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Pathogenicity of sisal brown leaf spot and associated fungal species in Tanzania: A multi-site investigation

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Abstract

Sisal (*Agave sisalana*) and aloe, members of the *Agavaceae* family, are economically important crops in Tanzania, with sisal primarily grown in coastal regions and aloe distributed as wild plants. Sisal brown leaf spot (BLS) disease, initially reported in 1982, poses a significant threat to the sisal industry, impacting fiber quality and productivity. This study aimed to investigate the pathogenicity of BLS and identify the associated fungal species. Eight symptomatic sisal plants and rhizosphere soil samples were collected from farms in Morogoro, Kilosa, and Korogwe, along with one wild aloe plant from Lake Eyasi in Arusha. Conventional and molecular methods were used for pathogen isolation and identification, including colony morphology, microscopic examination, and ITS Sanger sequencing. The results revealed the presence of *Alternaria* species in both sisal and aloe samples and *Fusarium* species in soil samples. Pathogenicity tests confirmed that *Alternaria* species induce typical BLS symptoms in inoculated sisal plants, while *Fusarium* species contribute to disease severity through synergistic interactions. The study highlights the influence of environmental conditions and agronomic practices on disease incidence and severity, emphasizing the need for region-specific management strategies. This multi-site investigation provides valuable insights into the management and control of BLS disease, contributing to the development of effective control measures to enhance the productivity and sustainability of sisal plantations in Tanzania. Future research should focus on integrated disease management, soil health improvement, and collaborative efforts among stakeholders to ensure the viability of the sisal industry.

Key words: Aloe, a*ternaria; brown leaf spot; fungal pathogens; Fusarium; sisal; Tanzania*

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Introduction

Sisal (*Agave sisalana*) is a vital agricultural commodity, primarily grown for its robust fibers, which are used in various industrial applications, including ropes, mats, and agricultural twines (Saxena *et al.,* 2011). In Tanzania, sisal cultivation is a significant economic activity, contributing to both local livelihoods and national income (Kilawe *et al.,* 2018). Tanzania is one of the world's leading sisal producers, with the crop being extensively cultivated in regions such as Tanga, Morogoro, and Kilimanjaro (Terrapon-Pfaff *et al.,* 2012). However, sisal production is increasingly threatened by various diseases, among which Korogwe Leaf Spot (KLS) has emerged as a predominant concern (Kimaro *et al.,* 1994).

Korogwe Leaf Spot, characterized by the appearance of brown, necrotic lesions on the leaves, can significantly impair the photosynthetic capacity of the plant, leading to reduced growth and lower fiber yield (Esh and Taghian, 2022). This disease not only affects the quantity of the sisal fibers but also compromises their quality, thereby impacting the overall profitability of sisal cultivation (Xie *et al.,* 2021). The etiology of KLS is complex, often involving multiple fungal pathogens that synergistically or independently infect the plant tissues (Lamichhane and Venturi, 2015).

The etiology of KLS disease remains inadequately understood, with limited comprehensive studies detailing its pathogenicity and the specific fungal species involved (Tadesse *et al.,* 2008). Previous reports have suggested the involvement of various fungal pathogens, and others have been reported to be caused by a virus, but a definitive understanding of their roles and interactions is lacking (Quintanilha-Peixoto *et al.,* 2021). Given the critical role of sisal in Tanzania's agricultural sector and the substantial impact of brown leaf spot (BLS) disease on fiber quality and yield, it is imperative to elucidate the pathogenic mechanisms and identify the species responsible for this disease (Mpunami, 1987)).

On the other hand, the spatial heterogeneity of environmental conditions across sisal-growing regions in Tanzania, it is imperative to conduct multi-site investigations to comprehensively understand the pathogenicity and prevalence of BLS and its associated species. Such studies are essential for identifying region-specific disease dynamics and developing tailored management strategies. A multi-site approach also facilitates the assessment of the impact of different agronomic practices and environmental variables on disease incidence and severity.

