Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Clonostachys rosea* Causing Root Rot on Garlic in Mexico

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Garlic (*Allium sativum*) is an important crop worldwide and it is widely grown and used in different industries to manufacture food, pharmaceutical, and insecticidal products (Shang et al. 2019; Velsankar et al. 2020). According to SIAP (2020), more than 87 ha of the crop were lost in Mexico due to various problems, including diseases that attack this crop such as basal rot, white rot, and root rot. During the 2019 fall/winter season, garlic plants of Perla and Piedra Blanca cultivars were collected from Aguascalientes and Zacatecas states in San Antonio Tepezala, Rincon de Romos, and Calera municipalities. The commercial fields encompassed 10 ha with approximately 20% disease incidence and 35% severity. The sampling focused on diseased plants with symptoms of root rot, foliar wilt, stunting, and small bulbs. The roots of 25 plants were cleaned, and portions of the diseased tissue were cut and disinfected in 1% NaOCl for 3 min. They were rinsed twice with sterile water and dried with paper towels. The plant tissue was plated onto potato dextrose agar (PDA) and incubated at 25°C in the dark for 72 h. Pure cultures were obtained after observing mycelial growth using monospore culture. Sixteen isolates were obtained, including 3 identified as *Fusarium oxysporum*, 1 as *F. solani*, and 12 as *Clonostachys rosea*. The latter isolates were white at the beginning before turning yellow. The mycelia had a felt-like cotton texture. The conidia formed verticillate and penicillate conidiophores. The primary conidia were

abundant, hyaline, smooth, and subglobose. They were 5.1 to 7.7 × 8.3 to 8.9 µm ($n = 50$) long and 2.0 to 2.9 × 3.2 to 3.5 µm wide ($n = 50$). The conidiophore stipe length ranged from 70 to 180 µm, and the base width was 3.3 to 5.4 µm. Secondary conidiophores were penicillate and stiped with a length of 58 to 106 µm; the base measured 3.3 to 6.1 µm. The secondary conidia measured 4.1 to 5 × 5.3 to 5.6 µm long and 2 to 2.3 × 2.6 to 2.9 µm wide ($n = 50$) (Sun et al. 2020). The identity of six isolates was molecularly confirmed by DNA extraction and PCR reactions using ITS1/ITS4 primers and gene TEF 1α EF1-728F/TEF 1α EF1-986R. The resulting products were sequenced and compared with the NCBI database using BLAST. The results showed 100% identity with *C. rosea* (accession nos. MT441901 and KX185000). The sequences were deposited in GenBank (accession nos. OK263088 and OL700031). Pathogenicity tests were carried out using the following procedure. A conidial suspension of five isolates (5×10^5 conidia/ml) in sterilized water was prepared from 1-week-old colonies. The garlic cloves were planted after being disinfected with 1% NaOCl in sterilized soil. When the healthy garlic plants were 30 days old, a spore suspension was inoculated in soil through irrigation to 10 plants. Likewise, 10 control plants were inoculated with sterile distilled water. After 25 days, the inoculated plants were wilted and had dry leaves; their root system showed light-brown lesions and rot. These plants were stunted compared to the healthy control plants. The inoculated strain was recovered and was morphologically and molecularly identified as *C. rosea*, thus confirming its pathogenicity toward garlic. There are reports of *C. rosea* causing root rot to Fabaceae crops such as *Glycine max* L. and *Vicia faba* L. (Afshari and Hemmati 2017; Bienapfl et al. 2012), in addition to affecting orchid crops (*Gastrodia elata*) in Korea (Lee et al. 2020). This is the first report of *C. rosea* causing root rot on garlic in Mexico, thus presenting a potential risk to this crop.

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