

First record of *Aspergillus niger* (OR365417) infecting *Musa paradisiaca* leaves

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Short Report

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Additional Declarations: No competing interests reported.

Abstract

Leaf spot disease was observed on the leaves of field-grown banana (*Musa paradisiaca*) between November 2022 and January 2023. The disease was first reported from Ramganj (Latitude: 25.640855; Longitude: 88.430628). There were yellowish brown spots all over the central part of the leaves. Fungus was blackish in color. The fungus as *Aspergillus niger* (OR365417) was identified via molecular identification (18S rDNA partial sequencing). Using the Koch postulation, the leaf spot disease was restored on banana plant leaves. This is the first record of *A. niger* (OR365417) affecting banana from Ramganj region of Uttar Dinajpur District, West Bengal.

Full Text

Leaf spot disease was observed on the leaves of field-grown banana (*Musa paradisiaca*) between November 2022 and January 2023. The disease was first reported from Ramganj (Latitude: 25.640855; Longitude: 88.430628) (Fig. 1). There were yellowish brown spots all over the central part of the leaves. Total number of 25 plants out of 27 plants had the similar symptoms with a percentage of 92.59. 0.1% HgCl₂ was used to surface sterilize the diseased leaf sample, which measured 1 to 1.5 cm. On potato dextrose agar supplemented with the antibiotic Monocef-O 100, a fungus coded as MUSLF-3 was isolated from the diseased leaf. The MUSLF-3 isolate's spore and mycelia were observed under a scanning electron microscope (Fig. 2). Using a Veriti® 96-well thermal cycler, isolated DNA was amplified using 18s rRNA Specific Primers (ITS1 and ITS4). A ~600 bp PCR amplicon band was found. ITS1 and ITS4 primers (ITS1- TCC GTA GGT GAA CCT GC GG; ITS4- TCC TCC GCT TAT TGA TAT GC) were used in a bi-directional DNA sequencing reaction of the PCR amplicon using the BDT v3.1 Cycle sequencing kit on an ABI 3500Dx Genetic Analyzer. Fungus was blackish in color. The fungus as *Aspergillus niger* (OR365417) was identified via molecular identification (18S rDNA partial sequencing) and the fungal rDNA sequence data was submitted to NCBI GenBank (Fig. 3). Using the neighbor joining approach, *A. niger* (OR365417) was phylogenetically analyzed alongside other ex-*Aspergillus* species (Fig. 4). As per NIH Nucleotide BLAST results this isolate MUSLF-03 (OR365417) have showing 99.83 and 99.50 identity with Srilankan isolate MZ331798.1 and Pakistan isolate KJ432863.1 respectively. Within three to five days, disease signs appeared on the infected banana leaves that had been detached. Yellow- brownish spots were appeared on the middle portion of the infected detached leaves after 10 days of inoculation of spore suspension (1X10⁸ spores/ ml suspension) of *A. niger* (Fig. 5). Infection was established in the internal structure of the infected leaves (observed through transverse section of infected leaves) (Fig. 6). Existence of spore of *A. niger* was found on infected detached leaf surface of banana through scanning electron microscopy (Fig. 7). Control leaves were intact, but *A. niger* was reisolated from the infected areas of leaves and the fungus was morphologically recognized. The symptoms were comparable to those seen in the field, despite the artificial nature of the inoculation technique. Using the Koch postulation, the leaf spot disease was restored on banana plant leaves under field settings following foliar application of a spore suspension (1X10⁸ spores/ml suspension) of *A. niger* (Fig. 8) in total of 8 plants. Out of 8 plants, similar symptoms were appeared in 7

plants after seven days of artificial inoculation. The disease reappearance was with a percentage of was 88. Infection was also established in the internal structure of the infected leaves, collected from the field (Fig. 9) and existence of spore of *A. niger* was found on infected leaf surface of banana (Fig. 10).

