

The first report of *Curvularia senegalensis* causing leaf and floral spots on *Zinnia elegans* in Sri Lanka

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Necrotic, reddish brown, circular to irregular-shaped, 1 - 2 mm diameter spots with a whitish center at advanced stage were found on the leaves and ray florets of Zinnias (*Zinnia elegans*) from nurseries in Peradeniya.



The fungus associated with the disease was identified as *Curvularia senegalensis* based on morphological and molecular data.

This is the first record of *C. senegalensis* causing leaf and floral spot on *Z. elegans* in Sri Lanka

Highlights

- Necrotic spots were found on leaves and ray florets of *Zinnia elegans* in Sri Lanka.
 - The spots were reddish brown, 1-2 mm diameter with a whitish center.
 - The causative fungus was identified as *Curvularia senegalensis*.
 - This is the first report of *C. senegalensis* causing spots on *Z. elegans* in Sri Lanka.
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SHORT COMMUNICATION

The first report of *Curvularia senegalensis* causing leaf and floral spots on *Zinnia elegans* in Sri Lanka

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Abstract: Nursery-grown Zinnias (*Zinnia elegans*) in Peradeniya, Central Province, Sri Lanka showed necrotic, reddish-brown, circular to irregular-shaped, 1-2 mm diameter spots scattered over the leaves and ray florets which developed a whitish center with time. At the advanced stage, the necrotic spots merged, and the whole leaf/flower was infected. The fungus associated with the disease was identified as *Curvularia senegalensis* based on morphological and molecular data. Inoculated leaves produced necrotic symptoms and subsequent pathogen recovery was confirmed through Koch's postulates. This study provides the first report of *Curvularia senegalensis* causing leaf and floral spots on *Z. elegans* in Sri Lanka.

Keywords: Hyphomycetes; leaf spot; pathogens; phylogeny; *Zinnia*.

INTRODUCTION

Zinnias (*Zinnia elegans*; Family Asteraceae) are popular as bedding plants and cut flowers due to their vibrant colours. Several phytopathogens have been reported to cause a wide variety of diseases on zinnia plants worldwide. Most of them are fungal diseases including powdery mildew caused by *Golovinomyces cichoracearum* and Alternaria blight caused by *Alternaria zinniae* that may result in severe plant loss and decrease of ornamental value (Szopińska, 2016). Other fungal pathogens such as *Cercospora zinniae*, *Botrytis cinerea*, *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani* (Szopińska, 2016), and *Curvularia* spp. (Stevens *et al.*, 1993) also have been reported to affect zinnia. In early 2018, leaf and flower spot symptoms were observed on zinnia plants in a nursery at the University of Peradeniya premises (Kandy District, Sri Lanka). The symptoms initiated as necrotic, reddish-brown, circular to irregular-shaped, 1-2 mm diameter spots scattered over the leaves and ray florets. With time, the spots developed a whitish center and the necrotic spots merged, and the whole leaf/flower was infected (Figures 1a-b). These symptoms were quite similar to those of *Cercospora* leaf spot, which is common in *Zinnia* (Szopińska, 2016). The objective of this study was to identify the pathogen responsible for this leaf/flower spot disease using morphological, pathological, and molecular data.

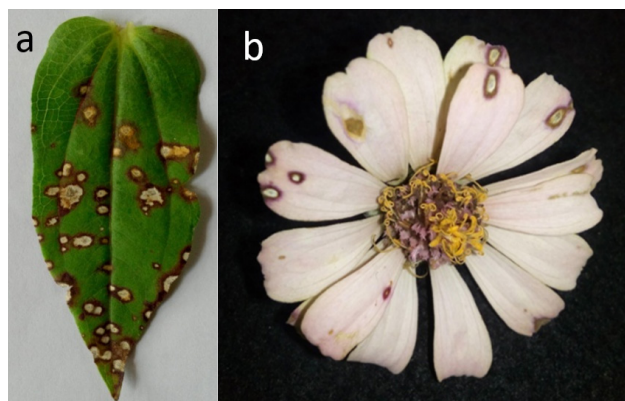


Figure 1: (a) Symptoms of spot disease on *Zinnia elegans* leaves and (b) Symptoms of spot disease on *Zinnia elegans* flowers collected from a nursery at the University of Peradeniya.

