Indian Journal of Experimental Biology
Vol. 64, May 2026, pp. 448-451
DOI: 10.56042/ijeb.v64i05.24213

First report of *Alternaria tenuissima* causing leaf spot in *Podophyllum hexandrum* in Kashmir valley, India

Mahrukh Irshad[1], Firdoous Ahmad Mir[1,2]* & Malik Muzafar Manzoor[1]

[1]Plant Science, CSIR-Indian Institute of Integrative Medicine, Sanatnagar, Srinagar, 190005, India

[2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

Received 25 September 2025; revised 13 March 2026

Podophyllum hexandrum (Royle), commonly known as Himalayan Mayapple is a medicinally important plant that thrives well in alpine Himalayas of Jammu and Kashmir. This study focused on the isolation of fungal endophytes from different tissues (roots, leaves) of *P. hexandrum.* Five endophytic fungi were isolated, of which one was found to be pathogenic. ITS primer gene sequencing using nucleotide BLAST identified four non-pathogenic isolates viz PdFRE1, PdFLE2, PdFLE3, PdFLE4 as *Xylaria karsticola, Sordariales sp., Nemania diffusa* and *Nodulisporium sp.,* respectively. The pathogenic isolate PdFLP was studied in detail. Microscopic and molecular analysis of the pathogenic fungus PdFLP was conducted. BLAST analysis showed 100% nucleotide identity with previously identified *A. tenuissima* isolate (ON712430.1). The pathogen thus isolated was identified as *Alternaria tenuissima* and the pathogenicity was established. To our knowledge, this is the first report of leaf-spot of *P.hexandrum*, caused by *Alternaria tenuissima* in Kashmir valley.

Keywords: Himalayan Mayapple, endophytes, pathogen, BLAST, microscopy, pathogenicity tests

Introduction

Podophyllum hexandrum (Royle), member of Berberidaceae family is an endangered rhizome bearing perennial herb[1]. Lower alpine regions of the Himalayan countries like India, Afghanistan, Pakistan, Nepal, Bhutan, and parts of South-west China are home to this medicinally important plant[2,3,4]. In India, *P. hexandrum* is found in Jammu and Kashmir, Himachal Pradesh, and Arunachal Pradesh[5]. Roots and rhizomes of this plant are a storehouse of many active metabolites, podophyllotoxin being the most significant as it acts as the main precursor of semi-synthetic anticancer drugs like etopophos, teniposide and etoposide. Podophyllotoxin is known to have various pharmacological properties like antiviral, antimicrobial, antihelminthic, and purgative[6,7]. Endophytic fungi harbouring many plants are rich sources of many bioactive compounds that are of great therapeutic use[8]. Besides being produced by the host plant, many endophytes isolated from *P.hexandrum* have been reported to produce podophyllotoxin[9]. *Fusarium oxysporum, Aspergillus fumigatus, Phialocephala fortinii and Alternaria spp.* are examples of endophytic fungi that have been reported to produce podophyllotoxin production. Many strains of these fungi yield higher amounts of podophyllotoxin than plant itself[10].

Methodology

Collection of Plant Material

Fresh above and underground parts of *P. hexandrum* were collected from the trans-Himalayan regions of Jammu and Kashmir, such as Apharwat (33°59′58″N 74°19′32″E), CSIR-IIIM Farm Station Verinag (33°32’4.8804"N 75°14’13.7616"E) and Bandipora, during the growing season (March to October).

Isolation and Characterization of Fungal Endophytes

Plant material was sterilized and processed for the endophyte isolation by the method developed by Ezra[11] with some modifications. To check the effectivness of surface sterilization 1 ml of final wash was spread on potato dextrose agar (PDA), Malt Extract Agar (MEA), and Sabouraud Dextrose Agar (SDA). Tissues were cut into smaller segments of around 0.5 cm (approx.) and plated on potato dextrose agar (PDA), then incubated at 25˚C for 1- 2 weeks and observed for any fungal growth. Pure cultures were maintained on PDA and preserved in 20% glycerol at -80˚C.