This is the first record of *A. niger* (OR365417) affecting banana from Ramganj region of Uttar Dinajpur District, West Bengal, as per USDA fungal database there were reports from Bangladesh and Brazil that *Aspergillus niger* causing fruit rot of banana (*Musa paradisiaca*), but there are no previous report of *A. niger* causing leaf spot disease from India. Similar results to the report of *Aspergillus niger* causing banana plant fruit rot disease were recorded. (Ali et al. 2021; Zakaria et al. 2021; Bashar et al. 2012).

Declarations

Acknowledgments

We acknowledge the Director of the USIC Scanning Electron Microscopy Unit at the University of North Bengal for taking the scanning electron microscopic images and M/s Edison Life Science, Kolkata, for molecularly identifying the isolate.

Data Availability Statement

This research work did not involve in the generation or analysis of any kind of separate datasets. All relevant data collected during the study are presented in the manuscript.

Clinical Trial

Not applicable

Ethical approval

The leaf samples used in the present study were procured from Ramganj, Uttar Dinajpur, between November 2022 and January 2023. samples are collected during survey programs of Banana cultivated fields in the local area, permitted and approved by the university and samples are documented properly. As an Assistant Professor of the Department of Botany of Raiganj University, the Corresponding author was fully authorised to collect, conserve, and study the local crop plant diseases and research the causal organisms. All the guidelines were followed as per the University research ethics for the collection, isolation, and documentation of the pathogens. No human or animal was harmed by the participants in the research that was presented. The experimental protocols were approved by the Raiganj University Research. The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation

Funding

No funding support was taken.

Conflict of Interest:

No conflicts of interest are disclosed by the author or authors.

Informed consent

No informed consent was required for this publication.

Consent of publication

I, Papan Chowhan performed the experiment and surveyed the cultivated fields. The photos belong to me. I have no objection if any image is used for publication in purposes of this article.

References

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2. Zakaria, L., Yan, C.Y., Mohd, M.H., Kamaruddin, N.A. and Azuddin, N.F. 2021. Characterisation and pathogenicity of *Aspergillus tamarii* causing banana fruit rot. **Trop Life Sci Res.** 32: 170-187.
3. Bashar, M. A., Shamsi, S., & Hossain, M. (2012). Fungi associated with rotten fruits in Dhaka Metropolis. **Bangladesh Journal of Botany.** 41(1):115-117.

Figures



Fig. 1: Isolation of fungal isolate – MUSLF-3 from diseased leaf sample collected from Ramganj, Uttar Dinajpur District

Figure 1

See image above for figure legend.

