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Research article

Preliminary report of *Epicoccum* spp. associated with brown leaf spot on cassava in Kenya

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A R T I C L E I N F O

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A B S T R A C T

Cassava brown leaf spot (BLS) is among the most damaging diseases that significantly reduce cassava root yields and quality. In this study, drought-resistant cassava varieties were screened on an experimental farm in Kitui County, Kenya. One variety, Kasukari, exhibited abnormal morphological aberrations suggesting BLS and necessitated systematic studies to establish the causal agents. Fungi were isolated from symptomatic leaves and purified on Potato Dextrose Agar (PDA) with antibiotics. Fungal pathogens were identified using morpho-cultural characteristics and molecular characterization through polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer (ITS). 162 Kasukari samples were used to determine the prevalence and severity of the disease, while 15 samples were used to determine the effects of the disease. Pathogenicity tests of ten isolates were conducted in vitro using detached leaves of the healthy Kasukari variety. The results indicated that the prevalence within the plots had no significant difference ($\chi 2 = 6$, p-value = 0.1991). However, there was a significant difference in severity ($\chi 2 = 53.013$, p-value = 1.166e-09). PCR with the ITS marker identified the fungal pathogens from the genera *Alternaria*, *Epicoccum*, *Preussia*, and *Cladosporium*. *Epicoccum* spp. had colonies of white clusters that formed concentric rings, while the reverse had grey-brown colonies and a regular margin. Conidia produced on PDA were oval, hyaline, unicellular, and aseptate, consistent with *Epicoccum* morphology. Based on ITS identification and pathogenicity assays, this study provides a preliminary report of an *Epicoccum* sp. associated with brown leaf spot (BLS) disease in cassava in Kenya. However, due to the absence of multi-locus sequence data and the limited number of isolates tested, the causal role of *Epicoccum* remains suggestive. These findings form the basis for future studies to confirm the causal role of *Epicoccum* spp. and clarify its epidemiology. The study will also help inform prevention and management strategies, including breeding programs and targeted control measures.

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1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most significant tropical root crops and a source of carbohydrates. Cassava is the only domesticated species of the genus that is native to the Western Amarizonian rim [1] and is a member of the family *Euphorbiaceae* of the perennial shrubs [2]. In Kenya, especially in the arid and semi-arid lands (Asals) of Eastern Kenya, cassava has been used as one of the traditional staple foods [3]. Common cassava preparation methods in Kenya involve roasting and boiling the 'sweet' cassava roots or making stiff dough by combining boiling water and cassava flour over a low-heat fire. Cassava is the world's fourth most important staple food behind rice, wheat and maize [4]. Cassava remains a climate-smart crop in Asals, which is characterized by fluctuating weather patterns and unsuitable terrain that results in irregular rainfall and prolonged drought. Biotic constraints impact cassava growth and yield, among which pathogenic diseases are significant [5]. Further, the global climate change phenomenon is differentially exacerbating disease occurrences in cassava.

Cassava brown leaf spot (BLS) is caused by *Cercospora henningsii* Allesch in China [6]. Lozano and Booth [7] also identified *Cercospora* as the causative agent of BLS in Asia. In Kenya, BLS has been reported to be caused by fungi from the genera *Alternaria*, *Cladosporium*, and *Colletotrichum*, working in synergy to cause the disease [8]. The key symptom of brown leaf spot disease is the appearance of a few to several brown spots on the upper surface of the leaves of diseased plants of susceptible cassava varieties [9]. The edges of the diseased leaves are brown and irregular, and the middle could split, creating little holes. Farmers mistake these for signs of crop maturity. Brown leaf spot leads to losses in root yield due to the extensive defoliation of the plants, which affects photosynthesis [10].

Since infected cassava leaves are the primary host of the fungus, it has been demonstrated that BLS is disseminated by cassava leaves that have fallen to the ground or onto other plants. In addition, planting materials that have been infected with the disease facilitate the spread of cassava disease [11]. Dissemination is further propagated by wind or rain splashing from plant to plant or weed to weed, and the new plants become infected from the sources; weeds that harbour the pathogen can also facilitate the spread [12]. The fungus thrives in soil or plant debris in winter, serving as an inoculum for subsequent infections. After planting, the disease can be observed after five to six months. The disease becomes more severe under warm and humid conditions in which the fungus produces spores on the underside of the leaves. Other cassava diseases that result from fungal infections include white leaf spot, cassava anthracnose disease (CAD), and root rot disease. The outbreak of white leaf spot disease caused by *Passalora manihotis* is characterized by white lesions that may appear circular or angular in shape [13]. Cassava anthracnose disease, caused by *Colletotrichum gloeosporioides*, is characterized by the development of cankers or sore-like features on the stem [14]. Root rot disease caused by *Polyporus sulphureus*, a parasitic mushroom, has been reported to be attacking cassava plants repeatedly [15].

While agronomically screening drought-resistant varieties in lower eastern Kenya, one variety in the plots, commonly called Kasukari, exhibited abnormal morphological aberrations whose cause necessitated systematic investigation. Very little information is known about the identity of pathogens associated with the brown leaf spot in this region, where cassava is cultivated to enhance food security, owing to the prevailing harsh climatic conditions that do not favour other crops. Identification of the causal agent(s) of BLS on cassava in lower eastern Kenya facilitates the development of effective management and control strategies and resistant breeding programs for BLS in cassava. Based on the fact that early identification of the causal agent(s) of any plant disease in the field is a critical initial step in the development of effective management and control strategies [16], it was important to initiate this study to determine the causative agent(s) of BLS on cassava and the effects of the implicated pathogen on the cassava.