Genomic DNA extraction and amplification of Inter transcribed sequence (ITS)

Genomic DNA from isolated fungal endophytes was isolated following a previously published protocol[12] which was further used for the amplification of ITS gene sequence. The thermal cycling program used is as follows: 3 min initial

Correspondence:
E-mail: firdosmir.iiim@csir.res.in

denaturation at 95°C, followed by 30 cycles of 15 seconds denaturation at 94°C, 30 sec primers annealing at 55°C, 30 sec extensions at 72°C, and a final 7 min extension at 72°C. PCR-amplified products (600bp) were visualized on 1% (w/v) agarose gel, purified using a DNA purification kit (Qiagen-USA) and then sent for Sanger sequencing.

Microscopy and phylogenetic analyses of PdFLP

In May 2024, leaves of *P. hexandrum* growing at Verinag field station of CSIR-IIIM (33°32'4.8804"N 75°14'13.7616"E; alt 2042.1m asl) were found to have brownish concentric necrotic spots with an average diameter of ~0.5-1cm. (Fig. 1). Symptomatic leaves were collected from the study site and surface-sterilized with 0.5% Sodium hypochlorite, followed by washing away the disinfectant with sterile water[13]. Leaves were then placed on PDA plates and kept at 28°C for seven days. After the incubation, the fungal colonies appeared on the plate. Microscopic identification of the culture was done.

For molecular identification, Internal transcribed spacer (ITS) region was amplified and sequenced using ITS4/ITS5 primers[14].

Pathogenicity tests

To confirm Koch's postulates, both *invivo* and *invitro* plant pathogenicity tests were conducted. For *invitro* confirmation, detached leaf assay was carried following standard protocol with little modifications[15]. Seven days old culture of *A. tenuissima* (PdFLP) isolate was used to perform the pathogenicity test. Small plugs of pathogen ~1–3 mm were placed on surface sterilized asymptomatic detached leaves of *P.hexandrum* which were kept on sterilized moist filter paper and incubated at 28°C and were then daily observed for leaf spot symptoms.

In vivo virulence of *A. tenuissima* was confirmed by growing four week old plants in the pots containing a mix of soil and vermicompost (2:1) under controlled conditions inside a growth chamber. Leaves of *P. hexandrum* were disinfected with 0.5% Sodium hypochlorite and small plugs of *A. tenuissima* (PdFLP) isolate were placed onto them. Leaves that served as control were sprayed with sterile distilled water and were daily observed for the disease symptoms.

Results

In the present study, five fungal endophytes were isolated from various tissues of *Podophyllum hexandrum*; of which one isolate (PdFLP) was pathogenic in nature. Morphology of the fungal isolate (PdFLP) was studied. Fungal colonies appeared white at the periphery and olivaceous at the centre on Potato Dextrose Agar (Fig. 2A). Microscopic identification of the culture was done which revealed that the conidia had both transverse and longitudinal septa and were obclavate with short conical beak. Size of conidia varied from 15.15 to 26.60 µm in length and 9.60 to 12.10 µm in width. Conidiophores were septate (Fig. 2B).

Fig. 1—Symptoms of leaf spot caused by *Alternaria tenuissima* on *Podophyllum hexandrum.*

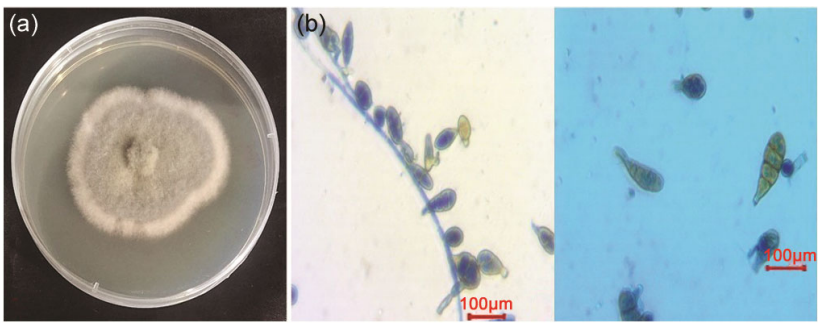

Fig. 2—Culture morphology of *Alternaria tenuissima* isolate PdFLP. (a) PdFLP colony growing on potato dextrose agar; (b) Micrograph of PdFLP; conidiophore and conidia; Scale bar (100 µm)