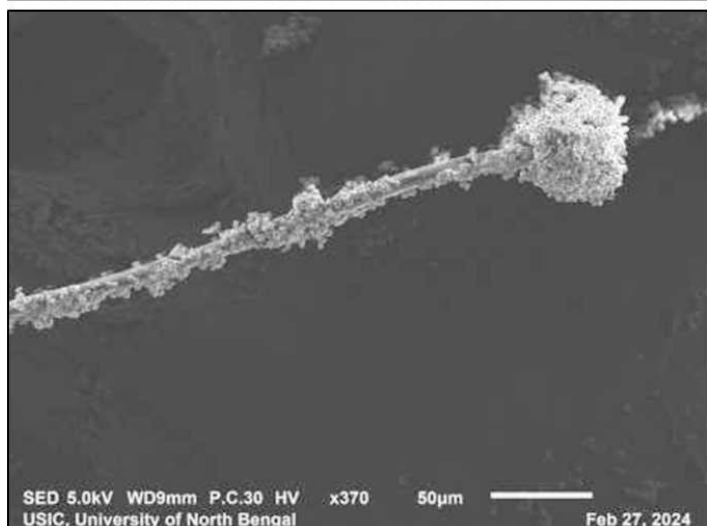
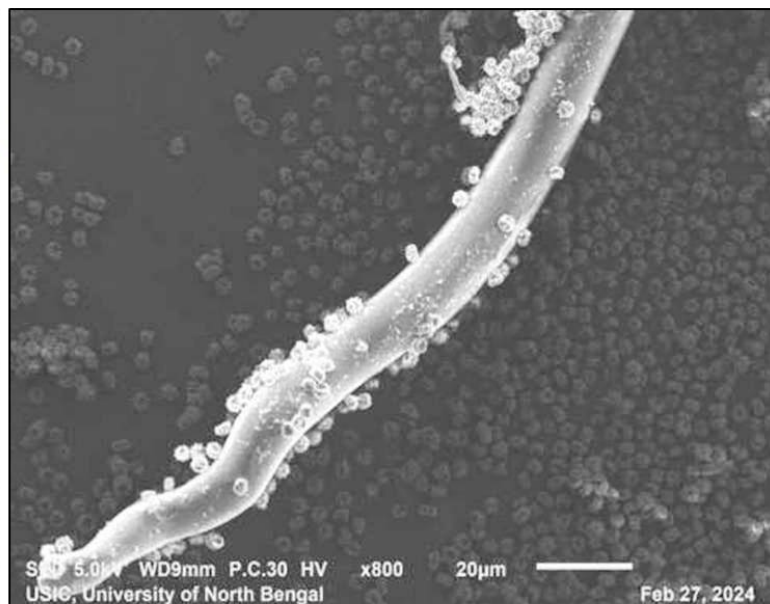
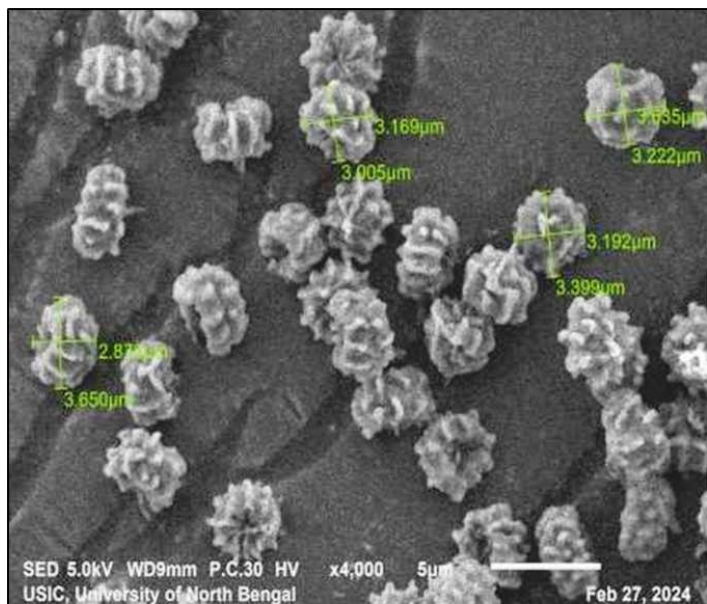


Fig. 2: Scanning Electron Microscopic view of isolated fungal isolate- MUSLF-3

Figure 2

See image above for figure legend.

Aspergillus niger isolate MUSLF 3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: OR365417.1

[FASTA](#) [Graphics](#)

LOCUS OR365417 599 bp DNA linear PLN 05-AUG-2023
DEFINITION *Aspergillus niger* isolate MUSLF 3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION OR365417
VERSION OR365417.1
KEYWORDS .
SOURCE *Aspergillus niger*
ORGANISM [Aspergillus niger](#)
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiomycetidae; Eurotiales; Aspergillaceae; *Aspergillus*; *Aspergillus* subgen. *Circumdati*.
REFERENCE 1 (bases 1 to 599)
AUTHORS Chowhan,P., Das,D. and Chakraborty,A.P.
TITLE Isolation of leaf pathogen- MUS LF 3- *Aspergillus niger* from leaf spot disease of banana
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 599)
AUTHORS Chowhan,P., Das,D. and Chakraborty,A.P.
TITLE Direct Submission
JOURNAL Submitted (31-JUL-2023) Department of Botany, Dr. Arka Pratim Chakraborty, Assistant Professor, Department of Botany, Raiganj University, College Para, Raiganj, Uttar Dinajpur, Raiganj, West Bengal 733134, India
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##

Fig. 3: Molecular Identification of fungal isolate was done by 18SrDNA sequencing, submitted to NCBI Genbank

Figure 3

See image above for figure legend.

