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NEW DISEASE REPORT

Leaf spot of *Ficus maclellandii* caused by *Penicillium crocicola* - a first report from Iran

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Banana-leaf fig, *Ficus maclellandii*, is a species native to China, India and Southeast Asia. In October 2018, a severe leaf spot was observed on five potted plants of *F. maclellandii* in Hamedan, Iran (34°52′ N; 48°32′ E). Symptoms began as necrotic ellipsoid spots, gradually becoming brown in the centre with a yellowish border (Fig. 1). No symptoms were seen on the branches.

Fungal isolates were recovered from the diseased tissues after surface-sterilisation of infected samples (dipped in 0.5% NaClO for five minutes), rinsing three times in sterile distilled water, plating on potato dextrose agar and incubating at 25 ±2°C for seven days. Four isolates were purified by single-spore isolation. Morphological observations on Czapek yeast extract agar (CYA; 1.0 g K_2HPO_4, 10 mg Czapek concentrate, 5 g yeast extract, 30 g sucrose, 15 g agar, 1 litre distilled water)



FIGURE 1 Leaf necrosis on Ficus maclellandii infected by Penicillium crocicola

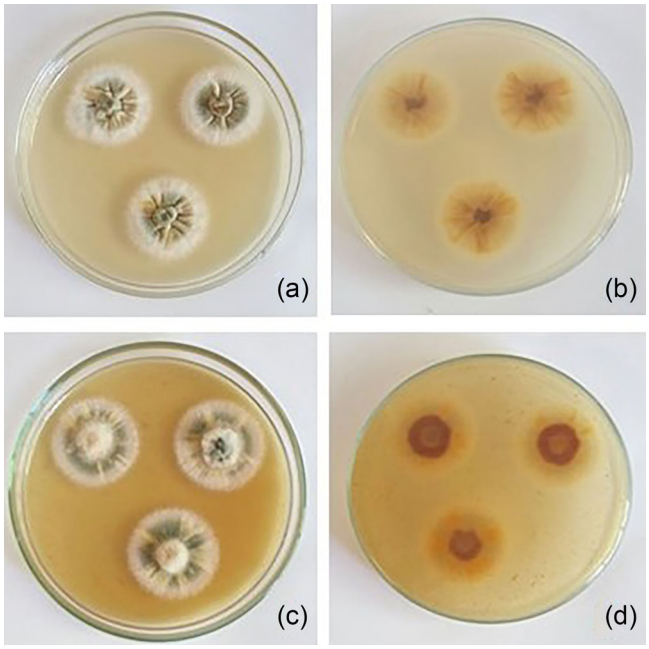

FIGURE 2 *Penicillium crocicola*: colonies grown on Czapek yeast extract agar (A: top, B: reverse views), and malt extract agar (C: top, D: reverse views) after seven days at 25°C

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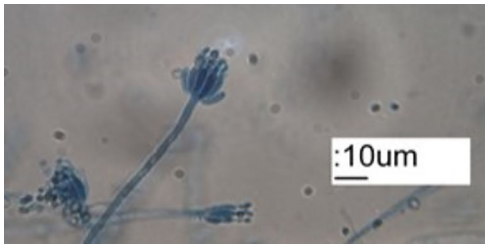