Microscopic and molecular analysis confirmed the close resemblence of PdFLP with *Alternaria* spp. DNA sequence was deposited in NCBI GenBank under accession no. PV069223. Amplified sequence showed 100% nucleotide identity with previously identified *A. tenuissima* isolate (ON712430.1) by BLASTn analysis. Phylogeny of the pathogen was established by ITS primer gene sequence using nucleotide BLAST and MEGA6 software[16] (Fig. 3).

Pathogenicity of PdFLP was confirmed by conducting both the *in vivo* and *in vitro* plant pathogenicity tests. In both the tests, virulence was confirmed after seven days of incubation of the pathogen on the host plant. Disease symptoms were seen on the leaves that were inoculated with the pathogen. No disease symptoms were found on the leaves that served as control. For confirmation, the fungus was reisolated from necrotic spots and growing on PDA (Fig. 4). Experiments were performed thrice (Fig. 5).

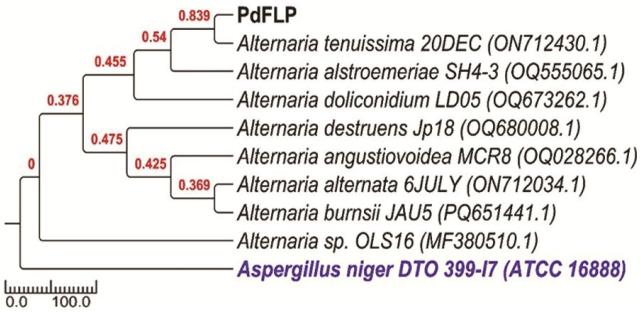

Fig. 3—ITS sequence based evolutionary position of the PdFLP.

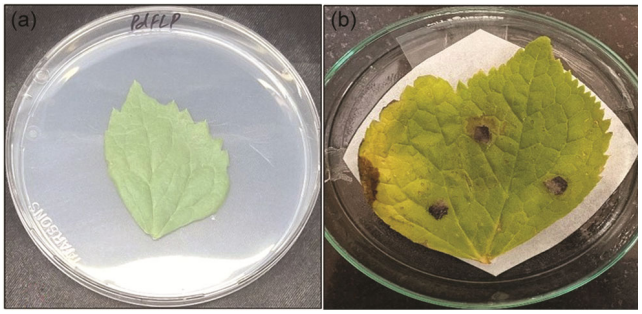

Fig. 4—*In vitro* pathogenicity test; (a) asymptomatic control leaf; (b) inoculated leaf bearing leaf spot symptoms.

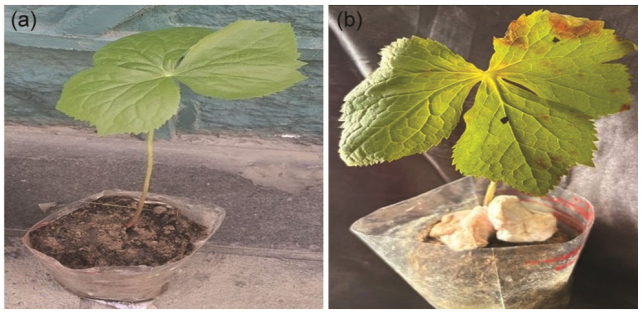

Fig. 5—*In vivo* pathogenicity test. (a) asymptomatic control plants; (b) inoculated plants bearing leaf spot symptoms.