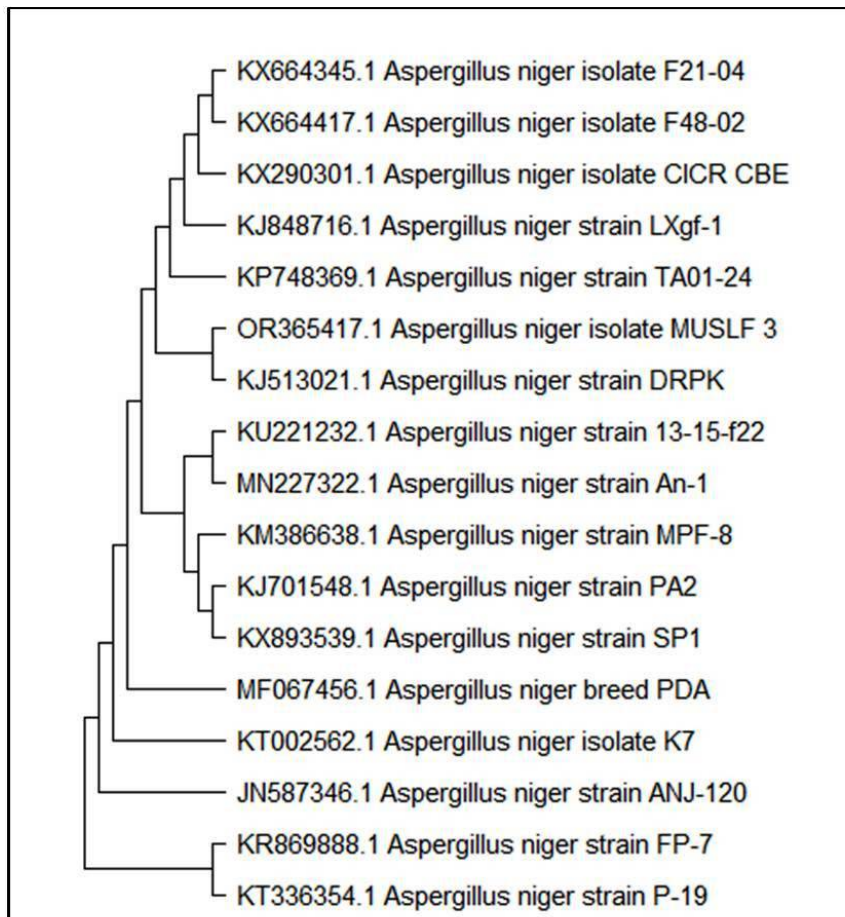


Fig. 4: Phylogenetic tree of *Aspergillus niger* (OR365417) by NJ method which is closely related to strain *Aspergillus niger* (KJ513021)

Figure 4

See image above for figure legend.



Fig. 5: Artificial inoculation on leaves of *Musa paradisiaca* with spore of *Aspergillus niger* with spore concentration of 1×10^8 spores/ml spore suspension: Healthy leaf (A); yellow-brownish spots appeared on leaves after 3 days of inoculation of spore suspension (B); Deep brownish spots appeared on leaves after 10 days of inoculation of spore suspension (C).

Figure 5

See image above for figure legend.