This study aims to fill this knowledge gap by conducting a thorough investigation into the pathogenicity of sisal brown leaf spot and identifying the BLS disease-causing organisms. The findings of this study will contribute to the development of effective control measures, enhancing the productivity and sustainability of sisal plantations in Tanzania in addition to enabling the formulation of targeted strategies for disease management. This research also underscores the importance of collaborative efforts among stakeholders, including farmers, researchers, and policymakers, to safeguard the future of the sisal industry.

Materials and methods

Sample Collection

To investigate the pathogenicity of sisal brown leaf spot (BLS) and identify the fungal species involved, sampling was conducted on the fields with typical symptoms of BLS. In September 2023, eight symptomatic sisal plants and corresponding rhizosphere soil samples were collected from eight different sisal farms located in Morogoro, Kilosa, and Korogwe. Additionally, one wild aloe plant with symptoms similar to BLS was sampled from Lake Eyasi in Arusha to assess potential cross-host pathogenicity. Each plant sample exhibited clear symptoms of brown leaf spot disease, characterized by dark spots and lesions on the leaves.

Sample processing and macroscopic description

On arrival to the laboratory soil samples were weighed and the plant samples were checked for the presence of vivid symptoms, signs or any abnormality from roots to leaf tip. Using the reference books and guides, the collected specimens were characterized based on the observable symptoms and features. During the process cross sections were made where necessary to check for any abnormalities on vascular bundles. All samples were photographed for reference of observed symptoms and signs.

Microscopy of Leaf Samples

All plants with disease symptoms were chosen for microscopy. From these samples, a tape mount was made by sticking a sticky part of clear sellotape on a lesion or area with symptomatic tissue. The tape was then mounted on a slide prefilled with a drop of lactophenol cotton blue stain. The slides were blotted for excess stain and placed on the compound microscope stage for observations. A compound light microscope (Leica ATC 2000, Leica Microsystems, Germany) with magnifications ranging from 100x to 1000x was used for specimen observations. The slide was scanned systematically to locate and study the Morphology of fungal structures such as hyphae, conidia, and sporangia. References Identification key was used for comparisons and identification of pathogen conidia/spores, mycelia or any other fruiting bodies Photographs of notable structures were taken and detailed sketches were made. The spore measurements were made and calibrated using the micrometer slide.

Pathogen Isolation

Potato dextrose agar (PDA) amended with 100mg of streptomycin was used as an isolation medium for fungi in both collected symptomatic plant leaf tissue and soil samples. For plant leaf tissue 2 mm-sized pieces of diseased tissue were chopped from lesion margins, sterilized with 70% alcohol for 1 min and rinsed with sterile distilled water. The sterile tissue was cultured onto sterile media and incubated at 25 °C for 7 days. Concurrently, rhizosphere soil samples were processed using soil dilution plating techniques on PDA to isolate soil-borne fungal pathogens. Emerging fungal colonies were subcultured onto fresh PDA plates to obtain pure cultures. This was achieved by transferring hyphal tips from the edge of growing colonies to new PDA plates, ensuring single-species growth. The purified isolates were incubated under the same conditions to obtain clean, uncontaminated fungal pure cultures.

Molecular Identification

For molecular identification, DNA was extracted from the purified fungal isolates using a commercial fungal DNA extraction kit (Quick-DNA fungal/bacterial kit (Zymo Research Corp. CA, United States) following the manufacturer's protocol. The extracted DNA was then subjected to polymerase chain reaction (PCR) amplification using fungal Internal Transcribed Spacer (ITS) primers, specifically ITS1 and ITS4 (White *et al.*, 1990). The PCR reactions were carried out using 2× OneTaq Quick Load Mastermix (New England BioLab) in a thermocycler (Agilent SureCycler 8800) under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes. The PCR products were subjected to gel electrophoresis using 1% agarose pre-stained with ethidium bromide. The gel was run at 120V for 1hr and viewed under UV light.