Discussion

The main objective of this study was to isolate and identify the causative organism of the leaf-spot of Podophyllum *hexandrum*. This finding thus significantly implys the need for the conservation and cultivation of this critically endangered medicinal plant. Isolation of both the pathogenic and non-pathogenic fungal isolates from *P. hexandrum* reveals the dual role of endophytes; as beneficial and harmful to their host[17,18].

Alternaria tenuissima has previously been reported as a pathogen on a large variety of crops like pigeon pea[19], bitter-gourd[20]. The morphology of the disease lesions on the infected leaves of the host plant resemble with those induced by *Alternaria alternata* infections[21]. The microscopic features of PdFLP are similar to the descriptions of *A. tenuissima* reported earlier[22].

Pathogenicity of *A. tenuissima* was established through *in vitro* and *in vivo* tests, thus fulfilling the Koch's postulates. The virulence of the pathogen was confirmed by the appearance of disease symptoms in the treated plants and the absence of symptoms in control plants. These findings go hand in hand with previously published reports of *Alternaria* species causing foliar diseases in medicinal plants, such as *Alternaria alternata* in *Withania somnifera*. In future, research should be aimed to investigate the role of abiotic stressors (e.g. temperature, humidity) on the host plant physiology and on the pathogenicity of *A. tenuissima* in *P. hexandrum.*

Conclusion

The present study isolated five fungal endophytes from *P. hexandrum*, among which one pathogenic isolate (PdFLP) was identified as *Alternaria tenuissima* through morphological and ITS-based molecular analysis. Pathogenicity tests under *in vitro* and *in vivo* conditions confirmed that *Alternaria tenuissima* causes characteristic leaf spot disease in *P. hexandrum* fulfilling Koch's postulates. To our knowledge, this is the first report of *Alternaria tenuissima* as a foliar pathogen of *P. hexandrum* in the Kashmir valley. The dual isolation of both pathogenic and non-pathogenic endophytes highlights the complex microbial interactions within this endangered medicinal plant. These findings underscore the urgent need for monitoring and

managing leaf spot disease in natural and cultivated populations of *P. hexandrum* to support its conservation. Future studies should explore the influence of abiotic factors on disease development and the potential role of non-pathogenic endophytes in biocontrol or podophyllotoxin production.

Acknowledgments

This study was possible by the support of the Division of Fermentation and Microbial Biotechnology Division, CSIR-Indian Institute of Integrative Medicine, Sanat Nagar and Srinagar 190005 India.

Conflict of Interest

Authors declare no competing interest to report.

References

1 Sharma N, Thakur M, Sharma P, Sharma YP & Dutt B. In vitro propagation from rhizomes, molecular evaluation and podophyllotoxin production in Himalayan May Apple (Sinopodophyllum hexandrum Royle TS Ying): an endangered medicinal plant. *Plant Cell, Tiss Org Cult.*149 (2022) 159.

2 Sharma A & Sharma P. The Himalayan May Apple (Podophyllum hexandrum): a review. *Asian J Adv Basic Sci*, 6 (2018) 42.

3 Wani ZA, Abdul Rahim PP, Dar JA, Lone AN & Siddiqui S. Predicting the potential distribution of Podophyllum hexandrum Royle in the Himalaya under CMIP6 climate projections. *Sci Rep*, 15 (2025) 25374.

4 Jagdish S, Joginder S & Tewari VP. Screening and evaluation of superior chemotypes of Podophyllum hexandrum Royle from different geographical locations of north-west Himalayas. *J Plant Chem Ecophysiol*, 3 (2018) 1021.

5 Qazi PH, Rashid AB & Shawl SA. Podophyllum hexandrum: a versatile medicinal plant. *Int J Pharm Pharm Sci*, 3 (2011) 261.

