

First report of Leaf Spot Disease Caused by *Exserohilum rostratum* on Bottle Gourd in India

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Bottle gourd (*Lagenaria siceraria*) is a medicinal and nutritious domesticated cucurbit vegetable having annual production of 2.37 million tons with a pantropical distribution in India. In August 2017, typical symptom of leaf spot disease was observed on 3 month old bottle gourd leaves sampled from open fields in Karnal, India of sporadic level of about 10% severity. During monsoon season, initial symptoms appeared mostly on young leaves with grayish white-

to-brown lesions surrounded by yellowish halo, measuring 0.5 to 1.7 × 0.4 to 0.8 cm; These lesions expanded gradually from circular to cylindrical, coalesced covering almost the entire leaf and ultimately led to blighting of foliage and defoliation. Symptomatic advancing lesions leaf bits of 4-6 mm, were surface sterilized with 0.1% HgCl₂ for 45s, rinsed 3 times in sterile distilled water, placed onto potato dextrose agar (PDA) medium and incubated at 27°C for 7-10 days. The emerging fungal colonies on plates were circular, gray to brown with abundant cottony aerial mycelium. Conidia were straight to slightly curved, ellipsoidal to narrowly obclavate or rostrate, brown, 5 to 14 septate, with basal septum darker and thicker than other septum, 22.4 to 154.4 × 8 to 14.7 μm, with a distinctly protruding basal hilum. Conidiophores were single, cylindrical, dark brown, geniculate, and 4 to 6 μm thick with a swollen basal cell. Further, it was identified by Indian Type Culture Collection, New Delhi (ITCC no. 10.643.17) as *E. rostratum*. For molecular identification, genomic DNA of isolate, Ex-1 was extracted from single conidial culture and the internal transcribed spacer regions of rDNA (ITS1-5.8S-ITS4) were amplified and sequenced with universal primers ITS1 and ITS4 (Lin et al. 2010) and deposited in GenBank (MG711857). BLASTn analysis revealed 100% identity with *E. rostratum* isolates (GenBank accession nos. JN711432.1, FJ949084.1, and KU204880.1). Partial sequence of glyceraldehyde-3-phosphate dehydrogenase-like (*Gpd*) gene of Ex-1 were also amplified using primers GPD1 and GPD2 (Liu et al. 2016; Berbee et al. 1999), sequenced and deposited in GenBank (MG918126). BLASTn analysis revealed 99% sequence similarity with an *E. rostratum* isolate (KU935741.1). Based on morphological characteristics and molecular analysis, the isolate was identified as *E. rostratum* (Drechs.) Leonard & Suggs (Lin et al. 2010; Ahmadpour et al. 2013).

Pathogenicity experiment was conducted using five bottle gourd plants (Three leaves inoculated in each plant). Newly matured leaves were inoculated by double spray at 24 hours

interval with isolate Ex-1 conidial suspension (10^5 conidia/ml). Leaves sprayed with sterile water served as negative control. Right after inoculation, plants were covered with plastic bags to maintain high relative humidity and incubated at $\sim 30^\circ\text{C}$ with a natural photoperiod. Seven days later, all pathogen inoculated plants showed symptoms identical to those observed in the field while control plants remained asymptomatic. Koch's postulate was proved with the re-isolation of the fungus from leaves. *E. rostratum* has been reported as causal agent of leaf spots on *Zea mays*, *Ananas comosus*, *Musa paradisiaca* and *Hevea brasiliensis* in China (Liu et al. 2016). To our knowledge, this is the first report of *Exserohilum rostratum* causing leaf spot disease in bottle gourd in India. This disease occurred in quite a large bottle gourd area and identification of pathogens was essential for developing IPM strategies.

References:

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Leaf spot of bottle gourd caused by *Exserohilum rostratum* A. Initial symptoms on leaf spot disease B. morphological features of *Exserohilum rostratum*