2. Materials and methods

2.1. Experimental site and plant materials

The study was conducted at an experimental site at the South Eastern Kenya University (SEKU) in Kitui County, Kenya. This county is located between longitudes 37040′ East and 39010′ East and latitudes 00 10′ South and 3010′ South [17].

Kitui experiences semi-arid climatic conditions with about 201 to 500 mm annual rainfall [18]. Rainfall is distributed over two rainy seasons: a short one around October, November, and December and another long one around March and April. Kitui experiences a varying temperature range between a minimum of 14°C to 22°C and a maximum of 26°C to 34°C, and it is in a sub-humid agro--ecological zone [19]. The soil dominant in the area is described as well-drained, sandy clay to clay, friable to firm, dark reddish brown to dark yellowish brown, moderately deep, with a topsoil of loamy sand to sandy loam.

The cassava variety used in this study was Kasukari, a drought-resistant cassava among the common varieties grown in the South-Eastern Kenya region. The Kasukari variety, along with other varieties used in this study, was sourced from local farmers in Kitui County. This variety was of interest since it displayed morphological aberrations that were suggestive of fungal disease manifestations. Other varieties—Makueni mixed, Machakos mixed, Kitwa, Makueni Kituaa, and Bitter cassava were used as controls because they appeared healthy. When planting, planting materials were only visually inspected, and no molecular or microscopic tests were performed due to logistical constraints.

2.2. Experimental design

The experiment was conducted between 2020 and 2023. Land preparation was carried out in 2020, planting occurred in 2021, data collection was conducted in 2022 and 2023, and data analysis was performed in 2023.

A randomized, complete block design was used to lay out the field experiment. The Kasukari variety was replicated three times

within the experimental site, making three Kasukari plots. The plots measured 10 m by 20 m, with ridges measuring 3 m apart. Each plot contained 18 rows with 200 seedlings and 1.0-m spacing between plants within the rows. Weeding was done uniformly in the three plots. Plants established in the experimental farm were regularly monitored using visual observations to assess for any disease infection. Six cassava plants were selected from every two rows using a simple random sampling (SRS) method to determine the prevalence of disease infestation in each block, and data were collected. Cassava leaves of selected plants were inspected based on the symptoms of BLS as described by Ng'ang'a et al. [20].

2.3. Prevalence and symptom severity of cassava BLS in the fields

Field observations for BLS foliar symptoms were performed in March 2022. Eighteen rows in each plot were grouped into two, forming nine rows. Data for the BLS prevalence were randomly collected from 6 plants within the nine rows, totaling 54 cassava plants per plot. The leaves were sampled from the three plots for the Kasukari variety, thus totaling 162 samples for the study. Disease prevalence (%) was calculated by dividing the total number of plants infected by the total number of plants observed, multiplied by 100 [21].

The same cassava plants used to collect data on disease prevalence were also used to estimate disease severity.

Brown leaf spot severity was assessed using a modified visual rating scale based on the proportion of infected leaves. Severity was scored as 1/S1 = no visible symptoms, 2/S2 = light infection (up to 25% infected leaves), 3/S3 = moderate infection (up to 50% infected leaves), 4/S4 = severe infection (up to 75% infected leaves), and 5/S5 = very severe infection (75–100% infected leaves). The scale was informed by established visual and diagrammatic disease rating methods for leaf spot diseases [22,23].

2.4. Sampling and fungal isolation

Necrotic lesions from diseased cassava leaves (Fig. 1a) were cut into small pieces and surface sterilized using 1.3% sodium hypochlorite for 3 min, then rinsed twice with sterile distilled water for 1 min. The cut sections (Fig. 1b) were air dried under sterile conditions and inoculated on a sterile Potato Dextrose Agar (PDA, HiMedia, Mumbai, India) that contained streptomycin sulphate 0.03 gL^{-1}. The inoculum was then kept at room temperature for 12 h of darkness and 12 h of daylight cycle (Fig. 1b). Morpho-cultural characteristics of pure fungal isolates were used for fungal identification, as guided by previous studies in the identification manuals [24–26].

2.5. Morphological identification of fungal isolates

Slides were prepared from pure colonies and observed under a light microscope (Leica DM5OO) for morphological characterization. A small piece of sterile PDA media was cut and placed on a sterile slide using a sterile needle. A little piece of mycelium was picked up using a sterile needle and smeared around the media within the slide. A clean cover slip was gently placed on top and kept in a dark room at room temperature. A little piece of mycelium was used to quickly introduce fungi into the starvation period after the depletion of nutrients. This procedure was repeated for all the fungal isolates. The inoculated samples were left for 7 to 21 days to produce conidia. A little piece of mycelia from each colony was then taken, and a drop of lactophenol cotton blue dye was placed on a sterilized glass slide using a sterile isolation needle. The mycelia were then entirely covered with a clean cover slip gently placed on top. For every sample, this process was repeated while sterilizing the needle. Each colony was first observed with an ×10 objective lens and subsequently at a higher magnification of ×40. The Features used for the morpho-cultural identification of the isolates were colony colour, surface appearance, margin, and shape. The observed microscopic characteristics included the type of hyphae, septate or aseptate, and the type of spore formed.

