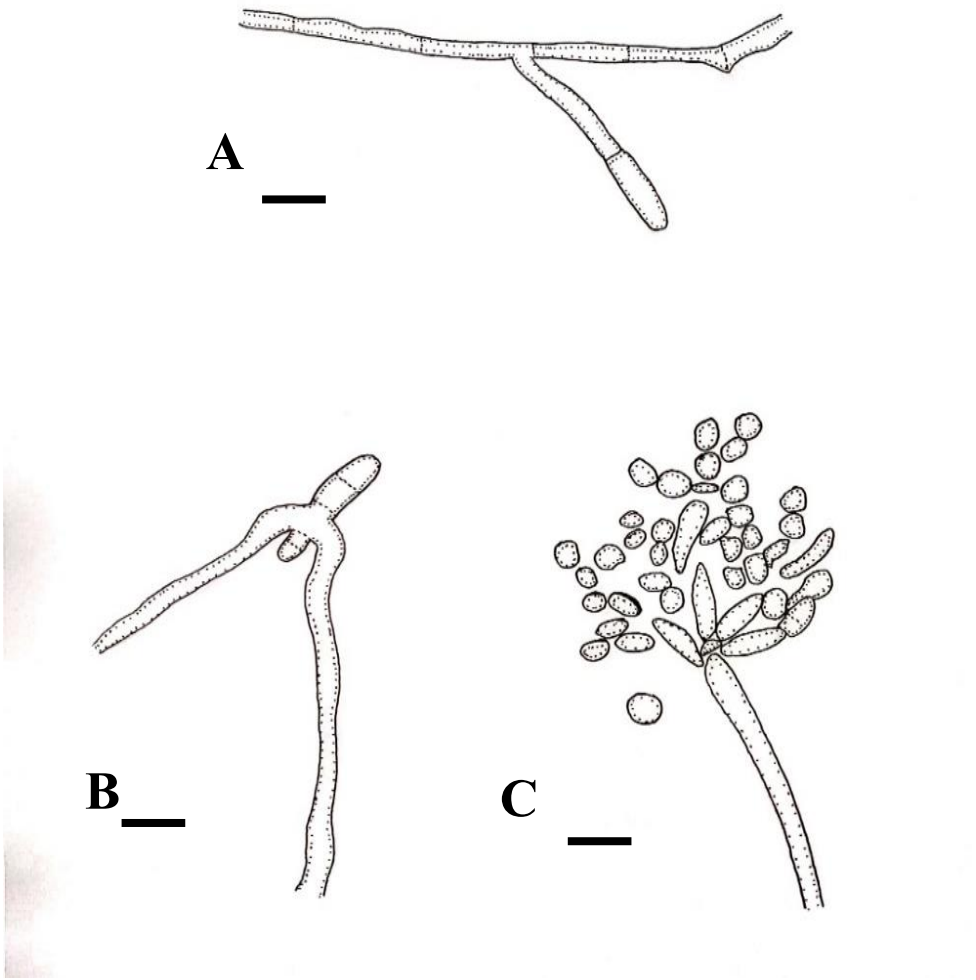

Plate 1(b): Line drawings of *Cladosporium cladosporioides* **A.** Hyphae; **B.** Conidium; **C.** Conidiophore with conidia. Scale bars: A=3 µm; B=5 µm; C=8 µm.