Note - Necrotic, reddish-brown, circular to irregular-shaped, 1-2 mm diameter spots scattered over the leaves and ray florets, which developed a grayish-white center with time.

MATERIALS AND METHODS

Isolation and characterization of the pathogen

Symptomatic leaves and flowers of zinnia were collected from a nursery at the University of Peradeniya premises during January-March 2018. Diseased tissues were observed for the presence of any fungal structures by taking scrapings and using the adhesive tape technique (Shivas and Beasley, 2005) on both upper and lower surfaces of the leaves and ray florets and observing them through the light microscope (Olympus CX21FS1, UK).

Segments of randomly collected diseased plant parts were surface sterilized in 2% sodium hypochlorite solution and placed on potato dextrose agar (PDA) plates and incubated for one week at room temperature (27±2 °C) under 12 h light-dark cycles. Fungal isolate CWj was recovered from the leaves and flowers of the infected plants. Single spore isolation was carried out to further purify the culture and it was maintained on PDA for further use. Morphological characteristics of the colonies were

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recorded, and the micro-morphology of fungal structures was examined under a light microscope.

Molecular identification

DNA was extracted according to a protocol described by Manamgoda *et al.* (2012) and PCR amplification for nuclear ribosomal internal transcribed spacers (ITS) 1 and 2 with 5.8S region was carried out using forward and reverse primers, ITS 1 and ITS 2 (White *et al.*, 1990) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) region was amplified using gpd1 and gpd2 primer pair (Berbee *et al.*, 1999). PCR products purification and bi-directional Sanger sequencing were carried out at Macrogen, South Korea. Raw sequences were assembled with the Bio Edit v7.0.5 program for Windows. Phylogenetic trees were constructed based on Maximum Parsimony (MP) with PAUP v. 4.0b10 (Swofford and Sullivan, 2003) and Maximum likelihood using the RAxML v.7.4.2 Black Box (Stamatakis *et al.*, 2008) in the CIPRES Science Gateway platform (Miller *et al.*, 2010).

Koch's postulates for confirming the pathogen

Pathogenicity of CWj isolate was tested using detached leaves (Akhtar *et al.*, 2011) and potted zinnia plants. For detached leaf assay, healthy and uniform size leaves at the third node from the apex of the branches/main stem were taken. Each leaf was surface sterilized with 70% alcohol and placed in plastic Petri dishes lined with moist paper. Two 10 μ L drops of conidial suspension (10^6 conidia/mL), harvested from 7-day-old cultures of the fungal isolate were

placed on each leaf and incubated at 27 ± 2 °C under 12 h light-dark cycles. For potted plant assay, 45-day-old zinnia plants were sprayed with the 10^6 conidia/mL suspension of CWj isolate and kept covered with plastic bags for 24 h to provide humid condition in a glasshouse. Plants and detached leaves treated with sterile distilled water served as controls. The pathogen was recovered onto PDA plates from the resulting symptomatic tissues and the colony morphology and microscopic features of the fungus were compared with those of the originally isolated pathogen.

RESULTS AND DISCUSSION

Isolation and characterization of the pathogen

Fungal colonies on PDA were fast-growing (average colony diameter 1 cm/day), effuse, velvety olive-brown to dark brown with time forming concentric rings (Figure 2a), reverse pale brown to dark brown (Figure 2b). Mycelia were branched and septate. Conidiophores were up to 160 μ m long, arising singly or in groups, pale brown to dark brown, simple or branched, septate, straight to flexuous, geniculate at the apex. Conidia measured were 15 - 21 $\times$ 8 - 11 μ m (av. = 18, SD = 2, n = 30; av. = 9, SD = 1, n = 30), smooth, straight to curved, mostly ellipsoidal, sometimes clavate, dark brown septa, 3 - 4-distoseptate, enlarged middle cells, brown-colored when mature, Apical and basal cells were hyaline or slightly brown, 3 - 5 celled and unequal sided. Hila flat, darkened. Fungal isolates recovered from both flowers and leaves showed identical colony and microscopic features.