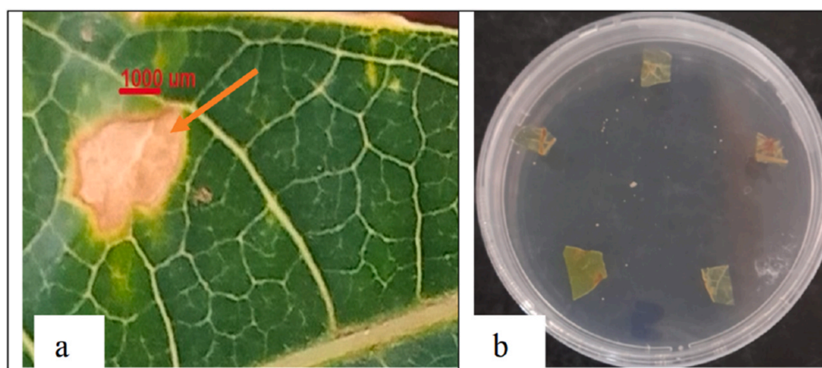


Fig. 1. Illustration of the necrotic lesion and cut sections from the symptomatic leaf of the Kasukari cassava variety plant: (a) part of the cassava leaf with a brown spot shown with a red arrow; (b) Petri dish containing cut leaf sections grown in PDA.

2.6. Molecular identification of fungal isolates

2.6.1. DNA extraction

The CTAB-SDS method of DNA isolation, as described by Osena et al. [27], was used. CTAB extraction buffer (0.2 M EDTA (pH 8), 1.4 M NaCl, 100 mM Tris-HCl (pH 8), 2% (w/v) CTAB, and 4% (w/v) PVP) was preheated in a water bath for 15 min at 60°C. Mycelia and fungal spores were scraped from the PDA medium and transferred into a centrifuge tube. In the presence of pre-warmed CTAB extraction buffer and 1% PVP, 200 mg of mycelia and spores were ground at room temperature using pre-chilled rods. The homogenate was incubated at 60°C in a water bath for an hour. The tubes were centrifuged at 4°C for 10 min at 10000 rpm, and the supernatant was transferred into new tubes. Samples were mixed for 15 min by inversion after adding the same amount of chloroform and isoamyl alcohol (24:1). The tubes were then centrifuged at 4°C for 10 min at 10000 rpm. This step was repeated, and the supernatant was decanted and transferred into new microtubes. To precipitate the DNA, chilled isopropanol was added, then incubated for 30 min at −20°C. The microtubes were centrifuged for 10 min at 10,000 rpm to pellet down the DNA. 70% ethanol was used to wash the pellet two times by centrifuging for 15 min at 14,000 rpm, then air drying at room temperature. Nuclease-free water (45 μl) was used to dissolve the DNA pellet. RNA was digested by adding 5 μl of RNAse to the DNA solution, incubating at 37°C for 30 min in a water bath, and stored at −20°C for further use.

2.6.2. Polymerase chain reaction

A 25 μl reaction volume containing 5 μl of 10 μm primers, 0.75 μl of 10 mM dNTP, 0.5 μl of 50 mM $MgCl_2$,5 μl of 10× buffer, 0.2 μl of Taq DNA polymerase, and 1 μl of genomic DNA was used for the PCR amplification [28]. The final volume was then adjusted using sterile distilled water. The internal transcribed spacer (ITS), amplified with primer pairs of ITS1 (F) (TCCGTAGGTGAACCTGCGG) and ITS4 (R) (TCCTCCGCTTATTGATATGC), was used for PCR amplification. The thermal cycling conditions were: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation, annealing, and extension at 94°C for 30 s; 54°C for 40 s; and 72°C for 30 s, respectively, and a final extension at 72°C for 5 min.

Other primers that were used for the amplification of fungal isolates targeted the partial sequence of the translation elongation factor 1α (EF1-α), EF1-688F (CGGTCACTTGATCTACAAGTGC) and EF1-1251R (CCTCGAACTCACCAGTACCG); glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), GDF (GCCGTCAACGACCCCTTCATTGA) and FDR (GGGTGGAGTCGTACTTGAGCATGT); and chitinase synthase (CHS), CHS-79F (TGGGGCAAGGATGCTTGGAAGAAG) and CHS-354R (TGGAAGAACCATCTGTGAGAGTTG). The primer pairs were expected to amplify about 500 and 600 bp regions.

2.6.3. Gel electrophoresis

The amplified PCR products were analyzed by electrophoresis on a 1% agarose gel and compared to a 1 kb DNA ladder (DNA Ladder Mix 1 kb, 1st Base Company, Singapore). The PCR products were post-stained with the ethidium bromide dye and loaded into the wells [29]. Aliquots of 3 μl PCR products with 1 × DNA loading dye and a 45-min run at 100 V were used for gel electrophoresis. The gels were examined under ultraviolet light using the Gel-Doc ™ XR + Imaging System (Bio-Rad, GmbH – FeldKirchen, Germany).

2.6.4. Sequencing and phylogenetic analysis

The amplified PCR products were sequenced by Macrogen Europe BV Laboratory (Amsterdam, Netherlands) using an Applied Biosystems 3730XL DNA Analyzer platform.

The obtained ITS sequences were edited using the BioEdit analysis software. The sequences were then compared against sequences available at the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLASTn) database. Phylogenetic analysis of sequences of fungal isolates, along with reference sequences obtained from the NCBI BLASTn database, was carried out in MEGA X Software, where the sequences for the top 10 similar species were used for phylogenetic analysis. The sequences were aligned using the ClustalW alignment tool in MEGA X version 11. The phylogenetic trees were rooted using the midpoint rooting method since there were no outgroups, and bootstrap replicates were set to 100. Phylogenetic trees were generated using the maximum-likelihood (ML) model in MEGA11 and edited in FigTree v. 1.4.4 software.