6 Castro MA, Miguel del Corral JM, Gordaliza M, García PA, Gómez-Zurita MA, García-Grávalos MD, de la Iglesia-Vicente J, Gajate C, An F, Mollinedo F & San Feliciano A. Synthesis and biological evaluation of new selective cytotoxic cyclolignans derived from podophyllotoxin. *J Med Chem*, 47 (2004) 1214.

7 Kalam MA, Malik AH, Ganie AH & Butt TA. Medicinal importance of Papra (Podophyllum hexandrum Royle) in Unani system of medicine. *J Complement Integr Med*, 18 (2021) 485.

8 Gunatilaka AL. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J Nat Prod*, 69 (2006) 509.

9 Liang Z, Zhang J, Zhang X, Li J, Zhang X & Zhao C. Endophytic fungus from Sinopodophyllum emodi (Wall.) Ying that produces podophyllotoxin. *J Chromatogr Sci,* 54 (2016) 175.

10 Biswas D, Biswas P, Nandy S, Mukherjee A, Pandey DK & Dey A. Endophytes producing podophyllotoxin from Podophyllum sp. and other plants: A review on isolation, extraction and bottlenecks. *S Afr J Bot*, 134 (2020) 303.

11 Ezra D, Hess WM & Strobel GA. New endophytic isolates of Muscodor albus, a volatile-antibiotic-producing fungus. *Microbiology*, 150 (2004) 4023.

12 Chi MH, Park SY & Lee YH. A quick and safe method for fungal DNA extraction*. Plant Pathol J*, 25 (2009) 108.

13 Meena M, Zehra A, Dubey MK, Aamir M, Gupta VK & Upadhyay RS. Comparative evaluation of biochemical changes in tomato (Lycopersicon esculentum Mill.) infected by Alternaria alternata and its toxic metabolites (TeA, AOH, and AME). *Front Plant Sci*, 7 (2016) 1408.

14 White TJ, Bruns T, Lee SJ & Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc*, 18 (1990) 315.

15 Twizeyimana M, Ojiambo PS, Ikotun T, Paul C, Hartman GL & Bandyopadhyay R. Comparison of field, greenhouse, and detached-leaf evaluations of soybean germplasm for resistance to Phakopsora pachyrhizi. *Plant Dis*, 91 (2007) 1161.

16 Tamura K, Stecher G, Peterson D, Filipski A & Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*, 30 (2013) 2725.

17 Kumari M, Tamang A, Swarnkar M, Kumar P, Kumar D, Warghat AR & Pandey SS. Deciphering the endomicrobiome of Podophyllum hexandrum to reveal the endophytic bacterial-association of in-planta podophyllotoxin biosynthesis. *World J Microbiol Biotechnol*, 41 (2025) 38.

18 Yi N, Junwei L, Yuan L, Na W, Caixia L, Libo W & Wenhua X. Diversity and Community Composition of Endophytic Fungi of Sinopodophyllum hexandrum (Royle) Ying on the Tibetan Plateau. *J Resour Ecol*, 16 (2025) 771.

19 Sharma M, Ghosh R, Mangla UN, Saxena KB & Pande S. Alternaria tenuissima causing Alternaria blight on pigeonpea in India. *Plant Dis*, 96 (2012) 907.

20 Iftikhar S, Anwar W, Akhter A, Ali S, Khan HA, Khurshid M & Haider MS. Genetic analysis and pathogenic characterization of Alternaria tenuissima induced fruit rot of bitter gourd. *Biodiversitas*, 22 (2021).

21 Anviksha A & Reddy MS. Isolation, structural and functional characterization of podophyllotoxin produced by an endophytic fungus Alternaria longipes isolated from Podophyllum hexandrum Royle. *S Afr J Bot*, 184 (2025) 1254.

22 Woudenberg JH, Seidl MF, Groenewald JZ, De Vries M, Stielow JB, Thomma BP & Crous PW. Alternaria section Alternaria: Species, formae speciales or pathotypes? *Stud Mycol,* 82 (2015) 1.