

Morphological and Molecular Characterization of Fungi Associated with Banana Leaf Spot Symptoms in Kenya

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Abstract

Banana production is threatened by numerous biotic and abiotic constraints, with pests and diseases as the major problems affecting it globally. Fungal diseases are very devastating and can cause up to 100% loss. This study aimed to address the lack of data on fungal diseases of bananas in Kenya's major producing zones (Kisii, Meru, and Taita Taveta) by studying the morphological and molecular diversity of fungi associated with banana leaf spot symptoms. Samples were collected from symptomatic banana leaves and sent for laboratory analysis at the International Livestock Research Institute in Nairobi. Fungi associated with banana leaf spot symptoms were isolated on Potato Dextrose Agar and characterized based on morphological features. Molecular analysis was performed by extracting DNA from the fungal isolates and amplifying the Internal Transcribed Spacer region. Sanger sequencing was performed on the extracted DNA templates, and the sequences were compared against the National Centre for Biotechnology Information GenBank database using the Basic Local Search Tool to confirm the species identity of the isolated fungi. Morphological characterization revealed *Fusarium* spp. as the most prevalent in Taita Taveta and Kisii, while *Alternaria* spp. dominated in Meru County. Out of 45 isolates tested, 29 yielded polymerase chain reaction products, 20 were selected for sequencing, and 15 yielded high-quality sequences for BLAST analysis. *Alternaria alstroemeriae* emerged as the most frequently detected fungal species among the sequenced isolates. Phylogenetic analysis confirmed the genetic diversity of the isolates and their taxonomic placement. These findings provide important information on the diversity and distribution of fungi associated with banana leaf spot symptoms in Kenya. However, the pathogenicity of these isolates was not confirmed, and further studies are required to confirm their role in banana leaf spot disease.

Categories: Agricultural and food economics, Plant-pathogen interaction, Disease & pests of crops

Keywords: molecular identification, fungi isolation, morphology, plant pathology, phylogenetic analysis, sequencing

Introduction

Banana (*Musa acuminata* Colla) is one of the most valued fruit crops in the world, ranked fourth among the most cultivated and consumed foods globally after rice, wheat, and maize [1]. With an annual global production of 155 million metric tonnes [2], bananas rank as the second largest fruit crop in the world. Over the years, they have held significant positions in agricultural production across the continent. Africa is one of the world's top banana-producing regions, with

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Tanzania, Uganda, Cameroon, Kenya, and Côte d'Ivoire leading the way [3]. Bananas are considered the most valuable fruit globally, primarily as a sweet dessert crop; however, in some regions, such as Eastern Africa, they are consumed as a starchy staple [4], underscoring their significance to local markets and food security. In Kenya, bananas (*Musa* spp.) play a vital role in food security, serving as a staple food and a key source of income for smallholder farmers [5].

Despite the economic and health benefits of bananas, their production has faced numerous constraints over the years. Banana production is affected by both biotic and abiotic factors, which have significantly reduced the average yield. Diseases and pests are the main factors that directly affect banana production, reducing the quality and quantity of the yield. Bacterial diseases such as banana *Xanthomonas* wilt (*Xanthomonas campestris* pv. *Musacearum*) and bacterial wilt (*Ralstonia solanacearum*) are becoming more common worldwide, decreasing productivity and increasing crop management expenses [6]. Pathogenic fungi and diseases are among the major biotic factors that cause serious crop diseases and, as a result, a decline in crop yield quantity and quality [7]. Black Sigatoka disease, caused by the fungus *Pseudocercospora fijiensis*, produces characteristic black streaks on banana leaves [8]. Improper or delayed diagnosis of diseases contributes to disease pandemics.

Over the past century, the spread of pathogens has resulted in significant disease problems in banana production [9]. The most destructive and limiting fungal diseases worldwide are *Fusarium* wilt, caused by the soil-borne fungus *Fusarium odoratissimum* (syn. *F. oxysporum* tropical race 4), and the Sigatoka disease complex, which is composed of three *Pseudocercospora* spp: *Pseudocercospora musae* (Yellow Sigatoka), *P. fijiensis* (Black Sigatoka), and *P. eumusae* (Eumusae leaf spot) [10]. These diseases are a serious threat to banana production worldwide because they reduce the photosynthetic capacity of plants through necrotic leaf lesions, which results in reduced crop yield and fruit quality [11].

More than 40% of banana crops consist of a single variety that is dependent on global markets for export, making bananas more vulnerable to rapidly spreading pathogens [9]. For instance, in the East African Region, the first report of Sigatoka was in the islands of Pemba, Tanzania, in 1987; afterwards, the disease rapidly spread to neighboring countries in the Lake region of Africa [12]. It is a heterothallic disease, producing conidia throughout the year, making it polycyclic and capable of causing multiple infections in a banana cycle, leading to substantial leaf damage and more than 50% yield loss [13]. *Fusarium* wilt is a highly devastating soil-borne disease found in all banana-producing regions of Sub-Saharan Africa [14]. Other important fungal diseases of bananas include root and rhizome rot, caused by the nematode *Radopholus similis*, often in association with the borer *Cosmopolites* spp. [15], and postharvest diseases like anthracnose, cigar end rot, stem end rot, and ripe rot, which contribute to approximately 25-30% postharvest losses in banana production [16]. Therefore, a proper understanding of the pathogenicity, host range, and modes of transmission of pathogens is very important for disease management and global food biosecurity [17]. The present study focused on identifying the pathogenic fungi affecting banana production in Kenya and documenting their morphogenic diversity.

Symptomatology and morphology are the most common methods for fungal identification used by farmers and scientists in Kenya. However, morphological identification alone can sometimes be inefficient, especially when identifying closely related genera [18]. Identification of pathogenic species based solely on morphological traits can lead to misidentifications due to phenotypic plasticity [17]; thus, the use of molecular techniques alongside morphological traits provides more accurate fungal identification during disease diagnosis. In addition, molecular identification techniques are highly specific and complement morphological approaches, as they rely on variations in Deoxyribonucleic Acid (DNA) sequences among closely related species and have been applied to fungal identification since the 1990s [19]. Genomic sequences of the short subunit (SSU), the long subunit (LSU), and the Internal Transcribed Spacer (ITS) have been widely used in fungal identification. Although ITS generally exhibits greater sequence variation than SSU and LSU, making it more suitable for species-level discrimination, its discriminatory power is limited in some fungal genera due to low interspecific variation, intragenomic variation, and high sequence conservation among closely related species [19]. Nevertheless, over the years, the ITS operon has been identified as a potential primary DNA barcode marker, owing to its ease of amplification and the abundance of reference sequences available in the GenBank [18].