2.7. Pathogenicity of fungal isolates on cassava leaves

2.7.1. Preparation of the inoculum suspension

Using a modified procedure of Aberkane et al. [30], the pure fungal isolates were utilized to create a spore solution for detached leaf pathogenicity testing. A tiny piece of the innermost fungal mycelia, about 1 cm by 1 cm, was carefully scraped off each plate using a sterilized nichrome wire loop. Care was taken to remove as little of the media as possible. One millilitre of sterile distilled water was added to each of the labelled sterile Eppendorf® tubes containing the scraped pieces. The fungus mycelia were macerated to make a suspension with sterile microfuge pestles. For 1 min, the suspension was thoroughly stirred using a vortex mixer. Next, the Eppendorf Minispin® Plus centrifuge was used to centrifuge the Eppendorf® tubes for 15 min at 500 rpm. A volume of 500 mL of the supernatant was cautiously and aseptically moved to an additional pair of sterile Eppendorf® tubes with the appropriate labels. Both hyphal and spore suspensions were present in the samples. In order to remove the hyphal fragments and preserve just the conidial suspension, each suspension was strained through a sterile syringe connected to a sterile filter with a pore diameter of 10 μm (Tisch Scientific) [30]. The concentration of the spore suspensions of 1×10^6 spores/mL of water, measured using a hemocytometer, was used for each fungal isolate.

2.7.2. In vitro detached leaf pathogenicity test

Fifty fully developed and healthy leaves were used for the pathogenicity test, with each fungal isolate replicated five times. The leaves were detached from a healthy, susceptible Kasukari variety established in the greenhouse and used to test for the pathogenicity of all the fungal isolates. The Kasukari variety, with fully developed and healthy leaves, was detached from the parent plants grown and maintained in a greenhouse at the University of Nairobi. The leaves were sterilized by running tap water on them first to remove any dirt on the surface, then washed for 30 s in 70% ethanol, and finally rinsed once with sterile distilled water. The leaves were then placed in 1.3% sodium hypochlorite for 1 min and subjected to three cycles of washing in sterile distilled water. For controls, the water from the last wash of each leaf was utilized.

Sterile paper towels were used to line sterile Petri plates. Six hundred microliters of sterile distilled water were applied to the paper towels using a micropipette. This was done to ensure that the relative humidity of the Petri dishes was more than 70%. After the leaves were sterilized, leaflets were cut and distributed to the petri dishes using a random number generator (RANDOM.ORG, True Random Number Service). With the adaxial leaf surface facing the bottom of the plates, the leaflets were positioned on a sterile wire gauze on top of the paper towels in the Petri dishes. Five tiny puncture holes were made transversely along the equator of each leaf using sterile inoculation needles. This was done to enable a possible fungal pathogen to invade the leaf's cuticle and cause an infection.

Ten microliters of the prepared 106 spores/mL spore suspension were added to each leaf puncture site. Additionally, sterile paper towels soaked in 400 µl of sterile distilled water were used to line the Petri dish covers. Following inoculation, the Petri dishes containing inoculated leaflets were wrapped in parafilm, sealed, and incubated for seven days at 23 ± 2°C with 12 h of darkness and 12 h of daylight. Two sets of controls were established with the leaves from the Kasukari variety, and they received the same treatment except that the first set of controls was inoculated with the last rinse water and the second with sterile distilled water. Any inoculated leaves that differed from the features of the control were considered to have been treated with pathogenic fungal isolates. Based on the descriptions provided by Imathiu et al. [31], the usual symptoms were chlorosis, necrosis, leaf colouration, and leaf discolouration on the affected leaves.

After an in vitro detached leaf pathogenicity test, Koch's postulate procedure was used to confirm the causative agent of the disease. Symptomatic in vitro infected leaves were used for fungal re-isolation and later inoculated on detached healthy Kasukari leaves. The resultant morphological characteristics were compared to the initial result and confirmed to be similar. Using a random number generator (RANDOM.ORG - True Random Number Service), representative pathogenic isolates of each type of symptom were chosen at random based on the unique symptoms that appeared on leaves following in vitro pathogenicity tests. These particular symptomatic