Sanger Sequencing and Bioinformatics Analysis

The positive PCR amplicons were purified using a PCR purification kit (DNA cleanup kit: DNA Clean & Concentrator (Zymo Research; Irvine, CA, USA) and subsequently sent to the Macrogen Europe facility for Sanger sequencing. The sequencing was performed using both forward and reverse ITS primers to ensure complete coverage of the ITS region. The resulting sequences were Bioinformatically analyzed using MacVector with Assembly Software Version 18.6 and clean sequences were blasted in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) to identify the fungal species that has the highest hit with the query sequence. Multiple sequence alignment was further carried out to observe variations in nucleotide arrangements and percentage similarities. Phylogenetic trees were constructed using Mega XI software set at 1000 bootstraps maximum Likelihood factors to learn the evolutionary inferences of the sisal-isolated fungi against the reference sequences obtained from the NCBI.

Pathogenicity Test

Pathogenicity tests were conducted to confirm the role of the identified fungal species in causing brown leaf spot disease. Healthy sisal plants were inoculated with spore suspensions of the purified fungi isolated from the plant and soil samples. The complete randomized pot experiment with five treatments was used to set up this experiment. Each of the treatments consisted of five seedlings that served as a rep making a total of 25 seedlings for the whole experiment. In the experiment, eight weeks old sisal seedlings were inoculated with 10^8 inoculum suspensions made from ten days old pure culture isolated fungi namely *Alternaria*, *Fusarium*, *Aspergillus* and *Penicillium* respectively. The control plants were inoculated with plain sterile water that was used to prepare the inoculum. Inoculated plants were raised in a screen house and observed for 8 weeks for classical lesion formation and symptoms. The disease and incidence were recorded once weekly. The incidence was done by recording the number of plants in the experiment and number of diseased plants in the experiment while severity was done by comparing the size of the lesion with the key by Perina *et al.* (2019) that has a 0-8 scale whereby 0 means no disease and 8 means up to 50% severity.

Data Analysis

The colony morphology of the purified isolates was documented, and a microscopic examination was performed to observe spore and hyphal structures. The sequence data obtained from Sanger sequencing were analyzed using bioinformatics tools to confirm the identity of the fungal species using MacVector and Mega XI software as explained above. The combination of morphological characteristics and molecular data provided a comprehensive identification of the pathogens involved in sisal brown leaf spot disease.

Results

Macroscopic Observation of Collected Samples

The sisal leaves exhibited small to large raised dark brown spots, which coalesced in some areas to form randomly distributed lesions across the leaf surface (Figure 1). The lesions displayed concentric rings, predominantly on the upper leaf surface, and lacked a halo at the margins. Some leaves had fewer lesions on the lower surface. Aloe leaves presented similar symptoms, although the lesions were smaller, more raised, and differed in shape. The stems, roots, and crowns showed no signs of necrosis, and cross-sections revealed no browning of the vascular bundles. However, the bulbils exhibited pale vascular bundles with some brown streaks.

Figure 1

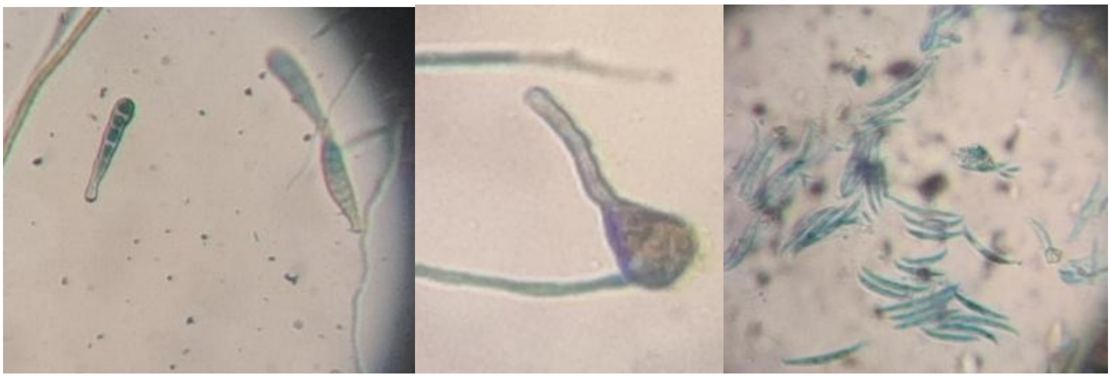
Symptomatic leaf with raised lesions with concentric rings, the lesions were distributed on the entire leaf (left) sisal and aloe plant (right)

