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NEW DISEASE REPORT

First report of *Alternaria destruens* causing leaf spot disease of *Prunella vulgaris* in India

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Prunella vulgaris (self-healing plant, family Lamiaceae) is a perennial herbaceous plant which grows in temperate regions of the world, and is used in traditional medicine (Wang *et al*., 2019). Huge losses have been observed in *P. vulgaris* due to a leaf spot disease.

In August 2023, fifty *P. vulgaris* leaves displaying symptoms of dark-brown to brown, circular to irregular necrotic patches were collected from three different areas of the Kashmir Valley, India (Fig. 1). The leaves were surface sterilised with 1% sodium hypochlorite, rinsed with distilled water and cut into small pieces, and placed on potato dextrose agar supplemented with chloramphenicol (50 µg/ml). The petri plates were incubated at 25 ±2°C. The fungal subcultures exhibited cotton-like, white and dark-brown to grey mycelium in the centre with circular edges (Fig. 2). Conidia were dark brown, measuring 14–53 × 6–18 µm, often obpyriform to obclavate or ellipsoid in shape, with a tiny conical beak at the tip. The conidia had one to two longitudinal and two to five transverse septa (Fig. 3). The morphological and microscopic characteristics resembled those of *Alternaria* sp.

Identification was confirmed by molecular and phylogenetic analysis based on the Internal Transcribed Spacer (ITS) rDNA. Genomic DNA was extracted using a GeneJET Plant Genomic Mini Kit (ThermoFisher Scientific, USA) and the ITS region was amplified using the ITS1F/ITS4R universal primers (White *et al*., 1990) and sequenced. The sequence was deposited in GenBank (Accession No. PP180006) and had 99.82% identity with *Alternaria destruens* ATCC 204363 (NR137143). A phylogenetic tree was constructed using the maximum likelihood method and RAxML (Fig. 4). The fungus isolate matched the morphological and molecular characteristics of *A. destruens* (Simmons, 2007).



FIGURE 1 *Prunella vulgaris* plant with leaf spot symptoms.

Confirmation of the pathogenicity of the fungus was performed on healthy mature leaves of *P vulgaris* which were sprayed with 2 mL

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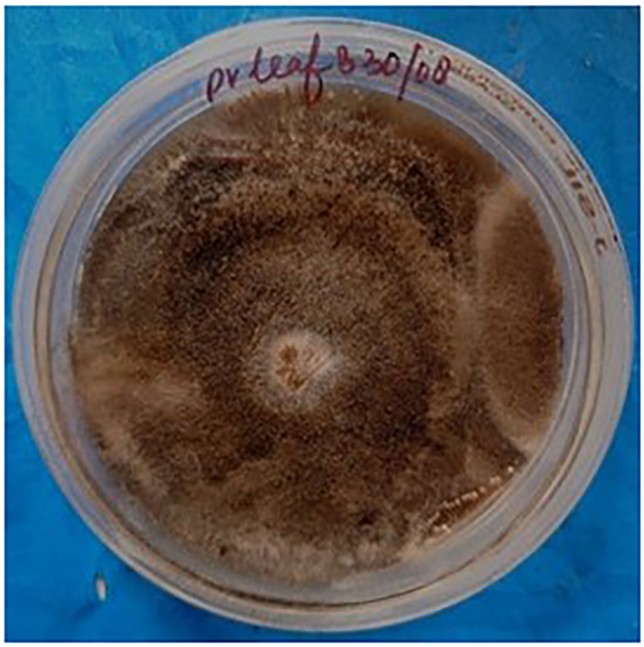


FIGURE 2 Culture of *Alternaria destruens* on potato dextrose agar.

