Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Epicoccum layuense* Causing Leaf Brown Spot on *Camellia oleifera* in Hefei, China

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Camellia oleifera is a major tree species for producing edible oil from China. Its oil, also called “eastern olive oil,” has high economic value due to its richness in a variety of healthy fatty acids (Lin et al. 2018). However, leaves are susceptible to leaf spot disease (Zhu et al. 2014). In May 2021, we found circular to irregular reddish-brown lesions, 4 to 11 mm in diameter, near the leaf veins or leaf edges on 30 to 50% of leaves of one-third of *C. oleifera* in a garden of Hefei City, Anhui Province, China (31.86°N, 117.27°E). To isolate the causal agents, symptomatic leaves were cut from the junction of diseased and healthy tissues (5 × 5 mm), treated with 70% alcohol for 30 s and 1% NaClO for 5 min, and inoculated onto PDA medium for culture. After 3 days, hyphal tips were transferred to PDA. Five isolates were obtained and cultured on PDA at 25°C for 7 days. The mycelia appeared yellow with a white edge and secreted a large amount of orange-red material to the PDA. Twenty days later, the mycelium appeared reddish-brown, and subcircular (3 to 10 mm) raised white or yellow mycelium was common on the Petri dish with occasional black particles. The colonies on the PDA produced abundant conidia. Microscopy revealed that conidia were globular to pyriform, dark, verrucose, multicellular, and 14.2 to 25.3 μm ($\bar{x}$ = 19.34 μm, n = 30) in diameter. The morphological characteristics of mycelia and conidia from these isolates were similar to those of *Epicoccum layuense* (Chen et al. 2020). To further determine the species, DNA was extracted from 7-day-old mycelia cultures and the PCR-amplified fragments were sequenced for internal transcribed spacer (ITS), beta-tubulin, and 28S large subunit ribosomal RNA (LSU) gene regions ITS1/ITS4, Bt2a/Bt2b, and LROR/LR5, respectively, followed by sequencing and molecular phylogenetic analysis of the sequence analysis (Glass and Donaldson 1995; Vilgalys and

Hester 1990; White et al. 1990). Sequence analysis revealed that ITS, beta-tubulin, and LSU divided these isolates into two groups. The isolates AAU-NCY1, representing the first group (AAU-NCY1 and AAU-NCY5), and AAU-NCY2, representing the second group (AAU-NCY2, AAU-NCY3, and AAU-NCY4), were used for further studies. Based on BLASTn analysis, the ITS sequences of AAU-NCY1 (MZ477250) and AAU-NCY2 (MZ477251) showed 100 and 99.6% identity with *E. layuense* accessions MN396393 and KY742108, respectively. The beta-tubulin sequences (MZ552310; MZ552311) showed 99.03 and 99.35% identity with *E. layuense* accessions MN397247 and MN397248, respectively. The LSU sequences (MZ477254; MZ477255) showed 99.88 and 99.77% identity with *E. layuense* accessions MN328724 and MN396395, respectively. Phylogenetic trees were built by the maximum likelihood method (1,000 replicates) using MEGA v.6.0 based on the concatenated sequences of ITS, beta-tubulin, and LSU. Phylogenetic tree analysis confirmed that AAU-NCY1 and AAU-NCY2 are closely clustered with *E. layuense* strains. To test pathogenicity, a conidial suspension of AAU-NCY2 (10⁶ spores/ml) was prepared and sterile water was used as the control. Twelve healthy leaves (six per treatment) on a *C. oleifera* tree were punched with a sterile needle (0.8 to 1 mm) and the sterile water or spore suspension was added dropwise at the pinhole. The experiment was repeated three times. By 10 days post inoculation, leaves infected by the conidia developed reddish-brown necrotic spots similar to those observed in the garden, while control leaves remained asymptomatic. DNA sequences from the strain reisolated from the infected leaves were identical to those of the original strain. *E. layuense* has been reported to cause leaf spot on *C. sinensis* (Chen et al. 2020), and similar pathogenic phenotypes were reported on *Weigela florida* (Tian et al. 2021) and *Prunus × yedoensis* Matsumura in Korea (Han et al. 2021). To our knowledge, this is the first report of *E. layuense* causing leaf spot on *C. oleifera* in Hefei, China.

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The author(s) declare no conflict of interest.

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