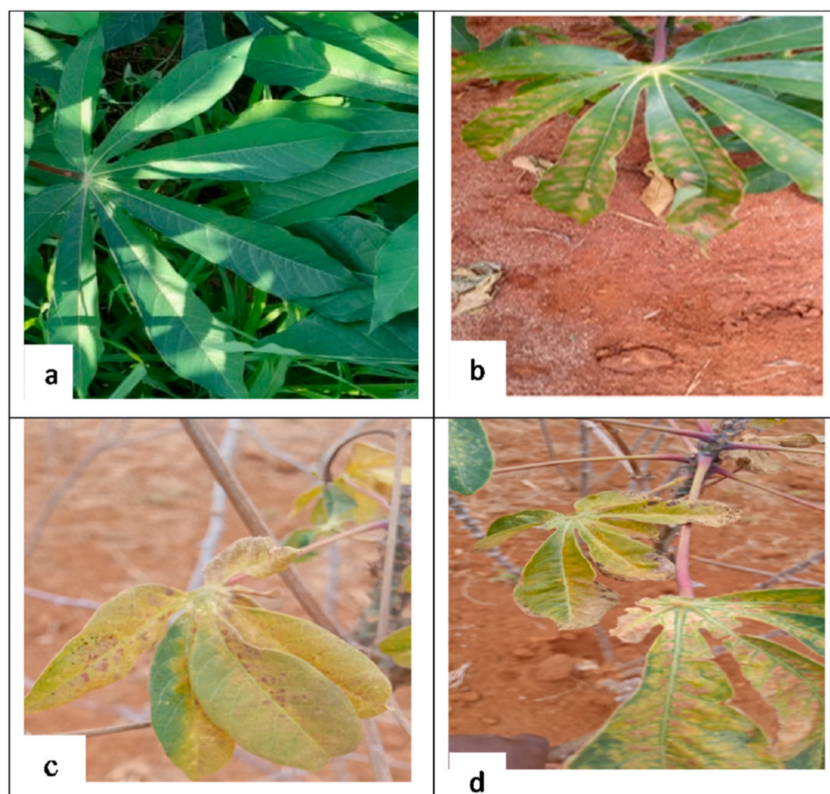


Fig. 2. Images of cassava leaves showing disease severity of brown leaf spot in the experimental farm at South Eastern Kenya University (SEKU), Kitui County. Leaf (a) represents a healthy leaf with no visible symptom (S1), leaf (b) shows moderate disease symptoms (S3), leaf (c) shows severe symptoms (S4), and leaf (d) shows very severe symptoms (S5).

leaves were utilized for the re-isolation of fungi for morpho-cultural characterization, whereby they were grown on new PDA Petri dishes and pure cultures inoculated on cassava leaves. The re-isolation procedure and confirmation of fungal pathogens were the same as those used in the inoculation.

2.8. Statistical data analysis

Microsoft Excel was used to summarize the data. R-GUI (version 4.2.2.0) software was used for the analysis of Chi-square for BLS prevalence and severity at a confidence interval of $p \leq 0.05$.

3. Results

3.1. Prevalence and severity of BLS on Kasukari germplasm in the Kitui experimental farm

From field observations, fungal disease infestations were identified upon comparing the symptoms with those found in the Pacific Pests and Pathogen Fact Sheets. Detection and inspection of brown leaf spots were done on the older leaves by checking for the appearance of round and angular light brown spots with yellow margins. Thus, the disease infection was identified as brown leaf spot disease (fungal) for all the assessed plants.

Severity scores were based on a 5-point scale S1 = no visible symptom, S2 = light, S3 = moderate, S4 = severe, S5 = very severe. Few to several brown spots on the upper surface of leaves, especially on old and mature leaves, were observed (Fig. 2 b, c, and d). In some leaves, the margins were irregular, and the middle of the leaves had small perforations, which further suggested brown leaf spot disease caused by a fungal pathogen. The identified infections matched the descriptions of fungal disease as described in the literature, in contrast to Fig. 2a, which illustrates healthy bitter cassava leaves.

Different prevalence levels were obtained in the three randomized blocks. For blocks 18, 9, and 2, there were prevalence levels of 27.7%, 32.9%, and 29.4%, respectively. However, these variations were not statistically significant (p-value = 0.1991, $\chi 2 = 6$).

Similarly, variations in severity scores within the blocks were also found (Fig. 3). However, the differences in this instance were statistically significant (p-value = 1.166e-09, ($\chi 2 = 53.013$).

3.2. Morphological characteristics of fungal isolates on PDA medium

Ten pure fungal cultures were obtained. Generally, the fungal colonies were from four genera: *Alternaria*, *Cladosporium*, *Epicoccum*, and *Preussia*. The morphological and cultural characteristics of the four cultures indicated that all four fungal pathogens had septate hyphae. The identified *Epicoccum* spp. had colonies of white clusters that formed concentric rings, with the reverse having grey-brown colonies and a regular margin (Fig. 4a–c). Conidia produced on PDA were oval, hyaline, unicellular, and aseptate, the typical morphology of *Epicoccum* species. One of the *Alternaria* spp. had muriform conidia (Fig. 5c and d), along with woolly, grey colonies that had a regular margin (Fig. 5a and b). Another *Alternaria* strain had woolly white colonies with red pigment on the medium (Supplementary Fig. S3). Another *Alternaria* spp. had a septate spore, as shown in the Supplementary Fig. S3. *Cladosporium* spp. Fig. 6d displayed macro- and microconidia with tapering ends; the colonies had a velvety to suede-like texture with an olive-green colour and

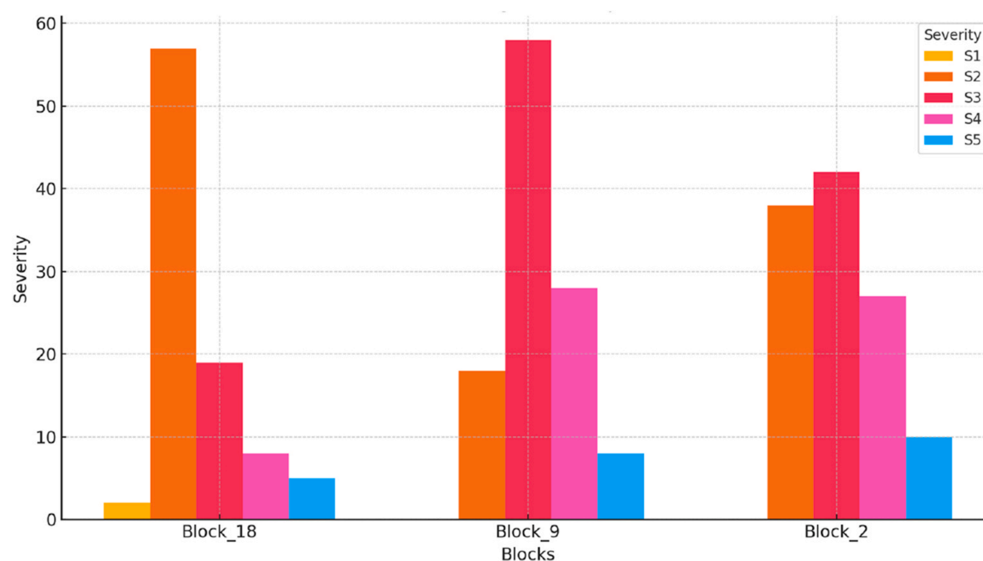


Fig. 3. Cassava brown leaf spot severity score within Kasukari plots [18,9,and2]] in the South Eastern Kenya University (SEKU) experimental farm in Kitui County.