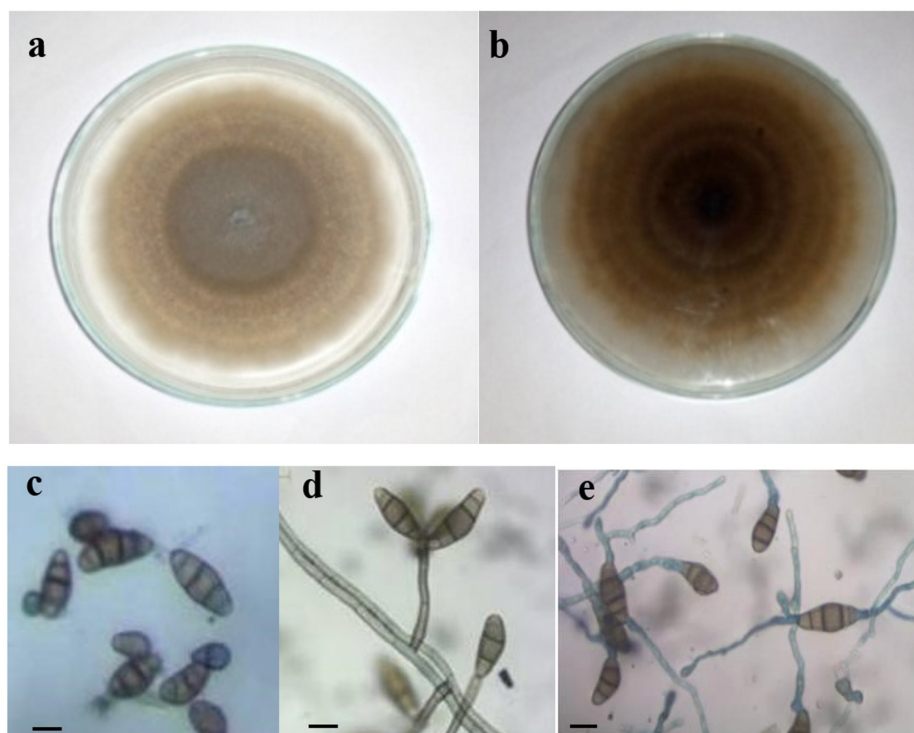


Figure 2: Morphological characteristics of pure colonies of CWj isolate on PDA. (a) Upper surface olive-brown to dark brown forming concentric rings; (b) Reverse pale brown to dark brown; (c) Conidia straight to curved, brown colored, mostly ellipsoidal, 3 - 4-distoseptate with enlarged middle cells; (d) Conidiophores bearing clusters of conidia and (e) Conidia (stained in cotton blue) showing monopolar and bipolar germination.

(c), (d), and (e) are light microscopy photographs. Scale bar = 15 μ m.

According to fungal morphological literature (Ellis, 1971), CWj closely resembled the morphological characteristics of *Curvularia senegalensis*. However, due to overlapping morphological characters, species-level identification of *Curvularia* and allied hyphomycetes genera is recommended to be performed using both morphological and molecular data (Manamgoda et al., 2015). Therefore, molecular data was utilized to confirm the identity of the species.

Molecular identification

Sanger sequencing produced approximately 550 bp and 540 bp sequence fragments for ITS and GAPDH regions, respectively (Genbank accession numbers: ITS-MW353711; GAPDH-MW358932). According to the NCBI BLAST database, ITS region of CWj showed 100% identity to several *Curvularia* species such as *C. senegalensis*, *C. asianensis*, *C. geniculata*, and *C. clavata* with 99% query coverage for each. Even though ITS is considered as the fungal barcode, GAPDH was reported to be the most

informative region for the genus *Curvularia* (Ferdinandez et al., 2021). To further confirm the species identification, multilocus phylogenetic analysis of ITS and GAPDH was performed for CWj with closely related ex-type isolate sequences. According to the phylogenetic tree (Figure 3), CWj clustered with reference isolate of *Curvularia senegalensis* (CBS 149.71) with high bootstrap support (Maximum parsimony 63 and maximum likelihood 99). Thus, the identity of CWj can be confirmed as *Curvularia senegalensis*.