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The present study assessed the morphological and molecular identities of fungi associated with banana leaf spot symptoms in major cultivation areas in Kenya using the nuclear ribosomal ITS of the rDNA. This is the most commonly used molecular marker for fungal identification, serves as the universal barcode endorsed by the Consortium for the Barcode of Life, and is the most frequently used marker in environmental DNA (eDNA) studies for assessing fungal diversity [20]. The ITS gene consists of repetitive, noncoding DNA sequences within the coding regions of ribosomal RNA genes and can reliably distinguish fungal taxa to the species level [21]. A key advantage of the ITS region is that organisms possess multiple paralogous copies, which enable effective amplification even from low-DNA samples like eDNA or unculturable fungi. Moreover, the ITS region's structure, comprising conserved regions flanked by highly variable regions, makes it ideal for designing reliable polymerase chain reaction (PCR) primers that target a wide range of fungal taxa [20]. This study encourages integration of both morphological and molecular techniques for accurate disease diagnosis.

Materials And Methods

Isolation of fungi from banana leaves

Fungi were isolated from banana leaves as previously described by Alsohaili et al. [22]. Infected banana leaves were cut into 5 × 5 mm pieces, disinfected by soaking in 1% sodium hypochlorite for 1-2 minutes, rinsed three times in sterile distilled water, and then blotted dry on sterile filter paper. Potato Dextrose Agar (PDA) medium was prepared by adding 49 g of PDA to 1000 ml of distilled water, autoclaved for 15 minutes, and dispensed into Petri dishes under sterile conditions. The banana pieces excised from symptomatic leaves were placed on PDA medium, sealed with parafilm, and incubated at 28°C for 7 days. After initial isolation of fungal colonies, single, well-isolated colonies were selected for subculturing to obtain pure cultures. A sterile inoculation needle was used to scoop a section of the fungal growth from the edge of the colony, which was then transferred to PDA under sterile conditions and incubated at 28°C for 7 days.

Morphological identification

Morphological identification of the isolates was done as previously described by Alsohaili et al. [22]. The isolates were observed to exhibit mycelial colours, textures, growth patterns, and sizes. A sterile scalpel was used to scrape a small portion of fungal mycelium and place the sample on a glass slide. This was followed by adding 1-2 drops of distilled water, gentle mixing, and then placing a coverslip to avoid bubbles. The mycelium was observed under the microscope at 400× magnification (40× objective lens × 10× eyepiece). The mycelia were observed to distinguish the hyphae size and septation, and the arrangement, shape, and size of the spores.

DNA extraction

The total DNA was isolated using the modified Cetyltrimethylammonium bromide (CTAB) protocol. Pure fungal isolates were grown on PDA for 7-10 days at ~25°C, and 100 mg of mycelium was scraped and placed into a 1.5 mL microcentrifuge tube. Liquid nitrogen was added to dry the mycelia, and the mycelia were ground into a fine powder. A total of 500 µL of pre-warmed CTAB buffer was vortexed, then incubated at 65°C for 30 minutes, with tubes inverted every 10 minutes. In all, 500 µL of chloroform: isoamyl alcohol (24:1) was added, and the mixture was inverted for 5 minutes. The mixture was centrifuged at 12,000 rpm for 10 minutes at 4°C, and then the upper aqueous phase (~400 µL) was carefully transferred into a new tube. The supernatant was discarded after centrifugation without disturbing the pellet, and 50 µL of 70% ice-cold distilled ethanol was added to the pellet, which was then centrifuged for 5 min at 12,000 rpm. The supernatant was discarded, and the pellet was air-dried. The dried pellet was dissolved in 40 µL of 0.1X TE buffer and kept at 65°C for 3 minutes (to suspend the pellet). The suspended DNA sample was stored at -20°C.

DNA amplification

The extracted DNA samples were used as templates for PCR amplification. The ITS region of the nuclear ribosomal DNA (rDNA), the formal barcode for molecular identification of fungi [23], was used. DNA amplification of the ITS region was carried out using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers in a total reaction volume of 20 µL. The PCR mixture consisted of 1-2 µL of DNA template, 0.4 µL of each forward and reverse

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primer (ITS1 and ITS4, respectively), 0.8 µL of dNTPs, 2.0 µL of 10× PCR buffer, 1.2 µL of 50 mM MgCl₂, 0.2 µL of Taq DNA polymerase (0.5 U/µL), and 14 µL of nuclease-free water. The PCR amplification was performed with the following thermal cycling conditions: an initial denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55–60°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 5 minutes, and the reaction was held at 4°C until further analysis. The samples were stored at -20°C, awaiting gel electrophoresis.

Agarose gel electrophoresis was used to assess the fragment length of DNA extracts qualitatively. A total of 1 g of agarose powder was added to 100 mL of 1X TAE buffer and heated in a microwave for 2 minutes in short bursts (20–30 seconds) until completely dissolved. The solution was cooled to ~50–55°C, EtBr (0.5 µg/mL) was added, mixed gently, and then poured into the tray. Combs were inserted to create wells, and the gel was allowed to solidify for 40 minutes at room temperature. The tank was filled with enough 1X TAE buffer; 5 µL of DNA ladder was loaded in one well for size reference, and 5 µL of PCR product was loaded. Electrodes were connected; the voltage was set to 80–100 V, and the run was 40 minutes. The PCR bands were observed under the UV transilluminator.

Genetic diversity and phylogenetic analysis of the fungal isolates

DNA templates were subjected to Sanger sequencing using ITS1 and ITS4 primers. The sequences were analyzed using the Staden package, yielding consensus sequences. To analyze the sequencing data, forward and reverse sequences were placed in separate folders. In the Staden package, TREV was used to view the sequences, and Pre-Gap4 was used to import and configure files for processing. After running the analysis, a new Gap4 file was created and opened, displaying both sequences. Internal joins were identified and aligned, with mismatches corrected using the Trace Display and confidence scores. Once all edits were complete, the sequences were joined, and the consensus sequence was saved. Species identification was done using the Basic Local Alignment Tool (BLAST), the Standard Databases, and the rRNA/ITS Databases. Species-level identification was accepted only for BLAST matches with an E-value $\leq 1 \times 10^{-5}$ and a minimum query coverage of 80%. Therefore, all sequences that did not meet these criteria were conservatively identified only to the genus level. Phylogenetic analysis was performed in Molecular Evolutionary Genetics Analysis (MEGA) software to determine distances between species and the genetic relationships among the recovered fungal isolates [24].