Microscopy: Symptomatic Plant samples

Examination of leaf lesions revealed septate, olive-brown, oval-shaped conidia with short to medium-sized beaks, containing 5 to 8 septa per conidium. These conidia were surrounded by brown septate hyphae. In bulbils, single-celled and septate (2-6) spindle-shaped conidia with curved ends were observed, resembling *Fusarium* species (Figure 2).

Figure 2

Micrograph showing slender conical-shaped conidia with short beak (left), vase-shaped conidia with medium-sized beak (center) and septate spindle-shaped conidia (right). (left) from sisal leaf (center) from aloe leaf (right) from bulbil lesions

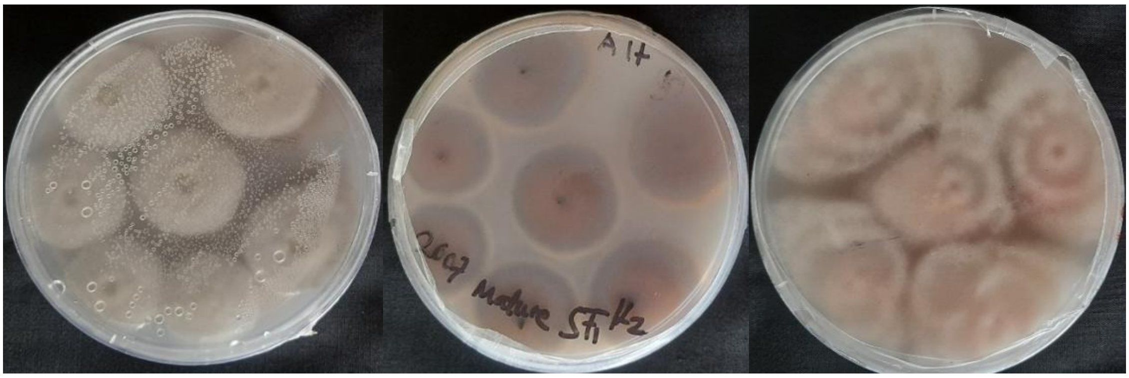


Fungal Cultures Colony Morphology

Cultures from sisal and aloe leaf samples exhibited light grey, fluffy fungal colonies with black-margined bottoms and deep grey colonies with completely black bottoms. These colonies were irregular in shape with button-like centers, measuring 1 to 2 cm in diameter. The bulbils displayed white, fluffy colonies isolated from tissue taken from the collar area, characterized by a cotton-like texture and a salmon-pink color, particularly at the centers (Figure 2) Fungi isolated from rhizosphere soil samples exhibited circular, white-margined green colonies and irregularly shaped black colonies with pinhead and fluffy surfaces. The green colonies with white margins were small and dominated the plates, while the black colonies were fewer. White, fluffy cotton-like colonies with patches of a salmon pink color were also isolated from rhizosphere soil (Figure 3

Figure 3

Pure colonies of fungi isolated from sisal/aloe leaves, the photos show the upper and lower look of the isolated colonies and purified white colonies isolated from soil and bulbils

