Disease Notes

First Report of *Colletotrichum siamense* Causing Leaf Spot in *Aloe vera* in Bangladesh

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Aloe vera (L.) Burm.f. is a perennial succulent plant commercially cultivated in tropical and sub-tropical regions for its medicinal, cosmetic, and ornamental uses. During November 2018, leaf spot symptoms were observed on approximately 2-3 years old *A. vera* plants grown in the experimental plots of the National Institute of Biotechnology Campus, Dhaka, Bangladesh. Nearly 100% of the plants were infected. The characteristic symptoms appeared as small (2-4 mm) circular dark green lesions found on the upper surface of the leaves. These lesions gradually turned into brown to dark brown spots surrounded by a watery zone. The infected tissue was cut into pieces (~4 mm^2), surface sterilized in 3% (w/v) sodium hypochlorite for 2-3 min, rinsed with sterilized water 3 times, plated onto potato dextrose agar medium and incubated at 28°C for 4 to 7 days in the dark. The fungal colonies were round in shape, cottony in texture, and white to grey in color. Conidia were single-celled, hyaline, smooth-walled, and cylindrical with rounded ends, measuring 10.0-17.0 μm (mean 14.2 ± 1.3 μm; n = 100) in length and 4.5–6.2 μm (mean 5.5 ± 0.8 μm; n = 100) in width. The morphological characteristics were consistent with the description of *Colletotrichum siamense* Prihast., L. Cai & K. D. Hyde (Weir et al. 2012). For accurate identification, genomic DNA was extracted from a representative isolate, and the internal transcribed spacer (ITS), actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta-tubulin (TUB2), microsatellite loci (Apn25L), and calmodulin (CAL) regions were amplified by PCR. The NCBI BLAST comparison of the sequenced PCR products revealed 99.3-100% similarity with the *C. siamense* (NCBI accessions and identity percentages are given in the e-Xtra). For the pathogenicity test, a small portion of healthy leaves were gently scratched using emery paper to slightly disrupt the cuticles and an agar block with the fungal culture was placed on it. The leaf of the inoculated plant was covered with a polythene bag and kept in the greenhouse at 25-30°C and 75-85% relative humidity. This experiment was performed on three leaves of each five healthy 2-3 years old plants. After 4 days, characteristic symptoms appeared on the inoculated plants similar to those seen in the field. However, symptoms did not appear on control plants treated with agar plugs without fungus. The re-isolation of the fungus from the inoculated plants showed identical morphology, which fulfills Koch's postulates. *C. siamense* exhibits pathogenicity to a number of plants across the world including cacao (Serrato-Diaz 2019), *Euonymus japonicas* (Wu 2020), Partridge tea (Liu et al. 2018), and cassava (Oliveira et al. 2018). Due to the fact that they are genetically very close and significantly differ in infectivity and fungicide sensitivity (Hu et al. 2015; Munir et al. 2016), accurate molecular identification of *Colletotrichum* species is essential in the management of the disease. To our knowledge, this is the first report of *C. siamense* causing leaf spots of *A. vera*.