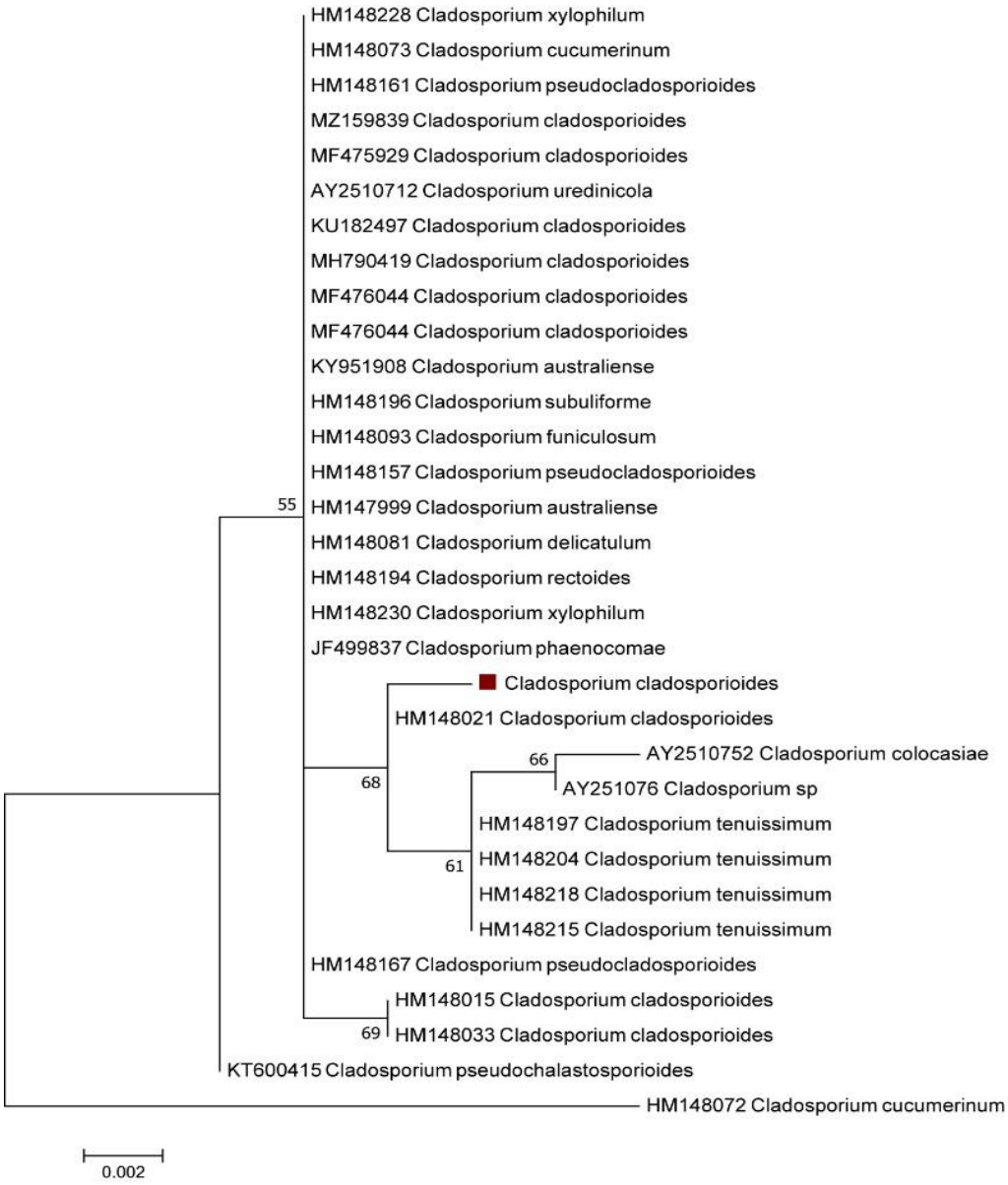

Figure 2. Phylogenetic analysis of 32 ITS sequences of genus *Cladosporium*. Maximum likelihood phylogram was constructed in MEGA 11. Bootstrap support values above 60% are shown above the nodes. Newly amplified sequences are highlighted.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest

AUTHOR'S CONTRIBUTION

Najam-ul-Sehar Afshan: validation, field tours, supervision of research work. Muhammada Jabeen: Corresponding author, methodology, microscopy, writing original draft, field tours, compilation of data phylogeny. Ramsha liaqat: Collection of data, microscopy.

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Not Applicable

REFERENCES

Abdollahzadeh J, J. Z. Groenewald, M. P. A. Coetzee, M. J. Wingfield and P.W. Crous 2020. Evolution of lifestyles in Capnodiales. *Studies Myco.,* 95: 381–414.

Ahmad, S. 1969. Fungi of West Pakistan. *Biol Soci Pak Monograph*., 5: 1-110.

Ahmad, S., S. H. Iqbal and A. N. Khalid, 1997. Fungi of Pakistan. *Sultan Ahmad Mycological Society of Pakistan,* 248.

Anonymous. 2005. Molecular Biology of Plant Senescence. *Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan.*

Bensch, K., U. Braun, J. Z. Groenewald and P. W. Crous. 2012. The genus *Cladosporium*. *Studies in Mycology,* 72*:* 1-401.

Bruns, T. D. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. *Plant and soil*, 170 (1): 63-73.

Crous, P. W., U. Braun, K. Schubert and J. Z. Groenewald. 2007. Delimiting *Cladosporium* from morphologically similar genera. *Studies Mycol.*, 58: 33-56.

Imathiu, S., S. Edwards, R. Ray and M. Back, 2014. Artificial inoculum and inoculation techniques commonly used in the investigation of Fusarium head blight in cereals. *Acta Phytopathologica et Entomologica Hungarica*, 49(2): 129–139.

Lee, W., J. S. Kim, C. W. Seo, J. W. Lee, S. H. Kim, Y. Cho, Y. W. Lim. 2023. Diversity of *Cladosporium* (Cladosporiales, Cladosporiaceae) species in marine environments and report on five new species. MycoKeys 98: 87-111. https://doi.org/10.3897/mycokeys.98.101918

Lenne, J. M. 1990. World list of fungal diseases of tropical pasture species. *Phytopath Papers,* 31: 1-162.

Nam, M. H., M. S. Park, H. S. Kim, T. I. Kim and H. G. Kim. 2015. *Cladosporium cladosporioides* and *C. tenuissimum* cause blossom blight in strawberry in Korea. *Mycobiol*, 43(3): 354-359.

Reyes, M. F. C., B. Auxier, B. Briggeman, and B. E. Snelders. 2025. High throughput culture and DNA] isolation methods for Aspergillus fumigatus. Current Protocols, 5(4): 1–14.

Vargas, W. A., J. M. S. Martin, G. E. Rech, L. P. Rivera, E. P. Benito, J. M. Diaz-Minguez, M. R. Thon and S. A. Sukno. 2012. Plant defense mechanisms are activated during biotrophic and necrotrophic development of *Colletotricum graminicola* in maize. *Plant Physiology*, 158(3): 1342–1358.