

# Disease Note

## Diseases Caused by Fungi and Fungus-Like Organisms

### First Report of *Nigrospora sphaerica* Associated with Leaf Spot Disease of *Crossandra infundibuliformis* in India

G. S. Tejaswini,<sup>1,2</sup> S. Mahadevakumar,<sup>3,4</sup> Josna Joy,<sup>5</sup> S. Chandranayaka,<sup>6,†</sup> S. Niranjan Raj,<sup>3</sup> Lakshmi Devi,<sup>5</sup> R. Sowjanya,<sup>5</sup> and R. Sowmya<sup>1,†</sup>

<sup>1</sup> Department of Botany, Yuvaraja's College (Autonomous University Constituent College), University of Mysore, Mysuru 570 005, Karnataka, India

<sup>2</sup> Integrated Regional Office (Southern Zone), Ministry of Environment, Forest and Climate Change, Kendriya Sadan, Bengaluru 560034, Karnataka, India

<sup>3</sup> Molecular Phytodiagnostic Laboratory, Department of Studies and Research in Microbiology, Karnataka State Open University, Mysuru 570 006, Karnataka, India

<sup>4</sup> Department of Forest Pathology, Division of Forest Health, KSCSTE – Kerala Forest Research Institute, 680653 Thrissur, Kerala, India

<sup>5</sup> Department of Studies in Microbiology, University of Mysore, Mysuru 570006, Karnataka, India

<sup>6</sup> Phytopathology Laboratory, Department of Studies in Biotechnology, University of Mysore, Mysuru 570 006, Karnataka, India

G. S. Tejaswini and S. Mahadevakumar contributed equally to this study.

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*Crossandra* (*Crossandra infundibuliformis* (L.) Nees.) is one of the main floriculture crops in Karnataka. In March to June 2020, a characteristic leaf spot disease of unknown etiology with an incidence of 10 to 12% (~30 ha area evaluated) was observed in Southern Karnataka (Mysore, Mandya). Initially, the symptoms developed as small specks (3 to 8 mm), characterized by circular to irregular shapes in the beginning and coalesced to form larger lesions. Ten samples were collected and the associated fungal pathogen isolated on potato dextrose agar (PDA) medium amended with chloramphenicol (60 mg/liter). Briefly, infected leaves were cut into small pieces and surface sterilized with 2% sodium hypochlorite solution, rinsed three times with sterile distilled water (SDW), blot dried, inoculated onto PDA medium, and incubated at room temperature (27 ± 2°C) for 3 to 5 days. Fungal colonies developed from the segments were subcultured through hyphal tipping to fresh PDA plates to get pure cultures. Twelve pure cultures were obtained. Mycelia were initially white and eventually turned gray. The conidia were black, single celled, smooth, spherical to subspherical, 9 to 18 μm in diameter ( $n = 50$ ), and borne singly on a hyaline vesicle at the tip of

each conidiophore. The identity was initially established as *Nigrospora* sp. based on culture and morphology (Deepika et al. 2021). To confirm the identity of fungal isolates, molecular sequence analysis was performed for two representative isolates (CIT1 and CIT2). ITS-rDNA, *tub2*, and *EF-1α* gene were amplified using primers ITS1/ITS4, T1/T22, and EF1-728F/986R (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997; White et al. 1990), then purified and sequenced. The BLASTn analysis of ITS, *tub2*, and *EF-1α* showed 99 to 100% similarity with reference sequences from GenBank of *Nigrospora sphaerica* (ITS: 520 bp, KX985935 – LC7312; MH854878 – CBS:166.26; *tub2*: 357 bp, MZ032030 – WYR007, 350 bp, KY019606 – LC7298, KY019522 – LC4278, KY019520 – LC4274; *EF-1α*: 472 bp, KY019397 – LC7294, KY019331 – LC4241; MN864137 – HN-BH-3) and the sequences were deposited in GenBank (ITS: OL672271 and OL672272; *tub2*: OL782120 and OL782121; *EF-1α*: ON051604 and ON051605; Wang et al. 2017). The associated fungal pathogen was identified as *N. sphaerica* (Sacc.) Mason (Chen et al. 2018; Deepika et al. 2021) based on the cultural, morphological, microscopic, and molecular characteristics. Pathogenicity tests were conducted on healthy plants (*crossandra* cv. Arka;  $n = 30$ ) grown under greenhouse conditions (28 ± 2°C; 80% RH). Inoculations were made with conidial suspension (18-day-old *N. sphaerica* isolate CIT1, 10<sup>6</sup> conidia/ml) prepared in SDW, and healthy plants sprayed with SDW ( $n = 10$ ) served as controls. All the plants were covered with polyethylene bags for 24 to 48 h and observations were made at regular intervals. Typical necrotic lesions developed on 16 plants 12 days after inoculation, but no symptoms were observed on control plants. The associated pathogen was reisolated from diseased leaves and its identity confirmed based on morphology and cultural characteristics. Earlier, *N. sphaerica* was associated with various tree species as an endophyte, and recently several reports have described it to cause disease on various crop plants (Deepika et al. 2021). However, there are no previous reports of *N. sphaerica* causing leaf spot disease on *C. infundibuliformis* from India. Early diagnosis of this leaf spot disease will help the floriculturist adopt suitable management practices to avoid significant economic loss.

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#### e-Xtra

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†Indicates the corresponding authors.  
S. Chandranayaka; moonnyak@gmail.com, and  
R. Sowmya; sow.ramaiah@gmail.com