Results And Discussion

Morphological characterization of the fungal isolates

Isolates from the three counties, Taita Taveta, Meru, and Kisii, were identified morphologically using a light microscope at 400× magnification (40× objective lens × 10× eyepiece) and recorded as shown in Figures 1, 2, and 3, respectively.

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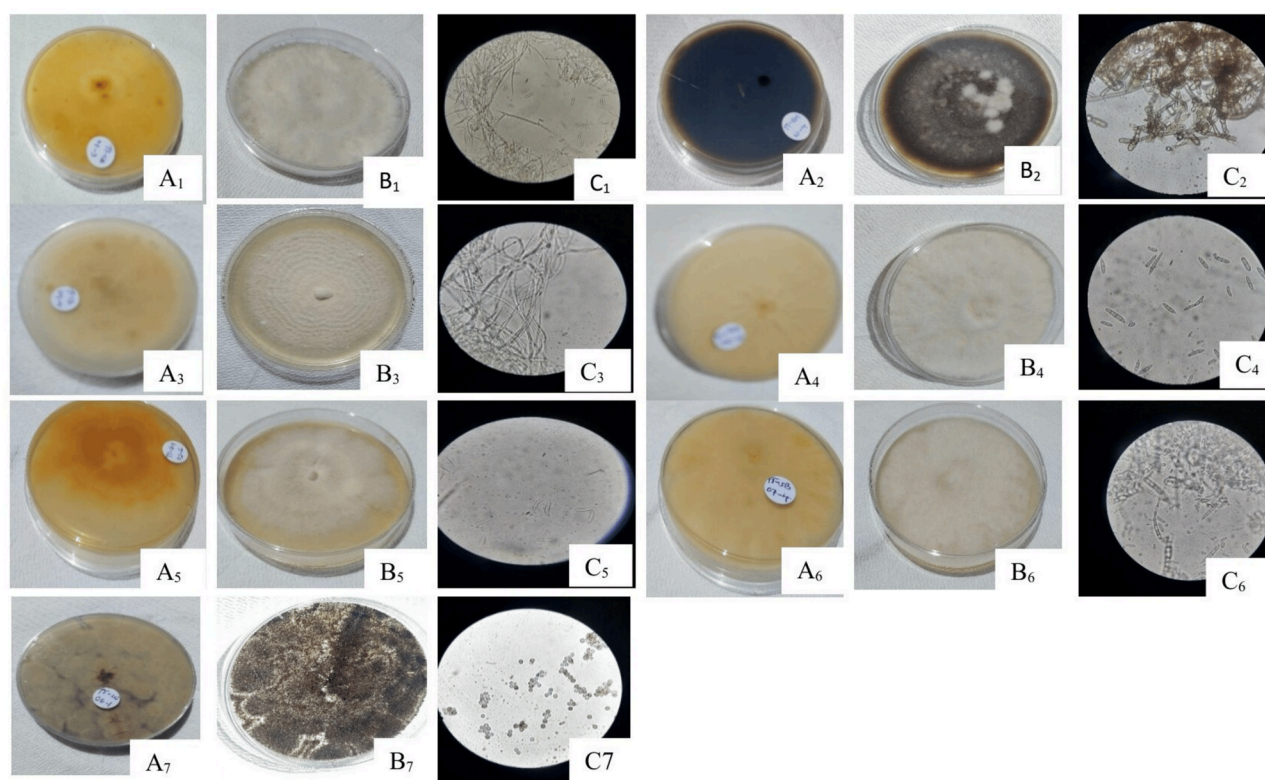


FIGURE 1: Morphological features of fungal isolates of banana from Taita Taveta County

For each isolate, panels are arranged as follows: A = colony morphology (obverse view), B = colony morphology (reverse view), and C = microscopic spore morphology observed at 400× magnification. Isolates are presented sequentially as: (A1–C1) TT-CB03-3 (*Fusarium* sp.); (A2–C2) TT-GN10-4 (*Alternaria* sp.); (A3–C3) TT-CB09-4 (*Fusarium* sp.); (A4–C4) TT-KL03-3 (*Fusarium* sp.); (A5–C5) TT-GN02-6 (*Fusarium* sp.); (A6–C6) TT-SB07-4 (*Fusarium* sp.); (A7–C7) TT-W06-1 (unidentified isolate)