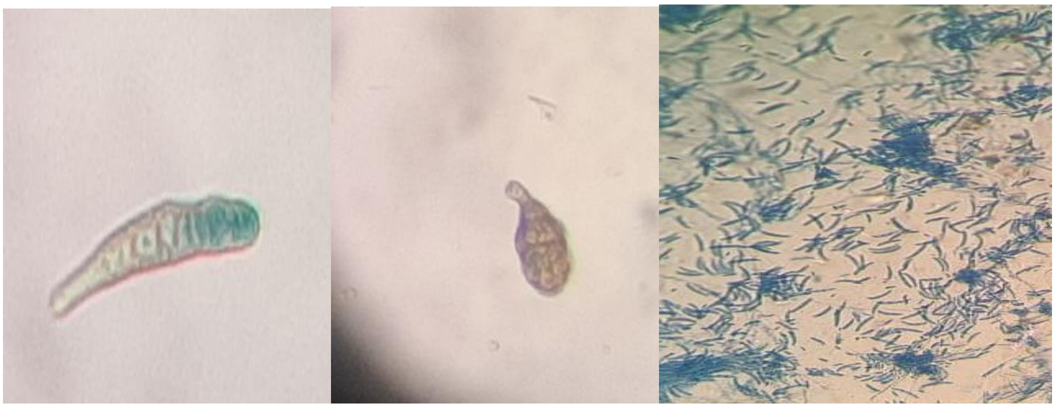


Microscopic Observation

Colonies isolated from leaves displayed oval, olive-brown, slender septate conidia with short beaks and five to seven septa (Figure. 4), consistent with characteristics of *Alternaria* species. Colonies from bulbils and rhizosphere soil revealed, single-celled and septate (2-6) spindle-shaped conidia with curved ends were observed, resembling *Fusarium* species. Microscopic examination revealed olive-brown, short-beaked, conical-shaped conidia (Figure. 4)

Figure 4

Olive-brown short-beaked conical-shaped conidia from sisal leaf lesion isolates and spindle-shaped macro-conidia from bulbils and soil-isolated cultures



Proof of Pathogenicity

After 8 weeks of observation, classical lesions with concentric rings were observed on seedlings inoculated with *Alternaria* species from both sisal and aloe (Figure 5). These lesions were small, irregularly shaped, and surrounded by halos. Seedlings inoculated with other fungi did not exhibit any symptoms or changes. The first symptoms appeared two weeks post-inoculation, with an average incidence of 20% (20 out of 100 seedlings) and severity of 2% (2 out of 100 seedlings). The symptoms did not intensify or increase in number by the end of the 8 wee

Figure 5

Symptomatic sisal seedlings showing brown spots in Alternaria inoculated sisal seedlings. The photos were taken 3 weeks and eight weeks post-inoculation respectively

PCR and Sequencing

The PCR amplicons produced the expected base pair sizes for ITS1 and ITS4 regions, mapping to reference ladders at 570 bp for *Alternaria* species and 540 bp for *Fusarium* species. Bioinformatics analysis of the sequenced PCR amplicons confirmed the presence of *Alternaria and Fusarium* species in both sisal and aloe leaves.

Sequences from *Fusarium* isolates from sisal (TZA10) and aloe (TZA08) were aligned with 24 different *Fusarium* species sequences that had the highest hits on the NCBI database based on e-value. The nucleotide similarity among these aligned *Fusarium* species with the sequences of isolates TZA10 and TZA08 ranged from 99.8% to 100% (Supplementary Figure 1a and Figure 1b). The phylogenetic tree indicated a nucleotide sequence divergence of 0.00050 in the ITS region for isolates from sisal and aloe plants in Tanzania (Figure 6). Similarly, the *Alternaria* species from Tanzania (TZA13) were aligned with 22 sequences of different *Alternaria* species retrieved from the NCBI database, showing nucleotide similarity ranging from 99.8% to 100%, with a divergence of 0.00020 based on phylogenetic analysis Figure7.

Figure 6

A phylogenetic tree depicting the evolutionary relationship of the Internal Transcribed Spacer (ITS) region for Fusarium species isolates collected in Tanzania (TZA8 and TZA10) about other published related species from the NCBI database