Koch's postulates for confirming the pathogen

Leaf spot symptoms were visible on detached leaves and leaves of potted plants within three days after inoculation with CWj conidial suspension. No symptoms were observed on control plants or detached leaves treated with sterile distilled water. Initially, the spots/lesions appeared as brown, irregular patches and the centre turned in to light brown to gray colour with time. Moreover, those spots did not have clear dark brown margins as seen in spots

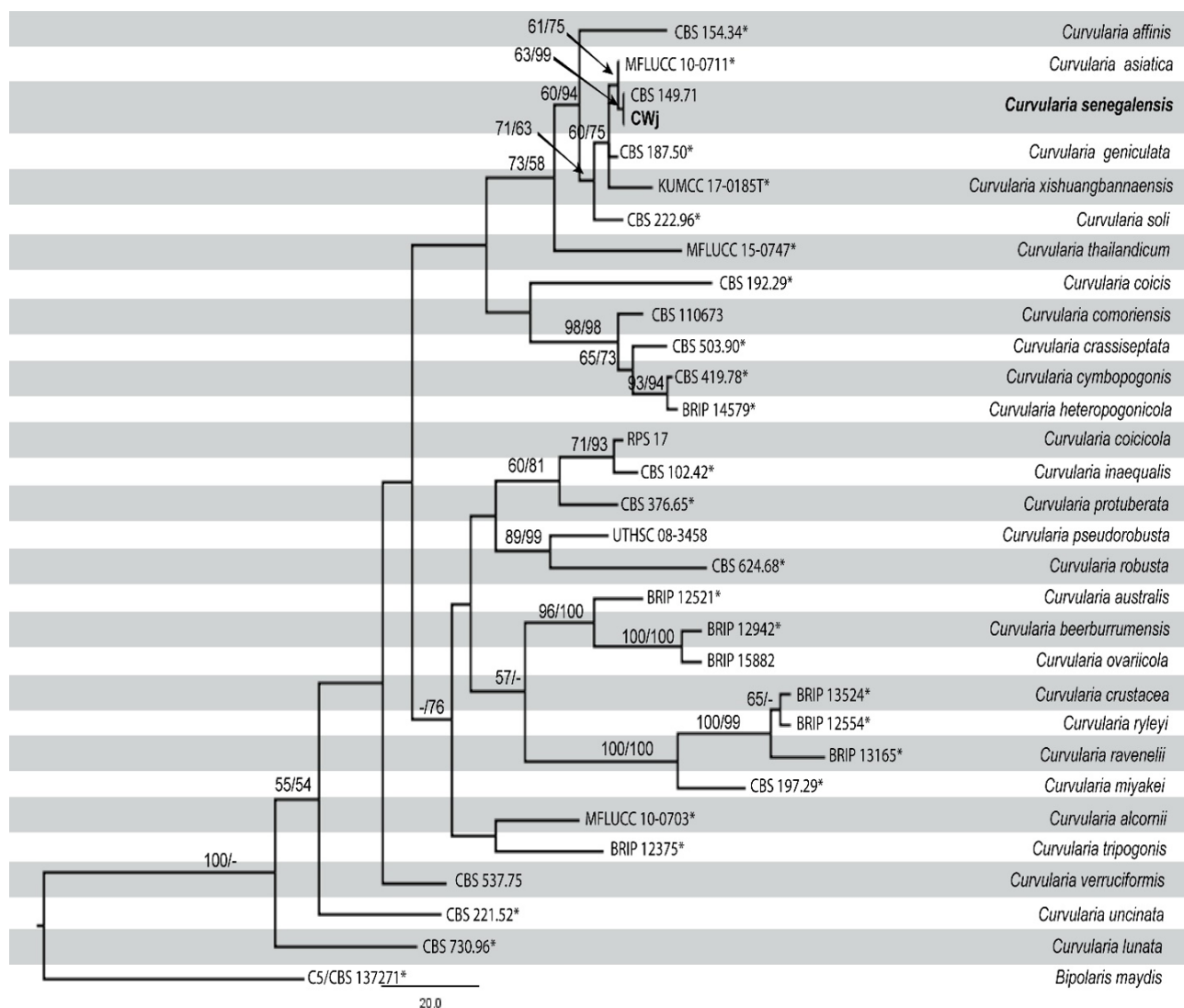


Figure 3: Maximum Likelihood phylogram to show the phylogenetic relationships of fungal isolate CWj and its closely related species based on the combined ITS and GAPDH alignment. Maximum parsimony (MP) and RAXML bootstrap support (BS) values above 70% are shown at the nodes respectively. Ex-type cultures are marked with an asterisk. The tree is rooted with *Bipolaris maydis* CBS 137271, the type species of the sister genus *Bipolaris*.