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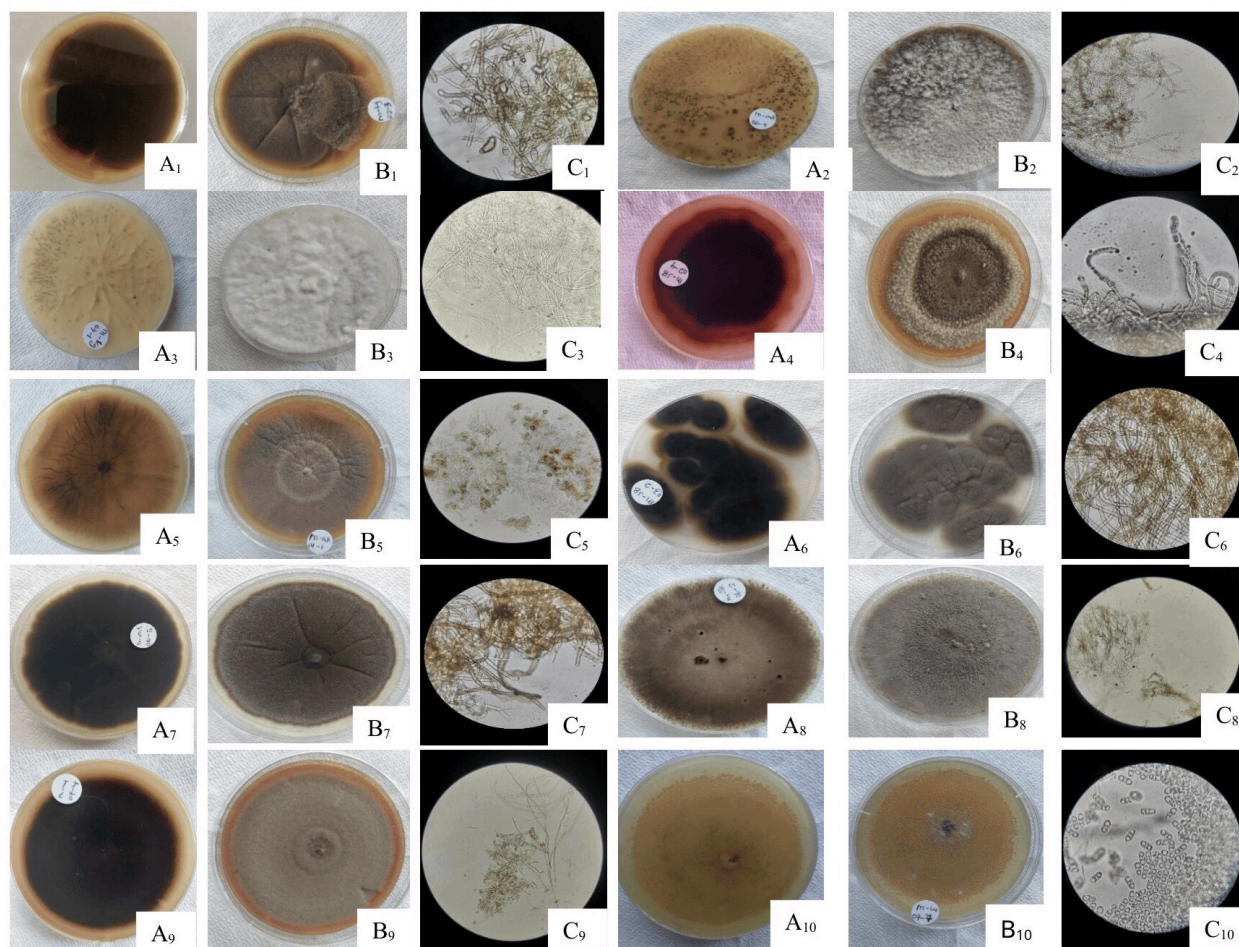


FIGURE 2: Morphological features of fungal isolates of banana from Meru County

For each isolate, panels are arranged as follows: A = colony morphology (obverse view), B = colony morphology (reverse view), and C = microscopic spore morphology observed at 400× magnification. Isolates are presented sequentially as: (A1–C1) M-KG02-3 (*Alternaria* sp.); (A2–C2) M-MT06-4 (*Alternaria* sp.); (A3–C3) M-KG04-1 (*Fusarium* spp.); (A4–C4) M-SB02-4 (unidentified isolate); (A5–C5) M-MK01-1 (*Alternaria* sp.); (A6–C6) M-SB03-2 (*Alternaria* sp.); (A7–C7) M-MT06-5 (*Alternaria* sp.); (A8–C8) M-TK06-2 (*Alternaria* sp.); (A9–C9) M-W07-4 (*Alternaria* sp.); (A10–C10) M-W07-7 (*Colletotrichum* sp.)

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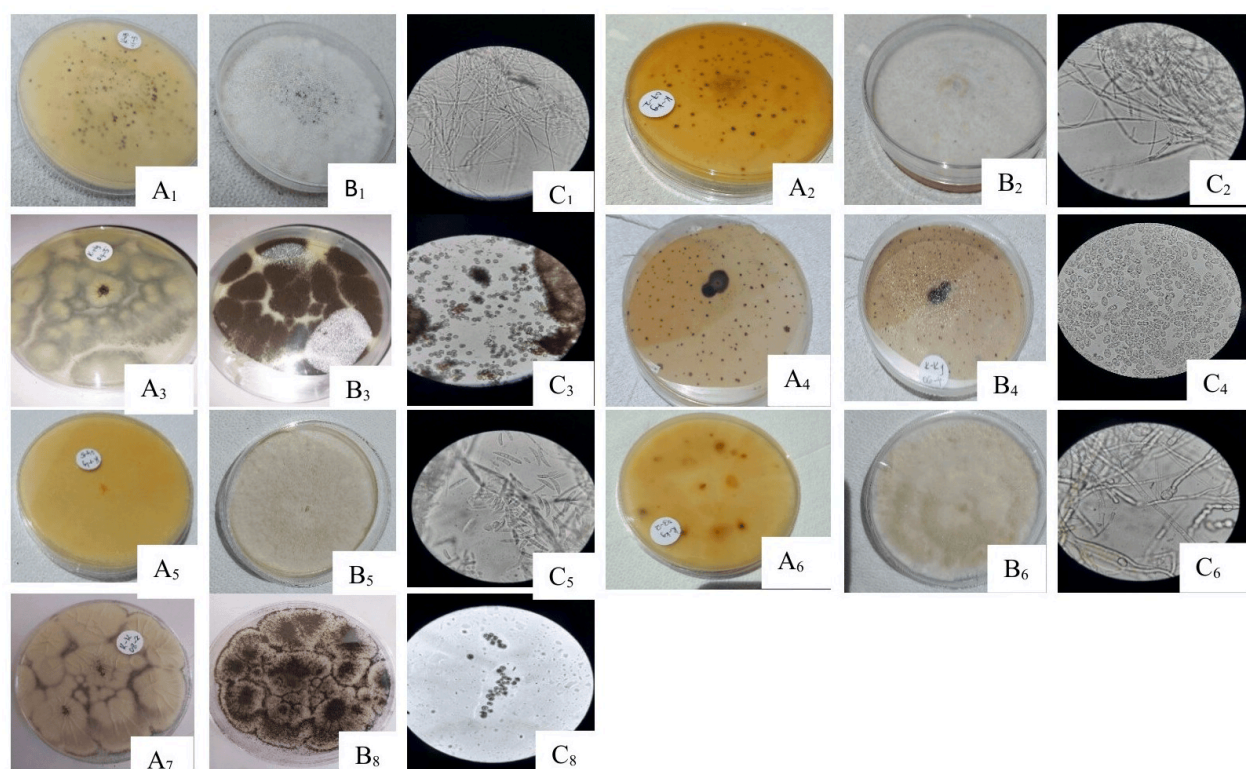


