

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Nigrospora sphaerica* Associated with Leaf Spot Disease of Cowpea (*Vigna unguiculata*) from India

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Cowpea (*Vigna unguiculata* [L.] Walp) is one of the main legume crops grown in arid and semiarid regions in the world. Brazil, Haiti, Myanmar, Nigeria, Sri Lanka, the United States, and India contribute to the substantial production of cowpea at the global level (Mahadevakumar and Janardhana 2012, 2014). Field surveys conducted during 2017 to 2019 (August to September) in major cowpea-growing regions of southern Karnataka revealed the occurrence of characteristic leaf spot disease of unknown etiology with an incidence ranging from 6 to 8%. Initially, the symptoms developed as small specks (1.5 to 3.5 mm), characterized by circular or irregular shape. These lesions began to develop from the leaf margin and regularly extended and coalesced to form larger lesions. After the successful manifestation of the symptoms on leaves, the associated fungal pathogen was isolated. In brief, the infected leaves were surface sterilized with 2% NaOCl for 2 min, rinsed thrice in sterile distilled water (SDW), and blotter dried. The leaf sections were placed on potato dextrose agar (PDA) in Petri plates and incubated at room temperature ($27 \pm 2^\circ\text{C}$) for 10 to 12 days. Mycelia that developed from infected tissues were transferred to fresh PDA plates, and pure cultures were obtained. Mycelia were initially white and eventually turned into gray. The conidia were black, single celled, smooth, spherical to subspherical, 10 to 22 μm in diameter ($n = 30$),

and borne singly on a hyaline vesicle at the tip of each conidiophore. Based on the cultural features and conidial morphology, the fungus was identified as *Nigrospora* sp. Further, to identify the pathogen to the species level, the internal transcribed spacer region of the ribosomal RNA gene was amplified using primers ITS1 and ITS4 (White et al. 1990). The amplified PCR products were purified and sequenced. The BLASTn analysis showed 100% similarity with reference sequences from the GenBank database for *Nigrospora sphaerica* (MT225783.1, MN795578.1), and the sequences were deposited in GenBank (accession nos. MT305812.1, MT305813.1, and MT305814.1). Based on the cultural, morphological, microscopic, and molecular characteristics, the associated fungal pathogen was identified as *N. sphaerica* (Sacc.) Mason (Chen et al. 2018; Wang et al. 2017), and a voucher specimen was deposited at the University of Mysore Herbarium with accession number UOM20-NS1. Further, pathogenicity tests were conducted on healthy cowpea plants grown under greenhouse conditions. Inoculations were made with conidial suspension (10^5 conidia/ml) prepared in SDW, and healthy plants sprayed with SDW served as a standard control. All the plants were covered with polyethylene bags for 24 to 48 h, and observations were made at regular intervals. Typical necrotic lesions developed after 12 days of inoculation, and no such symptoms were observed on the standard control set. The associated pathogen was reisolated from diseased leaves and its identity confirmed based on morphology and cultural characteristics. Leaf spots are becoming a major problem in cowpea-growing areas in recent years (*Dactuliophora* sp., *Pestalotiopsis* leaf spot, *Alternaria* leaf spot, and many others) (Mahadevakumar and Janardhana 2012, 2014). Recently, *Aplosporella hesperidica* causing collar rot on cowpea has been reported from the same region (Deepika et al. 2020). The seed-borne occurrence *N. sphaerica* on cowpea is reported from Brazil (Rodrigues and Menezes 2002), and there are no previous reports available on the occurrence of *N. sphaerica* on cowpea leaf spots. The present investigation is the first report of *N. sphaerica* causing leaf spot disease on cowpea from India.

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