of originally collected diseased leaves. These variations in symptoms may have partly resulted from higher spore concentrations used for artificial inoculation versus natural conditions in the field. Similar variations in symptoms have been reported when *C. senegalensis* isolated from *Hibiscus cannabinus* leaf blight tissues were artificially inoculated onto detached leaves or live plants under controlled conditions (Mullin *et al.*, 1993).

To satisfy Koch's postulates, the pathogen was re-isolated from symptomatic leaves of inoculated plants and the resulting colonies on PDA, and the microscopic features of the fungus were confirmed similar to those of the isolate originally recovered from diseased zinnia plants. Several *Curvularia* species have been reported in zinnia as a seed pathogen *i.e.* *C. protuberata* (Barreto *et al.*, 2011) and *Curvularia* sp. (Sriwastava and Gupta, 1983; Sing, 2017) and as a minor pathogen on leaves (Stevens *et al.*, 1993) and roots *i.e.* *C. ahvazensis* and *C. rouhaniai* (Mehrabi-Koushki, 2018). Any literature describing the symptoms of *Curvularia* leaf spot in zinnia is not available. *Curvularia* is also recorded as a pathogen in other ornamental plants in the family Asteraceae *viz.* *C. aerea* causing leaf blight in sunflower (Velázquez-del Valle *et al.*, 2017) and *C. clavata* causing leaf blight in gerbera (Yeasmin and Shami, 2013). A particular *Curvularia* species may produce leaf spots with distinctly different symptoms on different host plants. For instance, *C. lunata* produced purple-brown spots on the leaf surfaces of lotus leaves, with diameters ranging from 0.5 to 3.0 mm, which later developed grayish-white centers and a black-brown banding pattern on the edges (Cui and Sun, 2012), while the same pathogen species formed round to oval, light tan to light brown lesions (0.5 to 2.0 mm diameter) with reddish-brown margins often with chlorotic halos in corn leaves (Garcia-Aroca *et al.*, 2018).

Nevertheless, *C. senegalensis* has not been recorded as a pathogen on *Zinnia elegans* before. However, the same fungal species have been reported to infect many commercially important crops such as sugarcane (Byther and Steiner, 1972), banana (Wallbridge and Pinegar, 1975), avocado (Korsten *et al.*, 1988), wheat (Darvishnia *et al.*, 2007) and rubber (Herath *et al.*, 2019) at the field and/or postharvest level.

Out of curiosity arose from the work reported here, a follow-up study was conducted in mid-2018, where samples of zinnia leaf with similar spot disease symptoms were collected from nurseries in Anuradhapura (North Central Province), Peradeniya Botanical Gardens (Central Province), and Mawanella (Sabaragamuwa Province). Three isolates obtained from diseased leaves of all three localities were also identified morphologically as *Curvularia* sp. with the slightly varying colony and microscopic characteristics among them (data unpublished). The pathogenicity of the three isolates was confirmed according to the protocol described for *C. senegalensis* and the molecular characterization of them is being conducted at present. Since all *Curvularia* isolates were obtained from zinnia leaves showing symptoms similar to *Cercospora* leaf

spot, these observations provide evidence to the fact that different pathogen species can sometimes cause disease with similar symptoms in a particular host (Byther and Steiner, 1972).

CONCLUSION

Based on morphological features, molecular characterization, and Koch's postulates, the fungus CWj recovered from diseased zinnia leaves/flowers was identified as *Curvularia senegalensis* (Speg.) Subram. According to our knowledge, this is the first report of *C. senegalensis* causing leaf/flower spot disease of zinnia in Sri Lanka.

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DECLARATION OF CONFLICT OF INTEREST

The authors hereby declare that there are no competing interests for the manuscript.

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