FIGURE 3: Morphological features of fungal isolates of banana from Kisii County

For each isolate, panels are arranged as follows: A = colony morphology (obverse view), B = colony morphology (reverse view), and C = microscopic spore morphology observed at 400× magnification. Isolates are presented sequentially as: (A1–C1) K-SB11-03 (*Fusarium* sp.); (A2–C2) K-KG07-2 (*Fusarium* sp.); (A3–C3) K-NG04-5 (unidentified isolate); (A4–C4) K-KG06-4 (*Colletotrichum* sp.); (A5–C5) K-NG04-2 (*Fusarium* sp.); (A6–C6) K-KG03-2 (*Fusarium* sp.); (A7–C7) K-KO8-2 (unidentified isolate)

Morphological characterization of the fungal pathogens from Meru, Taita, and Kisii was done through the integration of both symptomatology analysis of the banana plant in the field and microscopic analysis of fungal isolates from symptomatic banana tissues, which enabled preliminary identification based on morphological features of the fungal cultures on PDA plates as well as the mycelial and spore characteristics under the microscope. A total of 7 isolates from Taita Taveta County, 10 from Meru County, and 7 from Kisii County were examined, out of which 21 were successfully identified to genus level. Nine isolates TT-CB03-3, TT-CB09-4, TT-GN02-6, TT-KL03-3, and TT-SB07-4, M-KG04-1, K-SB11-03, K-NG04-2, and K-KG07-2 exhibited characteristic features of the genus *Fusarium*. These included cottony or fluffy aerial mycelium and canoe-shaped macroconidia with distinct septation, which are typical of *Fusarium* species. Eight isolates, TT-GN10-4, M-KG02-3, M-MK01-1, M-MT06-4, M-SB03-2, M-MT06-5, MTK062, and M-W07-4 showed morphological traits consistent with *Alternaria* spp., characterized by darkly pigmented, multicellular conidia with both transverse and longitudinal septa. Two isolates, K-KG06-4 and M-W07-7, exhibited morphological traits of the genus *Colletotrichum*. The remaining isolates did not produce any distinguishable conidia or structures under microscopic observation and could not be identified at this stage.

The most frequently isolated fungi from Taita Taveta and Kisii counties were *Fusarium* spp. They presented a fluffy appearance, pink and white coloration, and sickle-shaped macroconidia with septation. Atakan and Ozkaya [25] discussed these as the morphological features of *Fusarium* spp. The pathogen had a low incidence and severity in the field; however, it was the most isolated fungus in the laboratory. *Fusarium* has been reported to persist in the latent period for a very long time and to show symptoms only when conditions are favorable, as discussed by Olivares et al. [26].

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Temperature and moisture influence symptom expression [27]. The latent period of pathogenic species decreases with increasing temperature [28]. Severity of *Fusarium* wilt increases with an increase in temperature between 26°C and 34°C [29]. Despite Taita Taveta being warm, the moisture level in the county is low, and this condition might favor the *in vitro* sporulation of *Fusarium* but suppress symptom expression.

Kisii County records high humidity and rainfall, an environment that supports the survival of disease inoculum while suppressing symptom expression on the banana plant, either through cultivar resistance or ecological buffering. Meru County recorded the highest frequency of isolation of *Alternaria* spp., accounting for 7 of the 9 identified isolates. The isolates had dark pigmentation and multicellular conidia bearing both transverse and longitudinal septa, typical morphological features of *Alternaria* spp. This finding corresponds with field data, which showed that Meru had a significantly higher severity of *Alternaria* leaf spot. This distribution may be associated with environmental conditions in the region, although the present study did not directly evaluate the effects of climatic variables on *Alternaria* infection or distribution. However, warm and moderately dry conditions can stress the plant and predispose it to infection, favor the sporulation, infection, and spread of *Alternaria* spp. [30].

Molecular identification of the fungal isolates

PCR Band Visualization

A total of 45 DNA templates extracted from fungal isolates using ITS1 and ITS4 as forward and reverse primers, respectively, were run on a gel. Although 29 of these samples yielded PCR bands, sequencing was conducted for 20 samples, and only 15 generated high-quality sequences for BLAST analysis. The remaining isolates produced poor-quality chromatographs and/or incomplete sequence reads that did not permit further analysis. Figure 4 shows the agarose gel electrophoresis results with lanes 1-10 displaying amplified DNA bands, primarily around 600-700 base pairs, and lane 12 containing a DNA ladder for size reference.

