Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot on White Chrysanthemum (*Chrysanthemum morifolium*) Caused by *Epicoccum sorghinum* in Hubei Province, China

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White chrysanthemum (*Chrysanthemum morifolium*), a perennial herb of the Compositae family, is used for traditional medicine. The planting area of white chrysanthemum in Macheng city, Hubei Province, is about 3,333 ha, and the annual output can reach more than 5,000 t. In 2019, leaf spot disease appeared on almost all middle and lower leaves of white chrysanthemum in most fields of Fengshumiao county, Macheng city (N31°29'57", E115°05'49"). This county has 33 ha of white chrysanthemum planting area, and most of the plants in the county were infected with the leaf spot disease. The average incidence of leaf spot disease was 65%, and incidence in some areas was 100%. In our observations, leaf spot disease can occur throughout the whole growth period of white chrysanthemum, and it will become more serious under the high temperature and humidity condition. Usually, the diseased leaves account for 30 to 80% of the total leaves on the plant. Leaf spot initially manifests as necrotic lesions on the edge and tip of the leaf, and then the lesions coalesce and gradually expand to form irregular light-brown to brown-black spots, eventually leading to necrosis and curling of the entire leaf. This disease seriously affects the growth and development of plants, resulting in the decline of yield and quality of white chrysanthemum. Ten symptomatic leaf samples were collected, the surfaces disinfected with 0.1% mercuric chloride (HgCl₂) for 3 min, and washed with sterile distilled water three times. Ten tissue samples at the junction of diseased and healthy areas (0.5 × 0.5 cm) were cut and placed on potato dextrose agar (PDA) medium containing 100 µg/ml of cefotaxime sodium and incubated in a dark chamber at 28°C. After 2 days, the hyphal tips from the edges of growing colonies were transferred to fresh PDA plates for further purification. Finally, eight isolates were obtained, and these isolates were similar in morphology. The color of purified isolates was initially white to pale yellow. After 6 days of incubation, colonies had a diameter of 8 cm, and the cultures were pale gray and starting to secrete scarlet pigment. After

15 days of incubation, the colonies were grayish brown, and the backside was reddish-brown. Gray to tan chlamydospores were observed, nearly spherical, with a wart-like surface. Unicellular chlamydospores were 7.91 to 32.23 × 12.03 to 38.42 µm (*n* = 30), and multicellular chlamydospores were 6.32 to 25.10 × 21.75 to 100.05 µm (*n* = 30). The morphological characteristics were similar to *Epicoccum sorghinum* (Kang et al. 2019). The isolate FDY-5 was chosen for molecular identification. The sequences of rDNA-ITS, TUB, and LSU of FDY-5 were amplified (GenBank MT800929, MT799852, and MT800935, respectively) (Carbone and Kohn 1999; Lumbsch et al. 2000; White et al. 1990). BLAST results showed that the rDNA-ITS sequences, the TUB gene sequences, and LSU gene sequences of strain FDY-5 shared 99% identity with the sequences of *E. sorghinum* (syn. *Phoma sorghina*) in GenBank (MN555348.1, MF987525.1, and MK516207.1, respectively). Moreover, a phylogenetic tree of the LSU gene sequence of FDY-5 was constructed based on the neighbor-joining method in MEGA6 software (Tamura et al. 2013) and revealed that strain FDY-5 was closest to *E. sorghinum*. Based on morphological and molecular characteristics, the fungus was identified as *E. sorghinum*. Pathogenicity tests were conducted on 2-month-old white chrysanthemum plants. The upper three leaves of three plants were randomly selected for stab treatment and were inoculated with 5 × 5 mm mycelial discs produced from a 15-day-old colony on PDA. The inoculated and control (treated with sterile PDA disks) plants were incubated in a moist chamber (25 ± 2°C, 85% relative humidity). The first lesions appeared 1 day after inoculation on leaves, and the necrotic lesion area expanded outward and showed typical symptoms 3 days later. To fulfill Koch's postulates, the pathogen was reisolated from nine inoculated leaves by repeating the above isolating operation, and it was confirmed as *E. sorghinum* by morphology. To the best of our knowledge, this is the first report of *E. sorghinum* causing leaf spot on white chrysanthemum in China. *E. sorghinum* has a wide host range worldwide and often causes crop yield reduction. This report will facilitate the diagnosis of leaf spot of white chrysanthemum, allowing control measures to be adopted to manage this disease in a timely manner.

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