Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Caused by *Colletotrichum acutatum* on Olive Fruits in Pakistan

H. H. Nawaz,^{1,†} Rafique Dogar,¹ Muhammed Ijaz,¹ Ashraf Sumrah,¹ Kamil Husnain,¹ Ramzan Ansari,¹ and W. G. Miao²

¹ Center of Excellence of Olive Research and Training, Barani Agricultural Research Institute, Chakwal, Punjab Province, 48800, Pakistan

² Hainan Key Laboratory for Sustainable Utilization of Tropical Bioresources, College of Environment and Plant Protection, Hainan University, Haikou, Hainan Province, 570228, P.R. China

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Anthracnose symptoms on olive (*Olea europaea*) fruits cv. Gamlick were found in three farmer orchards in Chakwal, Punjab, Pakistan (32° N and 72° E), with an average prevalence of 59%. During September 2020, 45 samples of diseased olive fruits were randomly selected from these orchards for further study. Fruit symptoms start as thin, black, sunken lesions with a watery appearance that grow in diameter and coalesce into a large sunken soft zone. Lesions on mature fruit become noticeable 5 to 6 days after infection if temperatures are favorable (28°C). On the lesion, orange conidial masses in a dispersed or concentric circle arrangement can appear. Fragments (5 mm) were taken from the margins of fruit lesions and surface sterilized with 70% ethanol (1 min) and 1% NaClO (2 min), cleaned with sterile purified water, blotted dry, and plated on potato dextrose agar (PDA) in Petri dishes. The Petri dishes were incubated at 27°C. A fungus was consistently isolated, and 35 isolates were characterized. Aerial mycelia from olive isolate colonies were compact, initially white or cream white, then gray, and eventually dark gray with conidium masses forming in the middle. Mycelium was branched, septate, and hyaline. Conidia were hyaline, aseptate, and fusiform or often cylindrical, with obtuse apices and tapering bases. Their mean size was 8.5 µm in length and 3.0 µm in width. Based on morphological features, the fungus was tentatively identified as *Colletotrichum acutatum* (Agosteo 2010). The identification was

confirmed by amplification and sequencing of a representative isolate's internal transcribed region (ITS), beta-tubulin region (TUB2), actin region (ACT), and glyceraldehyde 3-phosphate dehydrogenase region (GAPDH) with the primers ITS1/ITS4 (Gardes and Bruns 1993), TUB4/TUB5 (Woudenberg et al. 2009), ACT1/ACT3 (Carbone and Kohn 1999), and GDF1/GDR1 (Guerber et al. 2003), respectively. BLAST analysis revealed 100% identity for ITS, GAPDH, and ACT and 99% identity for TUB between the sequences of the olive fruit isolate (GenBank accession nos. MW647502, MZA36968, MZ714412, and MW810331, respectively) and sequences of *C. acutatum* reference isolates (GenBank accession nos. GO613492, KF975660.1, MT274752.1, and MH547616, respectively). Phylogenetic analysis based on the ITS, GAPDH, and TUB regions of the olive fruit isolates and reference isolates of various *Colletotrichum* species using the MEGA X software program confirmed the isolate from olive was *C. acutatum*. The fungal isolate was deposited as a living culture in the Barani Agricultural Research Institute's fungal culture collection center (BACA.9381). Pathogenicity tests were conducted with this isolate by placing a 20-µl drop of a conidial suspension (3×10^7 conidia ml⁻¹) on five healthy olive cv. Gamlick fruits. As a control, five noninoculated olive fruits were used. Fruits were placed at a temperature of 27°C with artificial light and a photoperiod of 12 h. Anthracnose symptoms developed after 7 days of inoculation. The fungus was reisolated from symptomatic fruits, and its identity was confirmed through morphological characteristics, thus verifying Koch's postulates. To the best of our knowledge, this is the first report of *C. acutatum* infecting olive fruits in the Chakwal region of Pakistan.

References:

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[†]Indicates the corresponding author.

H. H. Nawaz; h_husnain_012@yahoo.com