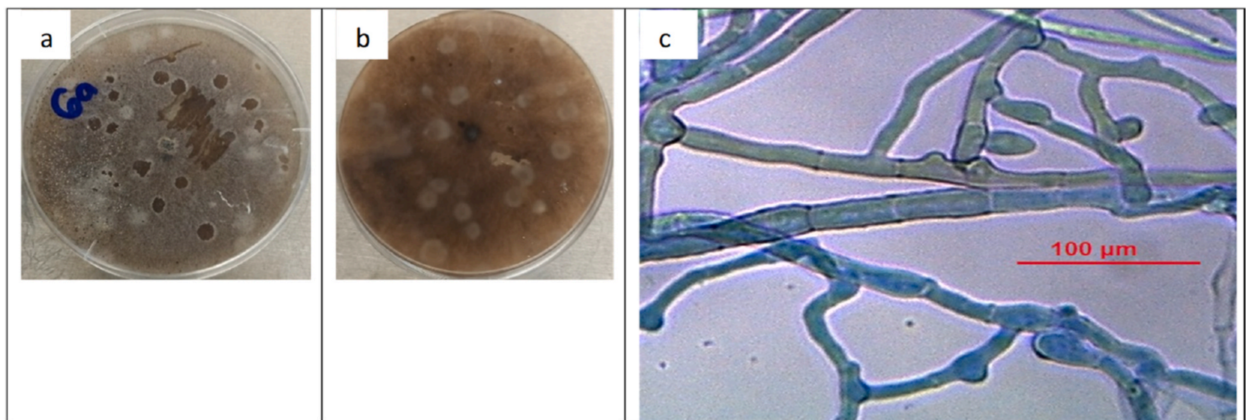


Fig. 4. Cultural and morphological characteristics of *Epicoccum* spp.: (a) and (b) top and bottom view of culture on PDA, respectively; (c) hyphal swelling.

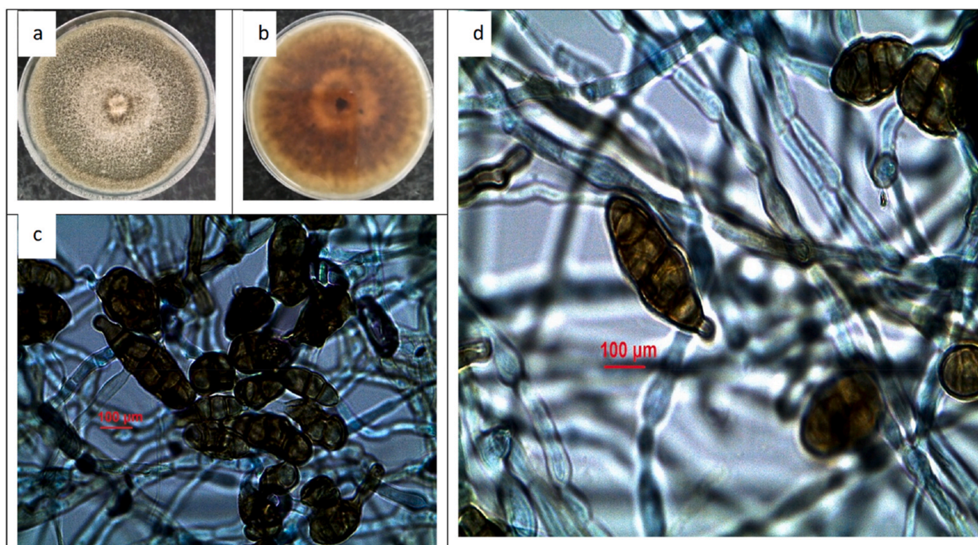


Fig. 5. Cultural and morphological characteristics of *Alternaria* spp.: (a) and (b) top and bottom views of culture on PDA, respectively; (c) and (d) show mature spores.

a regular margin (Fig. 6a and b). Micronidia were observed to be produced in chains (Fig. 6c). The other *Cladosporium* spp. are presented in Supplementary Fig. S4. *Preussia* species had brown to grey clusters with irregular margins and ellipsoidal to oval conidia (Fig. 7a–d). The other *Preussia* spp. is shown in Supplementary Fig. S2, which had a cream-white and brown colony at the centre.

3.3. Polymerase chain amplification of fungal isolates

The polymerase chain reaction performed by a universal primer, internal transcribed spacer (ITS) 1 and 4 partial sequences, gave the expected amplicon size of approximately 550 bp (Supplementary Fig. S1). A 1 Kb Plus DNA Ladder was used as a marker. A negative (-ve) control containing nuclease-free water was used to confirm specific amplification. The EF1-α, GAPDH, and CHS regions were also amplified and sequenced.

3.4. Sequencing and phylogenetic analysis

The ITS sequences identified 10 fungal isolates. The close fungal species identified belonged to the genera *Alternaria*, *Epicoccum*, *Preussia*, and *Cladosporium*. (Fig. 8, Table 1). Based on the nucleotide BLAST search, the similarity of the most 10 fungal isolates to the closest species available in the NCBI was 100% (Table 1). The nucleotide sequences of the ITS region for 10 fungal isolates were submitted to the NCBI database and were assigned the accession numbers from PP471615 to PP471624 (Table 1).