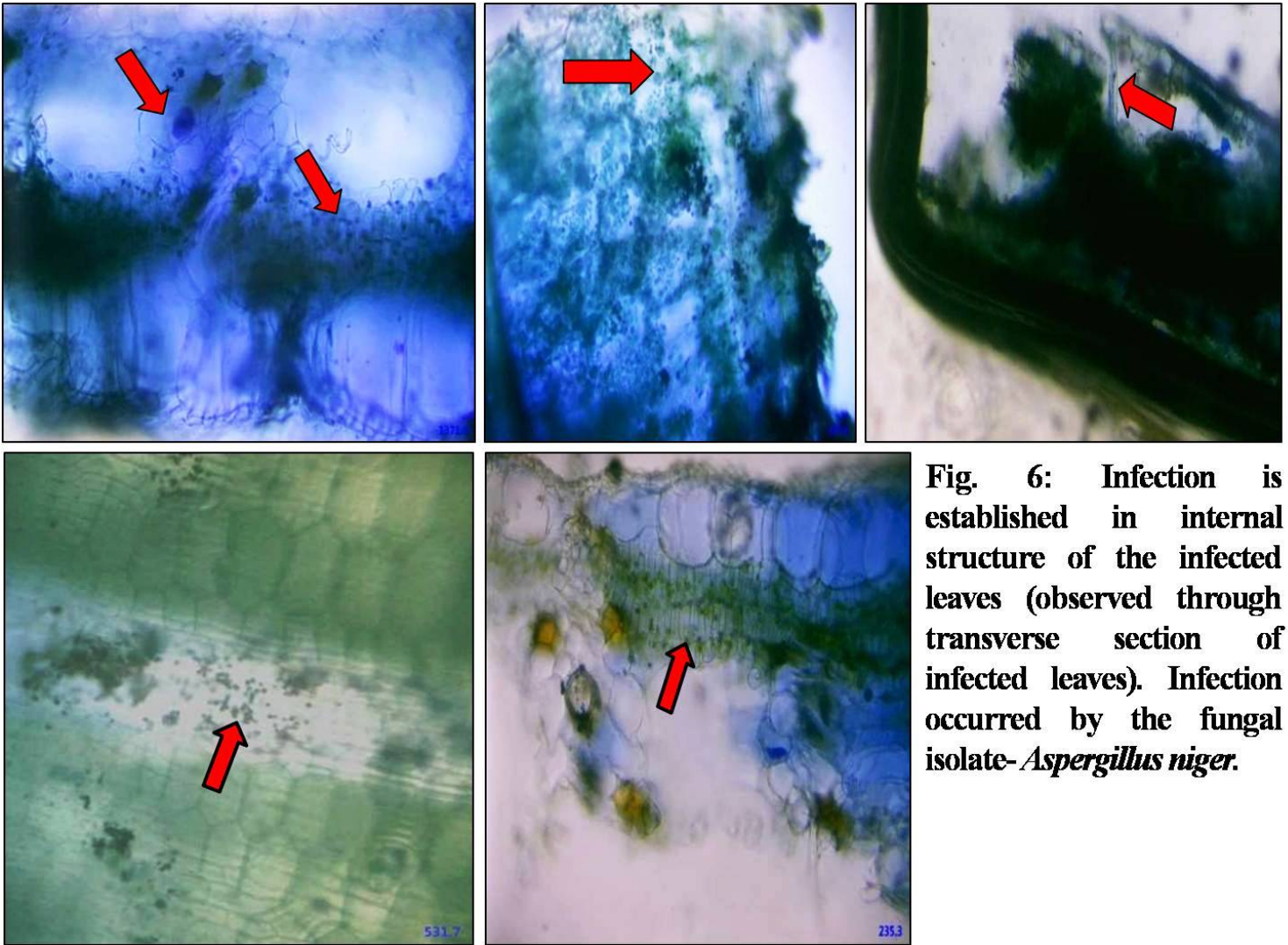


Fig. 6: Infection is established in internal structure of the infected leaves (observed through transverse section of infected leaves). Infection occurred by the fungal isolate- *Aspergillus niger*.

Figure 6

See image above for figure legend.