FIGURE 3 *Penicillium crocicola* conidiophores and conidia. Bar = 10 μm

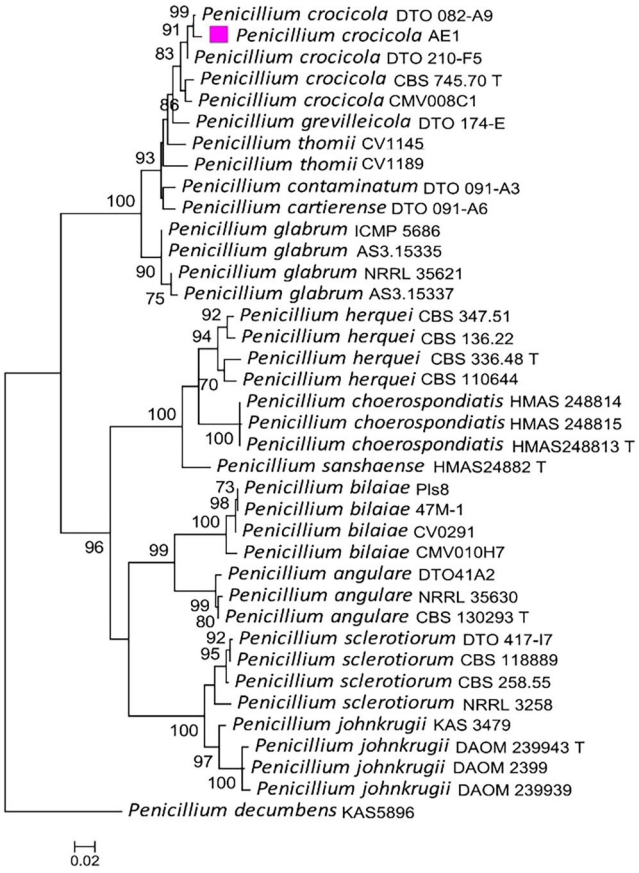

FIGURE 4 Phylogenetic tree of *Penicillium crocicola* combined sequences of ß-tubulin and calmodulin genes, along with the sequences from selected references. The analysis was conducted with MEGA 6.0 using maximum likelihood with 1000 bootstrap replications. *Penicillium decumbens* was used as the outgroup. Purple rectangle indicates the isolate from this study.

and malt extract agar (MEA) showed colony diameters of 42–45 mm on CYA and 33–37 mm on MEA after seven days' incubation at 25°C (Fig. 2). There was no growth on CYA plates after seven days' incubation at 37°C. On CYA, colonies were dark and there was no exudate. Conidiophores were 2.2-2.4 × 3-3.2 μm, monoverticillate and smooth. Phialides were 2–3 × 9–10 μm. Conidia were 2.0-3.5 μm, globose to obovoid, smooth-walled and green (Fig 3). The morphological characteristics consistently resembled *Penicillium crocicola* T. Yamam (Visagie *et al.*, 2014).



FIGURE 5 Leaf necrosis caused by *Penicillium crocicola* on inoculated *Ficus maclellandii* leaves after seven days' incubation at 25°C in plastic container.

Calmodulin and β-tubulin genes of a representative isolate were amplified using reference primers (Glass & Donaldson, 1995; Hong *et al.*, 2006; Houbraken *et al.*, 2014). A BLASTn search of the calmodulin sequence (GenBank Accession No. MG491671) and β-tubulin sequence (MN596864) revealed 97.51% and 99.52% identity to sequences of *Penicillium crocicola* (KM089155.1 and KM088770.1, respectively) (Fig. 4).

Pathogenicity tests were conducted on four healthy leaves of *F. maclellandii*. The leaves were surface sterilised with 70% ethanol prior to inoculation. A conidial suspension in sterile distilled water (10^6 conidia ml^{-1}) was sprayed onto the leaves, while another set of four leaves was sprayed with sterile distilled water as a control. The experiment was repeated three times. On inoculated leaves, necrotic spots appeared after seven days' incubation at 25°C and *P. crocicola* was isolated from all infected leaves and identified based on morphological and molecular characteristics, thus fulfilling Koch's postulates No symptoms appeared on the controls (Fig. 5). The isolate was deposited in the Iranian Research Institute of Plant Protection (Collection no: IRAN3158C) Tehran, Iran.

Penicillium crocicola has been reported from the corms of *Crocus sativus* in Japan, *Protea repens* infructescens in South Africa, sugarcane rhizosphere and grapevine plants in Iran, and elm-ash forest soil in the USA (Houbraken *et al.*, 2014; Ansari *et al.*, 2022). To our knowledge, this is the first report of *P. crocicola* as a plant pathogen, causing leaf spot on *F. maclellandii*. This finding signifies its pathogenic potential.

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