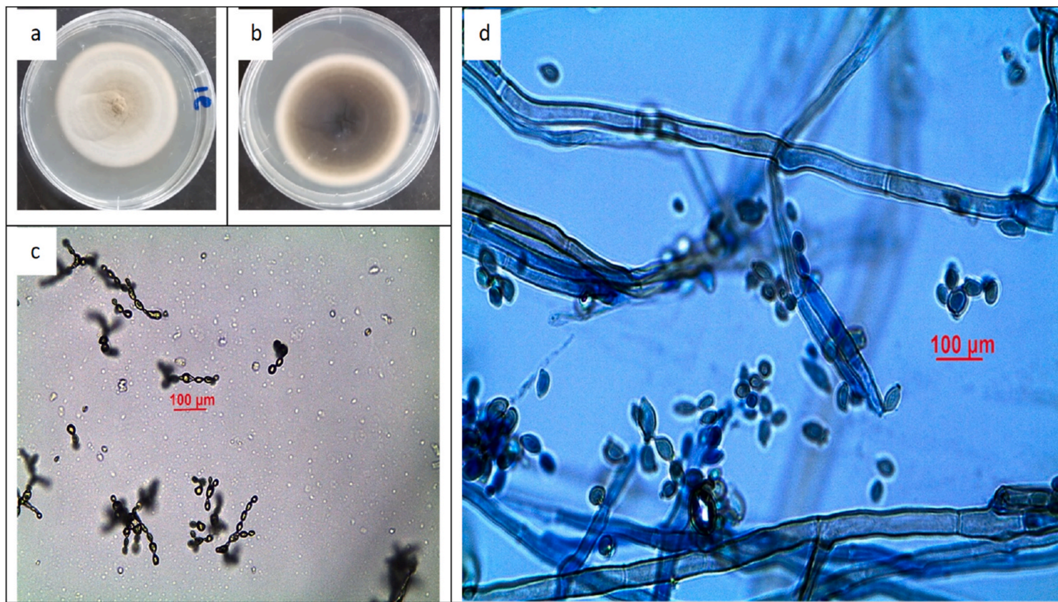


Fig. 6. Cultural and morphological characteristics of *Cladosporium* spp.: (a) and (b) top and bottom view of culture on PDA, respectively; (c) chains of microconidia; (d) conidiophores bearing conidia.

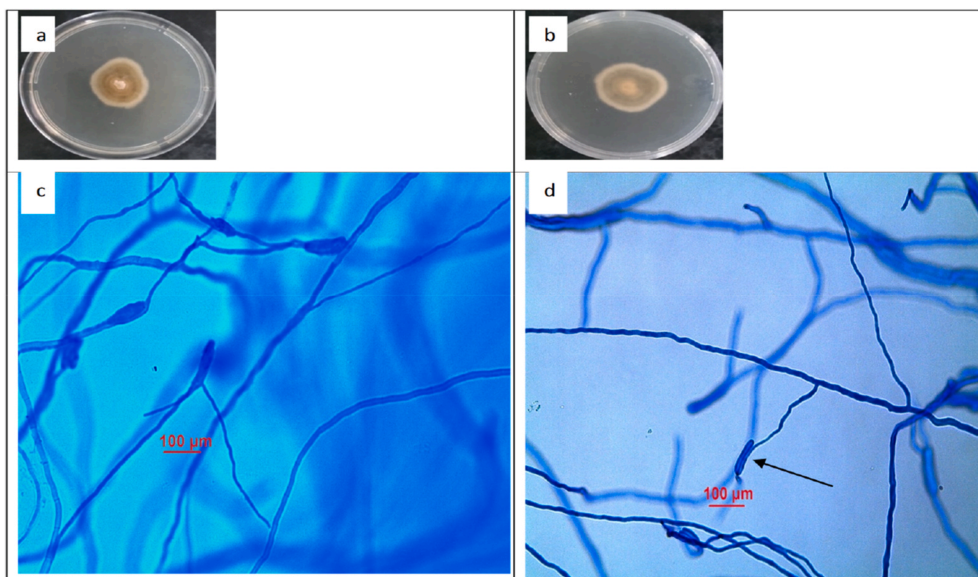
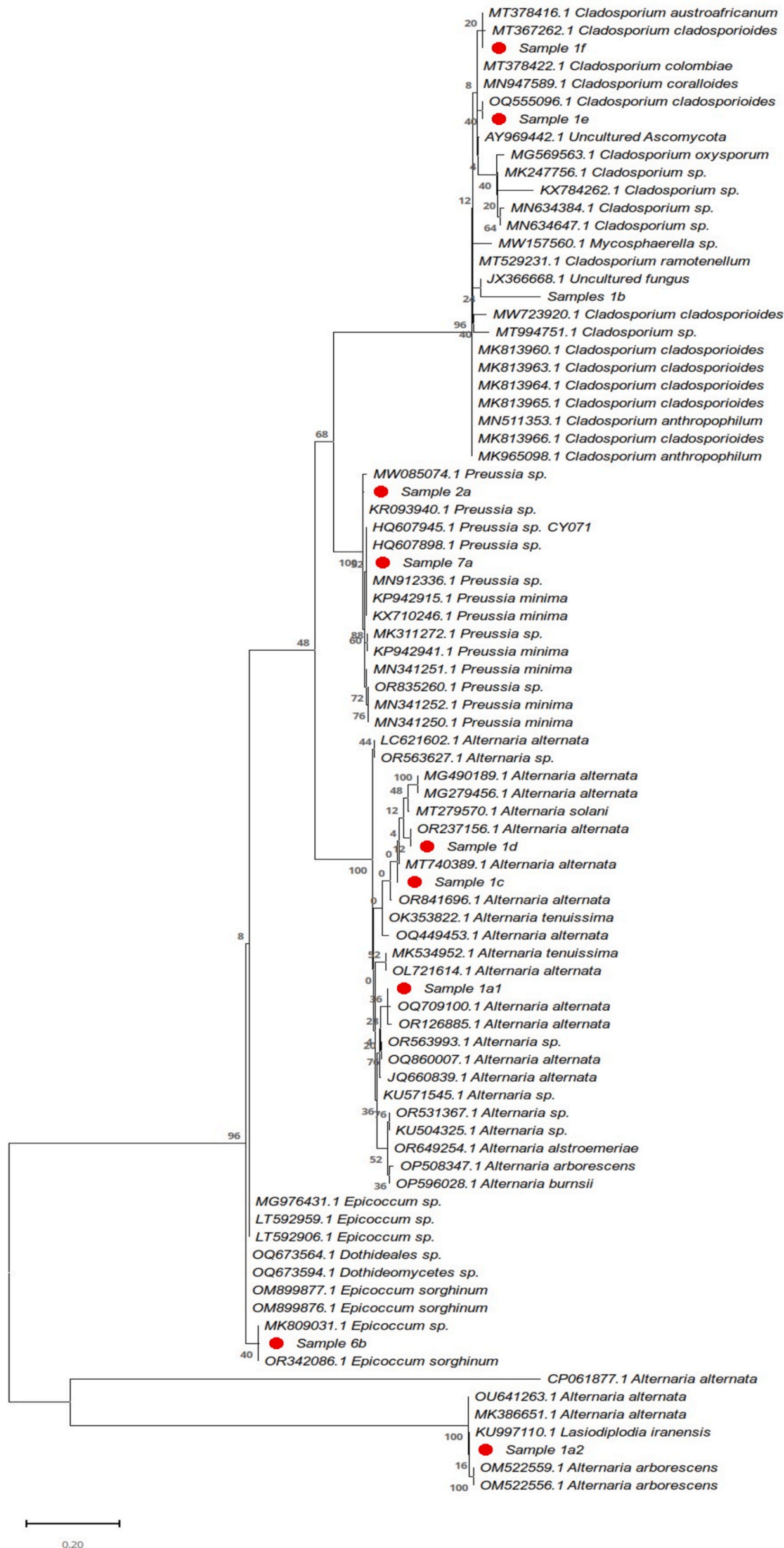