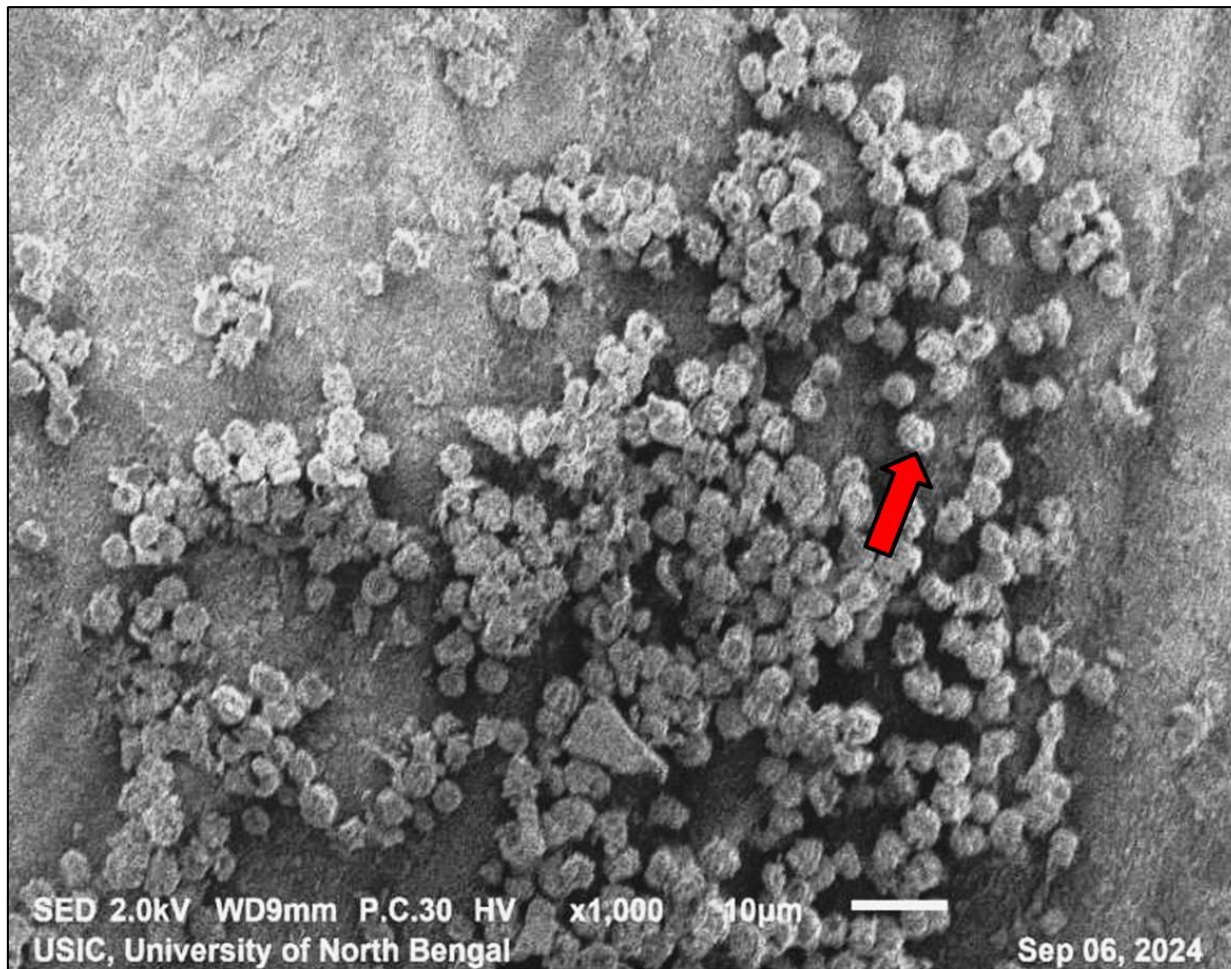


Fig.7: Existence of spore of *Aspergillus niger* observed through scanning electron microscopy on leaf surface of infected banana leaf collected from the *in vitro* study

Figure 7

See image above for figure legend.



Fig.8: Control banana plant in field (A & B) and establishment of disease- yellow brownish spots by *Aspergillus niger* on leaves of *Musa paradisiaca* in field condition (C) after inoculation of spore suspension of the *A. niger*.

Figure 8

See image above for figure legend.