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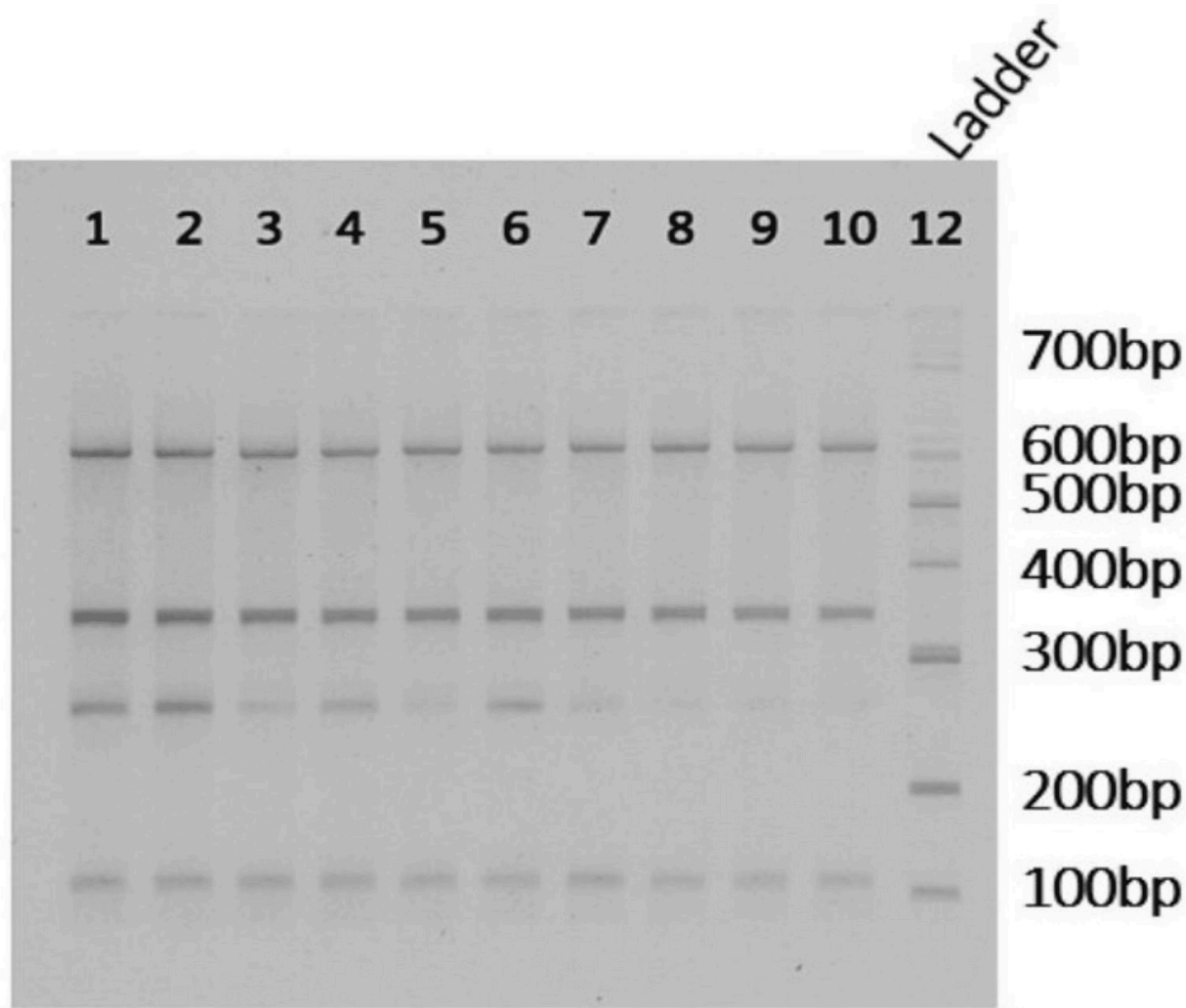


FIGURE 4: Fungal ITS PCR bands

ITS, Internal Transcribed Spacer; PCR, Polymerase Chain Reaction

Genetic identification using ITS universal primers (ITS1 and ITS4) successfully amplified fungal DNA from 29 of 45 isolates collected across the three counties. The presence of clear PCR bands confirmed the integrity and amplifiability of the fungal DNA, supporting the suitability of the ITS region for fungal barcoding in this study [31].

Species Identification Using the ITS Region Database in BLAST

Table 1 presents BLAST results for the ITS sequences recovered from fungal isolates, and their closest matches in the NCBI Nucleotide database, along with query coverage, E-values, percentage identity, and accession numbers. Standard error estimates (s) are shown above the diagonal and were obtained by using analytical formulas. This analysis involved 15 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd, and noncoding. All ambiguous positions were removed for each sequence pair by pairwise deletion. There was a total of 583 positions in the final dataset. A summary of the PCR, sequencing, and identification status of all fungal isolates is provided in Appendix Table 2. Evolutionary analyses were conducted in MEGA11 to establish the genetic relationships among the recovered fungal isolates [24].

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	Strain identity	Closest match from the Nucleotide suite	Query length	Query cover	E value	% Identity	Accession No
1	<i>Cyanoderma la oleoligni</i> Seq1	<i>Cyanoderma la oleoligni</i> CBS 140345	166	100%	3e-60	91.76%	NR_153930.1
2	<i>Alternaria alstroemeriae</i> Seq4	<i>Alternaria alstroemeriae</i> CBS 118809	189	100%	7e-92	98.41%	NR_163686.1
3	<i>Curvularia tamilnaduensis</i> Seq10	<i>Curvularia tamilnaduensis</i> SZMC 22226	119	85%	4e-18	89.86%	NR_165932.1
4	<i>Cladosporium chasmanthicola</i> Seq11	<i>Cladosporium chasmanthicola</i> CPC 21300	272	100%	2e-139	99.27%	NR_152307.1
5	<i>Alternaria alstroemeriae</i> Seq17	<i>Alternaria alstroemeriae</i> CBS 118809	223	100%	2e-112	99.10%	NR_163686.1
6	<i>Alternaria alstroemeriae</i> Seq18	<i>Alternaria alstroemeriae</i> CBS 118809	200	100%	6e-98	98.50%	NR_163686.1
7	<i>Alternaria alstroemeriae</i> Seq19	<i>Alternaria alstroemeriae</i> CBS 118809	356	100%	2e-179	98.32%	NR_163686.1
8	<i>Curvularia</i> sp Seq22	<i>Curvularia canadensis</i> CBS 109239	19	100%	2e-04	100.00%	NR_170004.1
9	<i>Alternaria alstroemeriae</i> Seq26	<i>Alternaria alstroemeriae</i> CBS 118809	180	99%	9e-91	100%	NR_163686.1
10	<i>Exserohilum mcginnisii</i> Seq27	<i>Exserohilum mcginnisii</i> CBS 325.87	207	100%	4e-105	99.52%	NR_157456.1

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11	<i>Alternaria alstroemeriae</i> Seq28	<i>Alternaria alstroemeriae</i> CBS 118809	426	100%	0.0	100%	NR_163686.1
12	<i>Alternaria alstroemeriae</i> Seq29	<i>Alternaria alstroemeriae</i> CBS 118809	174	100%	7e-87	99.43%	NR_163686.1
13	<i>Dasyscypha</i> sp Seq32	<i>Dasyscypha guianensis</i> ZT Myc 3413	18	72%	0.67	100%	NR_132857.1
14	<i>Alternaria alstroemeriae</i> Seq34	<i>Alternaria alstroemeriae</i> CBS 118809	353	100%	2e-165	96.33%	NR_163686.1
15	<i>Mucor lusitanicus</i> Seq38	<i>Mucor lusitanicus</i> CBS 108.17	161	100%	1e-79	99.38%	NR_126127.1

TABLE 1: Closest match to the ITS regions of the isolated fungi

Species-level identification was done only for sequences with E-values $\leq 1 \times 10^{-5}$ and query coverage $\geq 80\%$. Seq22 and Seq32 were not identified up to the species level because they did not meet these criteria, and were therefore conservatively assigned only to the genus level. ITS, Internal Transcribed Spacer

The evolutionary history was inferred using the Maximum Parsimony method. Tree #1 out of the 5 most parsimonious trees (length = 537) is shown in Figure 5. The consistency index is 0.880819 (0.813411), the retention index is 0.846523 (0.846523), and the composite index is 0.745634 (0.688571) for all sites and parsimony-informative sites (in parentheses). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm [32] with search level 1, in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 15 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd, and noncoding. There was a total of 583 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [33].

