Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Nigrospora oryzae* Causing Wilt in Summer Cypress (*Bassia scoparia*) in Pakistan

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Summer cypress (*Bassia scoparia*) is a large annual herb belonging to the family Amaranthaceae native to Eurasia. It has been introduced in many other countries. In Pakistan, summer cypress is also known as kochia and is grown as an ornamental plant for its red fall foliage for landscapes. During October 2017, a survey was conducted in Punjab Province, Pakistan, where 100 wilted plant samples were collected from 30 different plantations of Faisalabad district. Up to 50% loss of plantation was noted in all visited locations. Lower parts of the plants were affected first presenting with necrosis of leaf tips surrounded by a chlorotic zone. Then necrosis of apical margins of the plant parts occurred, followed by stem discoloration and wilting of entire herbaceous branches, leading to the partial wilting of the plants. Ultimately, whole plants wilted and died, appearing as though they had been scorched. Diseased tissues from lower stem (crown portion) were sampled, surface sterilized in 70% ethanol for 30 s, and cultured on to potato dextrose agar (PDA) medium. Petri dishes were incubated at 25°C with alternating 12-h periods of light and dark. Frequently observed, fast growing whitish gray fungal cultures with black pin head points were obtained after 7 days. Young conidia were one-celled, yellow to orange in color turning brown to black, ranged in size from 11 to 16 µm × 9.5 to 12 µm, and were ellipsoidal at maturity. Hyphae were branched, septate, and dark brown in color while conidiophores were flexuous, branched, and

were 3.5 to 4.5 µm in diameter and 14.5 to 26.5 µm in length. Based on morphology (Ellis 1971), the pathogen was identified as *Nigrospora oryzae* and submitted to the Westerdijk Collection of Fungi, The Netherlands (CBS 146145/RNOEG30). Total DNA of isolate EG30 was extracted and portions of the internal transcribed spacer (ITS) region and beta-tubulin (βt) gene were amplified using the universal primers ITS1F and ITS4 (White et al. 1990) and βt2a and βt2b (Glass and Donaldson 1995). The generated ITS (GenBank accession no. MG745331.1, 491 bp) and βt (MN629896, 408 bp) sequences were searched against GenBank using BLASTn and were 99% homologous to ITS (KX986074, 525 bp; MN341493, 550 bp) and 100% homologous to βt (MK262852, 409 bp) gene region from *N. oryzae* (Wang et al. 2017; Zhang et al. 2019). For pathogenicity tests, 10 healthy 2-month-old summer cypress plants were inoculated by soil drenching of a spore suspension (10⁶ to 10⁷ spores/ml) of the fungal isolate EG30 while five plants were treated with sterilized water and used as control treatments. Plants were incubated at 60 to 75% relative humidity (RH) and 25°C in a greenhouse. Leaf necrosis and partial to whole plant wilting were observed in the inoculated plants after 21 days. No symptoms appeared in control plants. A fungus was reisolated from the lower stem (crown portion) parts of the inoculated plants that was identical in morphology to isolate EG30. No fungus resembling EG30 was isolated from the control plants. To the best of our knowledge, this is the first report of summer cypress wilt caused by *N. oryzae* (Berk. and Broome) Petch, a known pathogen of several important crops in China, Australia, India, Canada, and Pakistan (Sharma et al. 2013).

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