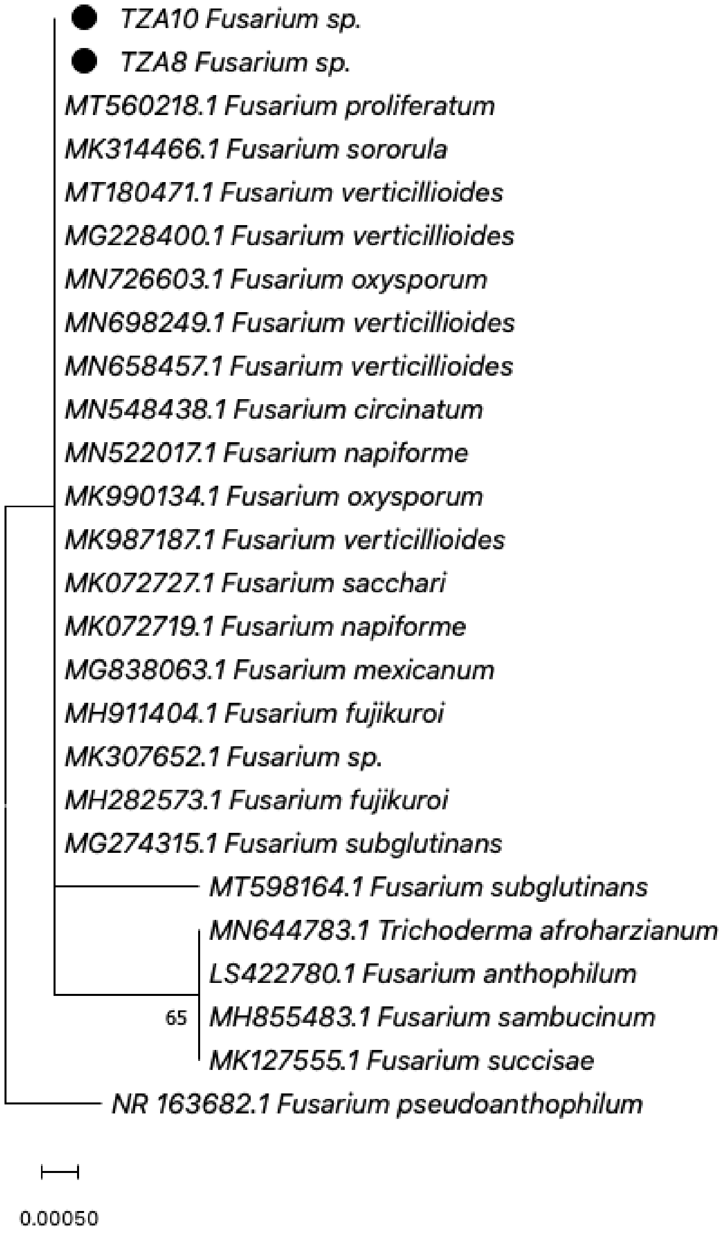
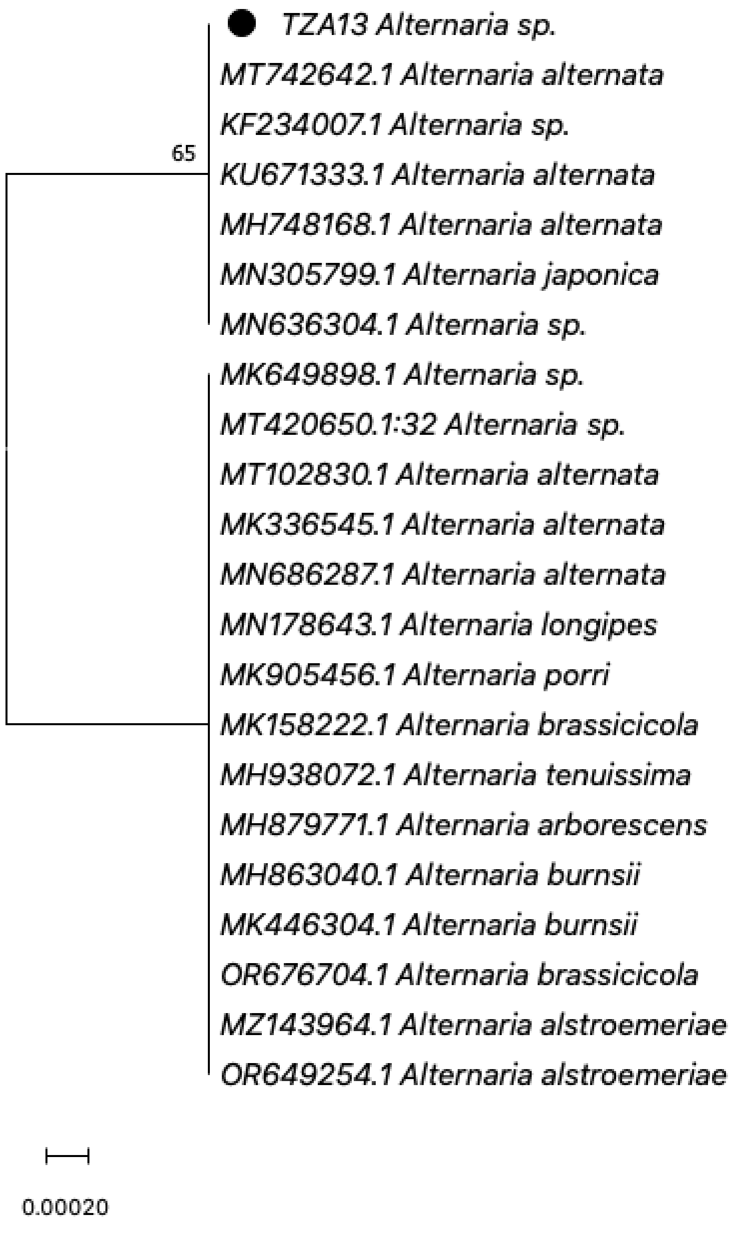