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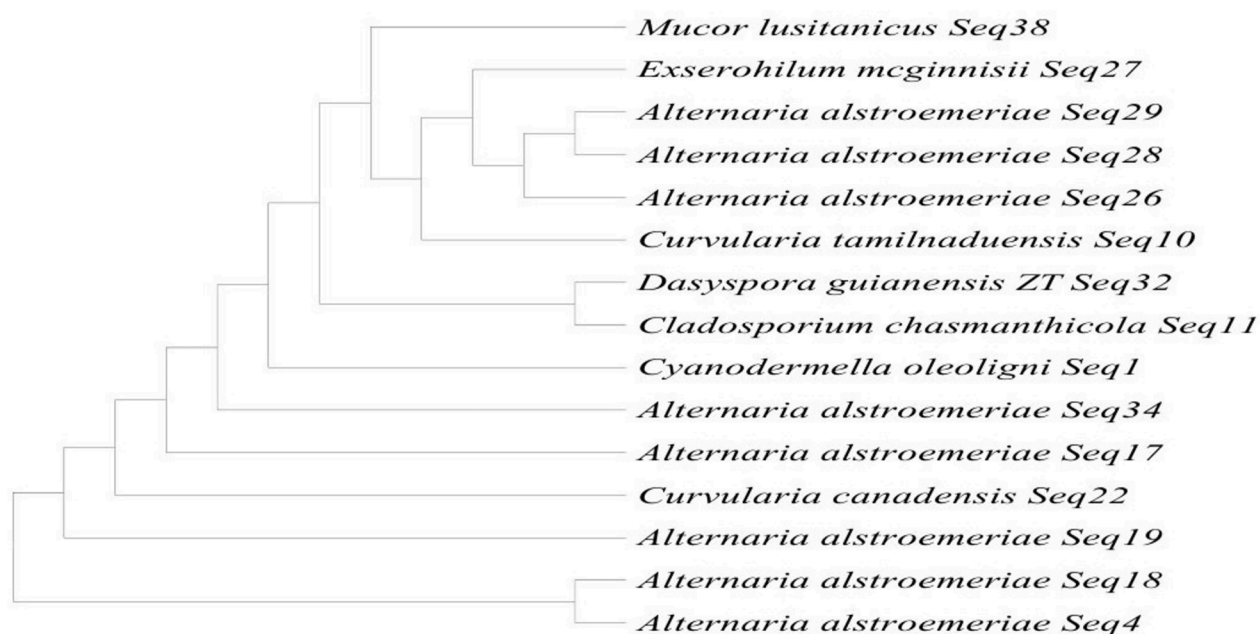


FIGURE 5: Evolutionary relations of the isolate fungi

Bootstrap support values and an outgroup were not generated for this phylogenetic analysis.

Eight out of 15 sequences were identified as *Alternaria alstroemeriae*. These sequences, Seq4, Seq18, Seq19, Seq17, Seq34, Seq26, Seq28, and Seq29, formed a large cluster that displayed a short evolutionary distance. This suggests that the eight sequences have close genetic relationships, but are not closely related to the other identified species.

Seq11 was identified as *Cladosporium chasmanthicola* (Seq 11). Previous studies have shown that *Cladosporium* species are beneficial endophytic fungi. They produce hormones and metabolites that protect the plant against biotic and abiotic stress [34]. However, *Cladosporium musae* has been reported to cause *Cladosporium* Leaf Freckle disease in banana crops across many banana-growing areas [10]. *Curvularia tamilnaduensis* (Seq10) and *Curvularia* sp. (Seq22) were also identified. *Curvularia* spp. have been reported in Pakistan as pathogenic fungi causing fruit rot in banana [35]. Their presence can be linked to postharvest rots in bananas in Kenya. Seq38 was identified as *Mucor lusitanicus*, Seq27 was identified as *Exserohilum mcginnisii*, and Seq32 was identified as *Dasyscypha* sp. The three species branched out independently on the phylogenetic tree. They formed separate clades that showed high divergence among the identified species.

Although ITS is the primary barcode for fungal identification, studies have shown that it might fail to detect some species, particularly within *Colletotrichum*, *Fusarium*, and *Pseudocercospora* genera, owing to its low intragenomic variation and high sequence conservation among closely related taxa [36,37]. This might explain the discrepancy between the morphological and molecular findings observed in the present study, particularly for *Fusarium* spp., which was established as the most dominant genus in the morphological identification but was not successfully sequenced. Thus, future studies should incorporate other molecular markers such as 1-alpha (TEF), IGS, together with ITS to improve the molecular identification and phylogenetic resolution of *Fusarium* isolates [38]. *Colletotrichum* species exhibit considerable genetic diversity and possess a wide range of survival strategies, influenced by both host specificity and environmental conditions [39]. Previous studies have shown that ITS alone is not sufficient for accurate identification of *Colletotrichum* sp., as discussed by Sharma et al. [39]. Although no studies have previously isolated *Alternaria* from banana in Kenya and other African countries, these fungi have been associated with banana leaf spot, fruit rot, and post-

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harvest diseases in other banana-producing countries like India, China [40], India [41], and Egypt [42]. Since *Alternaria* accounted for over 50% of the sequenced isolates in the present study, the results may warrant further investigation in relation to banana production and leaf spot symptoms in Kenya. This could be due to climate change and the rise in global temperatures, which might create new disease hotspots and alter disease dynamics [43]. This finding suggests that *Alternaria* sp., *Curvularia* sp., and *Cladosporium* sp. represent important fungal taxa associated with banana leaf spot symptoms in Kenya. However, since pathogenicity tests were not conducted in the present study, further tests are required to confirm their causal role in disease development.