References

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e-Xtra

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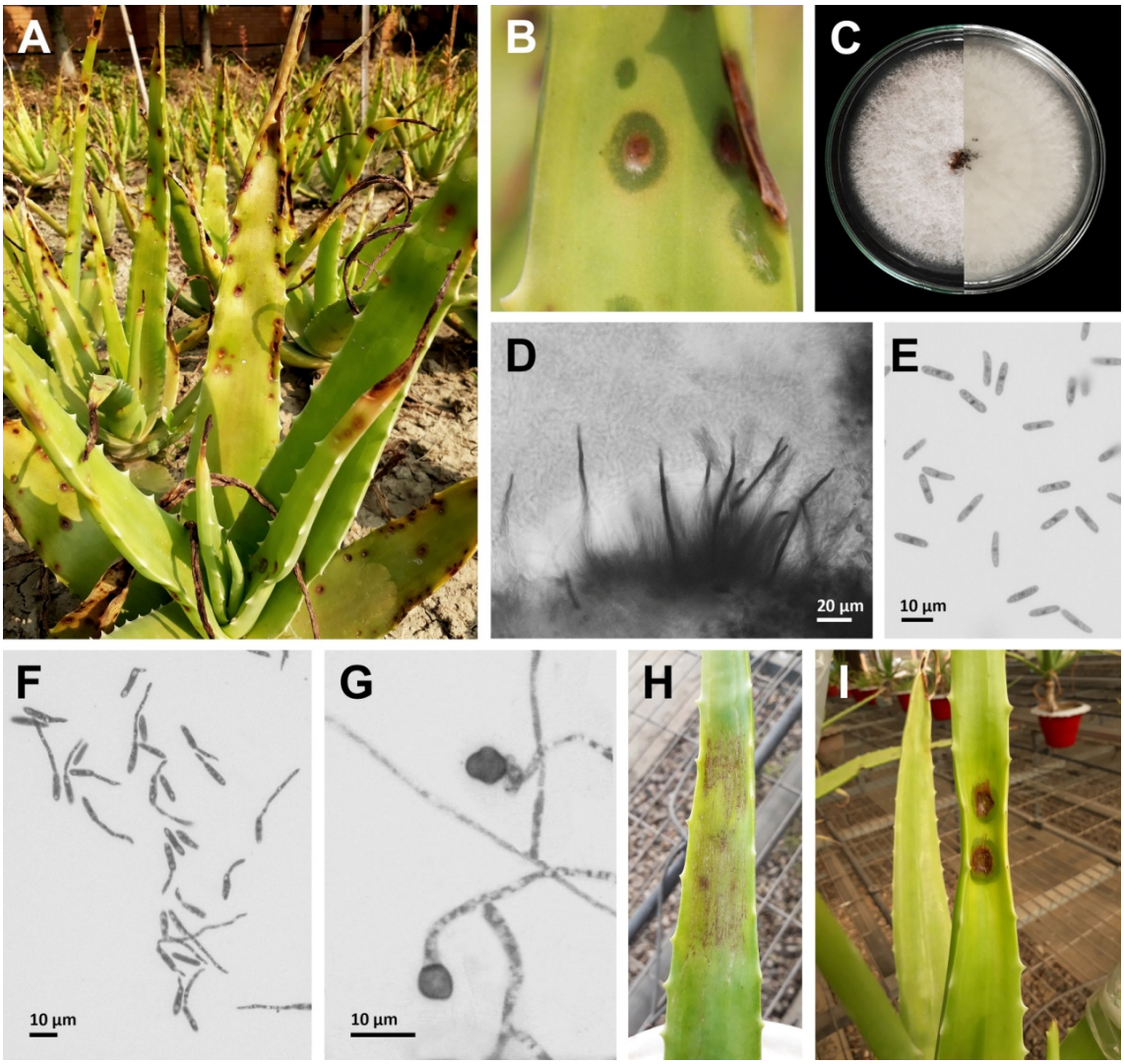

e-Xtra Figure 1. Leaf spot disease on *Aloe vera*. **(A)** Severe leaf spot disease observed on *Aloe vera* plants at the National Institute of Biotechnology campus, Bangladesh; **(B)** Characteristic brown spot surrounded by a watery zone; **(C)** Morphology of fungus grown on PDA medium after 7 days at 28°C; **(D)** Acervuli of *Colletotrichum siamense*; **(E)** Morphology of conidia observed under microscope; **(F)** Germinating conidia; **(G)** Appressoria; **(H)** No characteristic symptoms observed on control inoculated plant; **(I)** leaf spot symptoms observed on inoculated *Aloe vera* plants. Live fungal culture has been deposited to Centraalbureau voor Schimmelcultures and is publicly available.

Table: Primers used to identify DNA signatures, accession numbers and matched sequence in the NCBI database

Locus and NCBI accession number	BLAST identity and top match	Primer name	Primer sequences 5' – 3'	Primer reference
ITS (MT229369)	100% (MN075785)	fITS-1-F fITS-4-R	CTTGGTCATTTAGAGGAAGTAA TCCTCCGCTTATTGATATGC	Liu et al. 2016
Actin (MT241375)	100% (MN891768)	fACT-512F fACT-783R	ATGTGCAAGGCCGGTTTCGC TACGAGTCCTTCTGGCCCAT	Carbone and Kohn 1999
Glyceraldehyde-3-phosphate dehydrogenase, GAPDH (MT241374)	100% (MH681295)	fGDF1-F fGDR1-R	GCCGTCAACGACCCCTTCATTGA GGGTGGAGTCGTACTTGAGCATGT	Liu et al. 2016
Beta-tubulin (TUB2) (MT259946)	99.9% (KP703502)	fT1-F fBt-2b-R	AACATGCGTGAGATTGTAAGT ACCCTCAGTGTAGTGACCCTTGGC	Liu et al. 2016
Microsatellite loci, Apn25L (MT327811)	99.3% (HE657304)	fA5L-F fA5L-R	CAAGCGACGAAGTATACGAG GCATCACGGGAATAACTAGG	Liu et al. 2016
Calmodulin (CAL) (MT294136)	99.4% (MK387145)	fCL1-F fCL2-R	GARTWCAAGGAG GCCTTCTC TTTTTGCATCATGAGTTGGAC	Liu et al. 2016