Figure 7

A phylogenetic tree depicting the evolutionary relationship of the Internal Transcribed Spacer (ITS) region for Alternaria species isolates collected in Tanzania (TZA13) concerning other published related species from the NCBI database



Discussion

The findings of this study provide significant insights into the pathogenicity of brown leaf spot (BLS) disease affecting sisal (*Agave sisalana*) and aloe plants in Tanzania. The isolation and identification of *Alternaria* and *Fusarium* species as primary pathogens underscore the complexity of the disease etiology. These fungi were consistently associated with symptomatic plants across different geographical locations, indicating their widespread prevalence and critical role in disease manifestation.

The morphological and molecular characterization of fungal isolates revealed the presence of Alternaria species in both sisal and aloe samples, and *Fusarium* species in soil samples. This identification aligns with previous reports implicating *Alternaria* and *Fusarium* as key pathogens in various plant diseases (Lamichhane and Venturi, 2015). The pathogenicity tests confirmed that *Alternaria* species induce typical BLS symptoms in inoculated sisal plants, while *Fusarium* species contribute to disease severity through synergistic interactions.

The study bridged the gap on the exact cause of brown spots in sisal, previously speculated to be viral by Keswani and Mwenkaley (1982), and unresolved doubts from Mtung'e *et al.* (2014). The present findings provide definitive evidence that *Alternaria* spp. cause the brown spots, negating earlier virus-related theories (Keswani and Mwenkaley, 1982; Mtung'e *et al.,* 2014).

BLS disease significantly affects the quality and productivity of sisal fibers. The lesions caused by *Alternaria* species reduce the photosynthetic efficiency of the plants, leading to stunted growth and lower fiber yield. Similar impacts on aloe plants suggest a broad host range for these pathogens, posing a challenge for disease management (Xie *et al.,* 2021). The presence of *Fusarium* species in the soil complicates control measures, as these fungi can persist in the environment and infect new plants.

The multi-site approach of this study highlights the influence of environmental conditions and agronomic practices on disease incidence and severity. Variations in disease prevalence across different regions suggest that local climate, soil properties, and crop management strategies play crucial roles in disease dynamics. This finding emphasizes the need for region-specific disease management practices to effectively control BLS in diverse agroecological zones (Terrapon-Pfaff *et al.,* 2012).

Pathogenicity studies indicated that while *Alternaria* spp. caused brown spots in the sisal, the disease progression was limited. Factors such as weather, soil edaphic factors, and microbiomes might contribute to this limitation. For disease manifestation, a susceptible host, virulent pathogen, and conducive environment are essential. The observed symptoms in inoculated plants indicate a virulent pathogen and susceptible host, but the lack of symptom progression suggests non-conducive environmental conditions.