Conclusions

This study revealed that fungi associated with banana leaf spot symptoms in Kenya are diverse. Morphological analysis showed that *Fusarium* was most prevalent in Kisii and Taita Taveta counties. At the same time, *Alternaria* was prevalent in Meru County, which aligns with the region's climatic characteristics that favor disease infection and spread. More than 50% of sequenced isolates belonged to the genus *Alternaria*, suggesting its emerging significance in banana pathology in Kenya. These findings emphasize the need for integrated plant disease diagnostic approaches to ensure accurate identification. Further research should focus on conducting pathogenicity assays, evaluating host resistance, and developing specific genetic markers for pathogens such as *Fusarium*, Cigar End Rot, and Sigatoka diseases, as accurate identification of these diseases may require more targeted molecular techniques beyond the ITS region.

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Appendices

SN	Code	PCR result	SEQUENCING	Sequence No.	Sequence quality	Closest match (BALSTn)
1	M-SK17a	Positive	SEQUENCED	1	Good	a { text-decoration: none; color: #464feb; } tr th, tr td { border: 1px solid #e6e6e6; } tr th { background-color: #f5f5f5; } <i>Cyanoderma oleoligni</i>
2	M-SB12a	Positive	NOT SEQUENCED	N/A	N/A	N/A
3	M-TK24b	Negative	NOT SEQUENCED	N/A	N/A	N/A
4	M-SB12b	Positive	SEQUENCED	4	Good	<i>Alternaria alstroemeriae</i>
5	M-SK25	Positive	NOT SEQUENCED	N/A	N/A	N/A
6	M-TK24a	Positive	NOT SEQUENCED	N/A	N/A	N/A
7	M-SK24	Negative	NOT SEQUENCED	N/A	N/A	N/A
8	M-MK9a	Negative	NOT SEQUENCED	N/A	N/A	N/A
9	M-MK8a	Negative	NOT SEQUENCED	N/A	N/A	N/A
10	M-SB02a	Positive	SEQUENCED	10	Good	<i>Curvularia tamilnaduensis</i>
11	M-SB1a	Positive	SEQUENCED	11	Good	<i>Cladosporium chasmanthicola</i>
12	M-MT13a	Positive	SEQUENCED	12	Bad	N/A

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13	M-SK-01a	Positive	SEQUENCED	13	Bad	N/A
14	M-SB02	Positive	SEQUENCED	14	Bad	N/A
15	M-SB01b	Positive	SEQUENCED	15	Bad	N/A
16	M-MK9b	Positive	SEQUENCED	16	Bad	N/A
17	M-TK24c	Positive	SEQUENCED	17	Good	<i>Alternaria alstroemeriae</i>
18	M-SK17b	Positive	SEQUENCED	18	Good	<i>Alternaria alstroemeriae</i>
19	M-U01	Positive	SEQUENCED	19	Good	<i>Alternaria alstroemeriae</i>
20	M-U02	Negative	NOT SEQUENCED	N/A	N/A	N/A
21	M-SB12c	Negative	NOT SEQUENCED	N/A	N/A	N/A
22	M-TK6	Positive	SEQUENCED	22	Good	<i>Curvularia</i> sp.
23	M-MK8b	Negative	NOT SEQUENCED	N/A	N/A	N/A
24	TT11a	Negative	NOT SEQUENCED	N/A	N/A	N/A
25	TT-GN1b	Negative	NOT SEQUENCED	N/A	N/A	N/A
26	TT-GN08a	Positive	SEQUENCED	26	Good	<i>Alternaria alstroemeriae</i>
27	TT-GN1b	Positive	SEQUENCED	27	Good	<i>Exserohilum mcginnisii</i>
28	TT-GN1a	Positive	SEQUENCED	28	Good	<i>Alternaria alstroemeriae</i>
29	TT-KL6a	Positive	SEQUENCED	29	Good	<i>Alternaria alstroemeriae</i>
30	TT-GN11	Positive	NOT SEQUENCED	N/A	N/A	N/A
31	TT-KL2	Positive	NOT SEQUENCED	N/A	N/A	N/A

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32	TT-KL10	Positive	SEQUENCED	32	Good	<i>Dayspora</i> sp.
33	TT-KL7	Negative	NOT SEQUENCED	N/A	N/A	N/A
34	TT-GN8a	Positive	SEQUENCED	34	Good	<i>Alternaria alstroemeriae</i>
35	TT-GN8b	Positive	NOT SEQUENCED	N/A	N/A	N/A
36	TT-GN4	Positive	NOT SEQUENCED	N/A	N/A	N/A
37	TT-KL6b	Negative	NOT SEQUENCED	N/A	N/A	N/A
38	TT-GN09a	Positive	SEQUENCED	38	Good	<i>Mucor lusitanicus</i>
39	TT-KL01	Positive	NOT SEQUENCED	N/A	N/A	N/A
40	TT-GN09b	Positive	NOT SEQUENCED	N/A	N/A	N/A
41	TT-KL03	Positive	NOT SEQUENCED	N/A	N/A	N/A
42	TT-KL6c	Negative	NOT SEQUENCED	N/A	N/A	N/A
43	TT-GN1a	Negative	NOT SEQUENCED	N/A	N/A	N/A
44	TT-MT1	Negative	NOT SEQUENCED	N/A	N/A	N/A
45	TT-MT2	Negative	NOT SEQUENCED	N/A	N/A	N/A

TABLE 2: Summary of status of fungal isolates obtained in the study

A total of 29 samples tested positive for PCR; however, only 20 were sequenced due to budgetary constraints, and 15 of these yielded high-quality sequences for BLAST analysis. However, the BLAST statistics for Seq22 and Seq32 did not support reliable species-level identification. Thus, the identification of these two isolates was confined to the genus level as *Curvularia* sp. (Seq22) and *Dasyscypha* sp. (Seq32), respectively. PCR, Polymerase Chain Reaction

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Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Acquisition, analysis, or interpretation of data: Stellah M. Amakove, Javan Omondi Were, Becky Nancy Aloo, Samwel K. Muiruri, Rimnoma O. Serge

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Critical review of the manuscript for important intellectual content: Stellah M. Amakove, Javan Omondi Were, Becky Nancy Aloo, Samwel K. Muiruri, Rimnoma O. Serge

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Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen.

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

Conflicts of interest: In compliance with [the ICMJE uniform disclosure form](#), all authors declare the following:

- **Payment/services info:** USAID funded this study under the Current and Emerging Threats to Crops Innovation Lab (CET-CIL) and the Feed the Future program. .
- **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.
- **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

All data generated or analyzed during this study are included in this published article and/or its appendices.

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