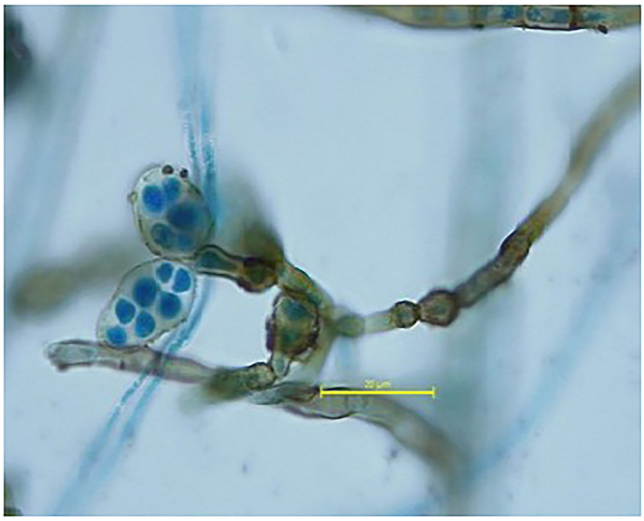


FIGURE 3 *Alternaria destruens* conidiophores and conidia. Bar = 10 µm.

of a conidial suspension of *A. destruens* (10^7 conidia/mL). Plants were sprayed with sterile water as a control. The plants were grown in 15 × 15 cm pots containing a 3:1 (v/v) mixture of soil and manure for 25 days, in a greenhouse at 25 ±2°C with a relative humidity of over 85%. The inoculated leaves produced leaf spot symptoms while the control plants remained symptomless (Fig. 5). *Alternaria destruens* was re-isolated from the inoculated leaves and confirmed by microscopic examination, satisfying Koch's postulates,

Alternaria destruens was reported to be pathogenic on *Cuscuta gronovii* (Simmons, 1998), aubergine, barley, mango and sunflower from Iran (Ershad, 2009) and on *Ligustrum sinense* from China (Yang *et al.*,

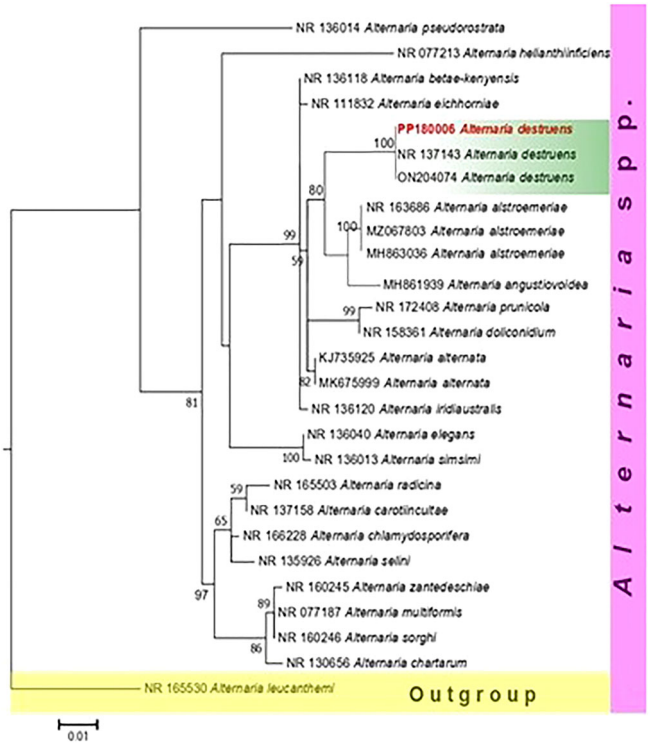


FIGURE 4 Phylogenetic analysis of *Alternaria destruens* as inferred by ITS-rDNA sequence based on the Maximum Likelihood method (sample from this study is shown in red font). Bootstrap support ≥ 50 are shown above or beneath the individual branches.



FIGURE 5 In-vivo pathogenicity test of *Alternaria destruens* on *Prunella vulgaris* leaves.

2019). This is the first report of *A. destruens* causing leaf spot of *P. vulgaris* in Kashmir, India.

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