Conclusion

This study elucidates the pathogenic mechanisms of brown leaf spot disease in sisal and aloe plants, identifying *Alternaria* and *Fusarium* species as primary pathogens. The multi-site investigation underscores the complexity of the disease and the need for integrated management strategies. The findings contribute to a deeper understanding of BLS and provide a foundation for developing effective control measures to enhance the productivity and sustainability of sisal plantations in Tanzania.

References

Saxena, M., Pappu, A., Haque, R., & Sharma, A. (2011). Sisal fiber-based polymer composites and their applications. Cellulose Fibers: Bio- and nano-polymer composites: *Green chemistry and technology. 589-659*

Kilawe, C. J., Mertz, O., Birch-Thomsen, T., & Maliondo, S. M. (2018). Transformation of shifting cultivation: Extent, driving forces and impacts on livelihoods in Tanzania. *Applied Geography. 94: 84-94*

Terrapon-Pfaff, J. C., Fischedick, M., & Monheim, H. (2012). Energy potentials and sustainability the case of sisal residues in Tanzania. *Energy for sustainable development. 16(3): 312-319*

Esh, A., & Taghian, S. (2022). Etiology, epidemiology, and management of sugar beet diseases. In *Sugar beet cultivation, management and processing* 505-540 pp. Singapore: Springer Nature Singapore

Xie, H. H., Long, L. Y., Huang, S., Mao, L. Y., Huang, Q. W., Wang, L. P., & Li, J. X. (2021). The first report of black spot caused by *Neoscytalidium dimidiatum* on sisal in Guangxi, China. *Plant Disease. 105(3): 701-701*

Kimaro, D., Msanya, B. M., & Takamura, Y. (1994). Review of sisal production and research in

Tanzania. *African Study Monographs. 15(4): 227-242*

Lamichhane, J. R., & Venturi, V. (2015). Synergisms between microbial pathogens in plant disease complexes: a growing trend. *Frontiers in plant science*. *6*: 1410-1448

Mpunami, A. (1987). Purification and partial characterization of a virus associated with korogwe leaf spot disease of sisal. (MSc. Thesis in Botany and Plant Pathology of Oregon State University). 124 pp

Quintanilha-Peixoto, G., Fonseca, P. L. C., Raya, F. T., Marone, M. P., Bortolini, D. E., Mieczkowski, P., & Aguiar, E. R. G. R. (2021). The Sisal virome: Uncovering the viral diversity of agave varieties reveals new and organ-specific viruses. *Microorganisms*. *9*(8): 1704

Tadesse, A., Ayalew, A., Getu, E., & Tefera, T. (2008). Review of research on post-harvest pests.In *Increasing crop production through improved plant protection*, Pp 475-562. Tadesse, A. (ed.). Plant Protection Society of Ethiopia (PPSE), 19-22 December 2006. Addis Ababa, Ethiopia. PPSE and EIAR, Addis Ababa, Ethiopia

White T. J., Bruns T., Lee S., & Tailor S. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics PCR protocols. *A guide to methods and applications.* Innins M. A., Gelfand D. H., Sninsky J. J., and White T. J.(eds). 315 -322pp. Academic Press, Inc. San Diego, Califonia

Ferrer, C., Colom, F., Frasés, S., Mulet, E., Abad, J. L., & Alió, J. L. (2001). Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8S ribosomal DNA typing in ocular infections. *J. of clinical microbiology. 39(8): 2873–2879.* https://doi.org/10.1128/JCM.39.8.2873-2879.2001

Perina, F. J., Belan, L.L, Moreira, S., Nery, E.M., Alves E. & Pozza, E. A, (2019) Diagrammatic scale for assessment of *Alternaria* brown spot severity on tangerine leaves. *J. of Plant Pathol.101:981–990*. https://doi.org/10.1007/s42161-019-00306-6