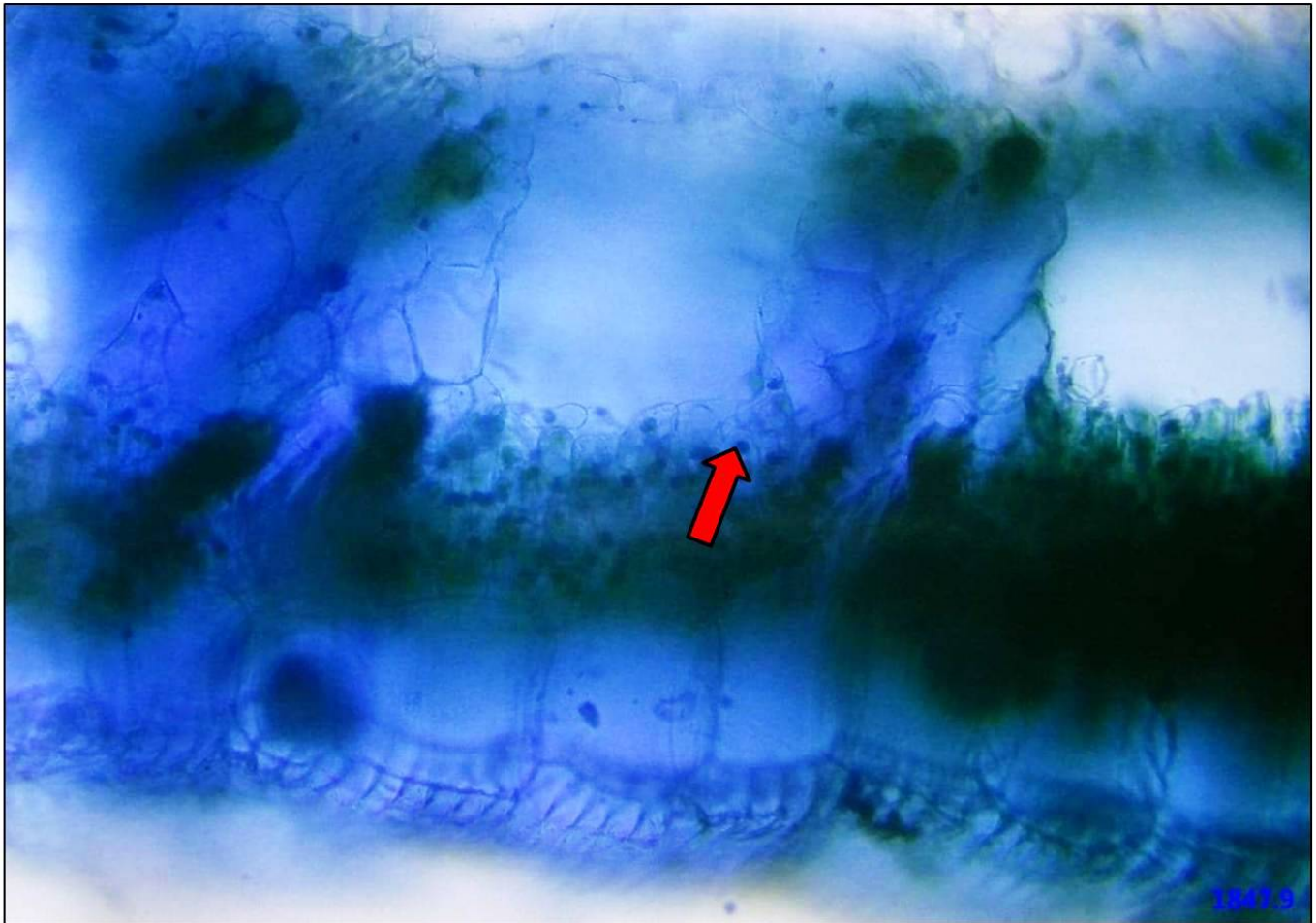


Fig. 9: Infection is established in internal structure of the infected leaves from the field condition (observed through transverse section of infected leaves). Infection occurred by the fungal isolate- *Aspergillus niger*.

Figure 9

See image above for figure legend.

