

Disease Notes

First Report of leaf spot disease in *Aloe vera* caused by *Nigrospora oryzae* (Berk. & Br.) in Bangladesh

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Aloe vera is an economically important plant because a gel contained in its leaves is used in pharmaceutical, food, and cosmetic industries as well as in traditional medicine. During November 2016, leaf spot symptoms began to appear in 1-3 year old *A. vera* plants grown in an open field of the National Institute of Biotechnology Campus, Dhaka, Bangladesh. By January 2017, 100% of the plants were infected. Symptoms began as small (1 to 4 mm in diameter), circular and dark green lesion on the leaves. The spots became brown to black with age and were surrounded by a yellow halo. In some cases, the spots expanded and fused into irregularly shaped lesions. Symptomatic zones lacked gel inside the leaf and the plants became severely stunted, destroying the plants' commercial value. Small pieces (4 mm diameter) of symptomatic leaf tissue were surface sterilized with 1.5% (w/v) mercuric chloride solution for 1 min, rinsed 4 to 5 times with sterilized distilled water, placed on potato dextrose agar (PDA), and incubated at 28 °C in the dark for 3 to 5 days. Mycelia growing from the tissues were sub-cultured onto fresh

PDA to obtain pure cultures. The whitish gray mycelium was fast growing and formed a white margin after 3-5 days, then become blackish with the onset of sporulation. The hyphae were multicellular. The conidiophores were short and simple; conidia were aseptate, spherical (8-10 $\mu\text{m} \times 10-13 \mu\text{m}$) and black in color, and grow on hyaline vesicles at the tip of conidiophores. Morphological characteristics of the isolate was consistent with *Nigrospora* sp. (Hudson 1963). To determine species identity, genomic DNA was extracted from three representative isolates. The internal transcribed spacer (ITS) region was amplified by PCR and sequenced using ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primer pair. In a NCBI BLAST search, a 521 bp amplicon (GenBank Accession MF806592) revealed 99% similarity with *Nigrospora oryzae*. Pathogenicity of the isolate was confirmed by fulfilling Koch's postulates. Agar blocks containing a fungal isolate were inversely placed on healthy *A. vera* plants with the culture side facing the leaves; the leaves were then wrapped with sterile polyethylene and incubated in a greenhouse (19-28 °C, 50-60% RH). Within 7 to 12 days, symptoms appeared on inoculated leaves that were similar to spots observed in the field, whereas controls (sterile agar blocks) remained symptomless. Re-isolation of the fungal pathogen from leaf lesions showed morphology identical to the original isolate. Based on the pathogenicity tests coupled with phylogenetic analysis, *N. oryzae* was determined as the causal agent of the leaf spot (CBS accession 143706). The disease in *A. vera* caused by *N. oryzae* had been reported in China (Zhai et al. 2013), Iraq (Shaker 2016) and Pakistan (Alam et al. 2017). To our knowledge, this is the first report of *N. oryzae*-causing leaf spots of *A. vera* in Bangladesh. The contribution of *N. oryzae* to leaf spot outbreaks in *A. vera* needs to be investigated further in order to develop management plans.

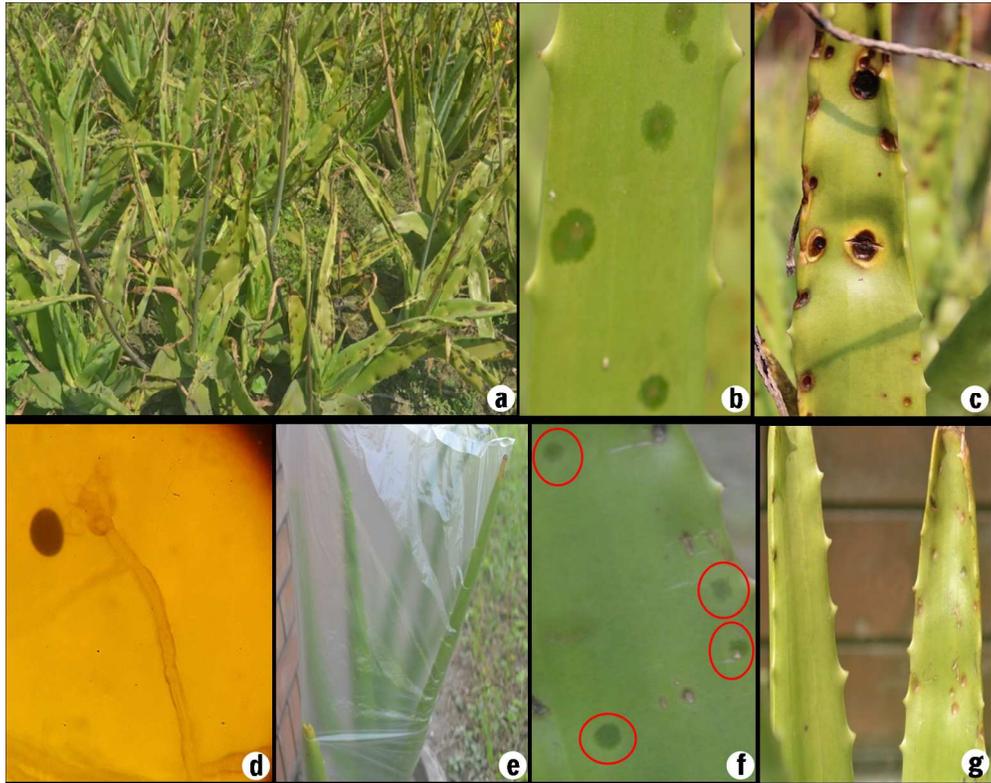
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e-Xtra Figure legend

Nigrospora oryzae-induced leaf spot disease in *Aloe vera*. (a) An *Aloe vera* field severely affected with leaf spot disease (b, c) progression of disease symptoms from small greener lesion

to tissue large cracks (d) characteristic spore of *Nigrospora oryzae* seen under microscope (e) artificial inoculation of the fungal isolate to fulfill Koch postulation (f) occurrence of disease followed by artificial inoculation of *N. oryzae* isolate (g) leaf spots become darker after artificial infection (right) while mock inoculated leaf remained symptomless (left). Live fungal culture has been deposited to Centraalbureau voor Schimmelcultures (CBS 143706) and is publicly available.



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