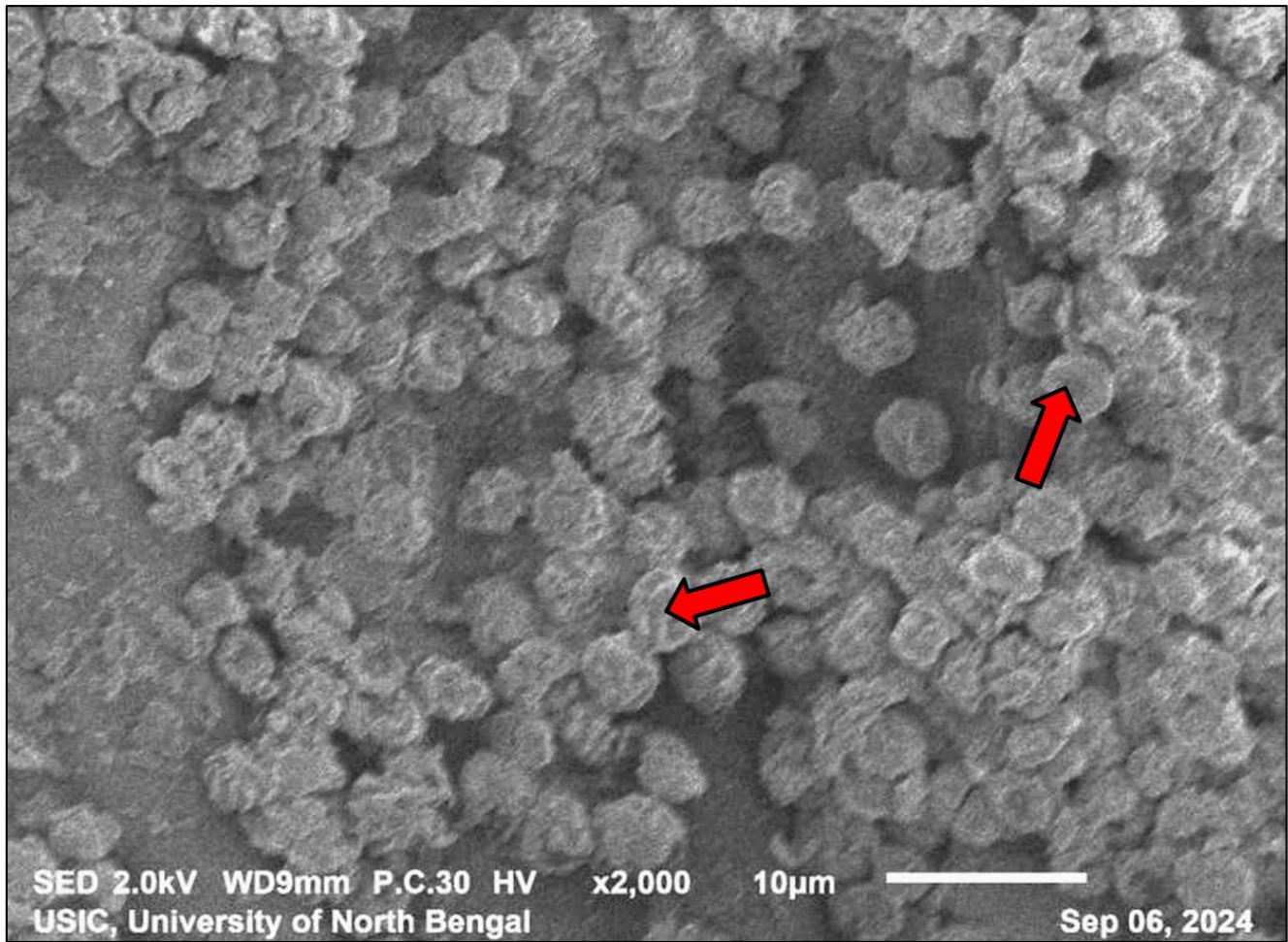


Fig.10: Existence of spore of *Aspergillus niger* through scanning electron microscopy on the surface of infected leaf of banana, collected from the field.

Figure 10

See image above for figure legend.