Fig. 7. Cultural and morphological characteristics of *Preussia* spp., (a) top view; (b) bottom view; (c) and (d) conidiophore bearing conidia with an arrow showing conidia born from the conidiophore on PDA medium.

The GAPDH and EF1-α sequences had less than 50% coverage and, therefore, were not used in genetic analysis to identify the fungal isolates. The CHS sequences had coverage of about 75% but the sequences were not used in the identification of the isolates since the ITS sequences had the highest coverage of 100%.

For phylogenetic analysis, the maximum-likelihood (ML) tree based on the datasets of ITS sequences was constructed and includes the 10 isolates from this study and several referenced fungal strains from the database. Phylogenetic analysis revealed that the 10 isolates clustered with the *Alternaria* spp., *Epicoccum* spp., *Preussia* spp., and *Cladosporium* spp. (Fig. 8), which were consistent with the homology search results that were conducted using BLASTn.



(caption on next page)

Fig. 8. A phylogenetic tree shows fungi identification using the ITS marker. The support values of the associated taxa are shown on the branches. The isolates and the identified genus are highlighted in red. The accession numbers of the samples are: Sample_1a1 = PP471615; Sample_1a2 = PP471616; Sample_1b = PP471617; Sample_1c = PP471618; Sample_1d = PP471619; Sample_1e = PP471620; Sample_1f = PP471621; Sample_2a = PP471622; Sample_6b = PP471623; and Sample_7a = PP471624.

Table 1
Similarity of ITS partial sequences of fungal species isolated from cassava plants of Kasukari variety with BLS in lower eastern Kenya, compared with that of the accessions in the GenBank database.

Isolate ID Code	ITS Accession Number	Closest Match in BLASTn	Similarity (%)
1a1	PP471615	*Alternaria alternata*	100
1a2	PP471616	*Alternaria alternata*	100
1b	PP471617	*Cladosporium spp.*	100
1c	PP471618	*Alternaria alternata*	99.41
1d	PP471619	*Alternaria alternata*	99.20
1e	PP471620	*Cladosporium cladosporioides*	99.62
1f	PP471621	*Cladosporium colombiae*	100
2a	PP471622	*Preussia spp.*	99.03
6b	PP471623	*Epicoccum sorghinum*	100
7a	PP471624	*Preussia minima strain*	100

3.5. Pathogenicity of fungal isolates on cassava leaves

Results of in vitro detached leaf assays delineated the eight isolates as fungal pathogens of cassava BLS (Fig. 9). *Preussia* spp. did not produce disease symptoms as the inoculated leaves remained the same as the controls inoculated with distilled water. The onset of the disease symptoms was characterized by enlarged holes, which were followed by the manifestation of grey lesions on the leaf surface. After 5 days, most inoculated leaves developed necrotic lesions, except inoculated samples with *Preussia* spp. As the days progressed, two samples that were inoculated with *Alternaria* spp. developed a dark colour around the centre of inoculation. The controls remained symptom–free during the entire period of inoculation.

4. Discussion

Cassava has been mainly used as a staple food in arid and semi-arid lands (ASALs), as demonstrated by El-Sharkawy [32], with a

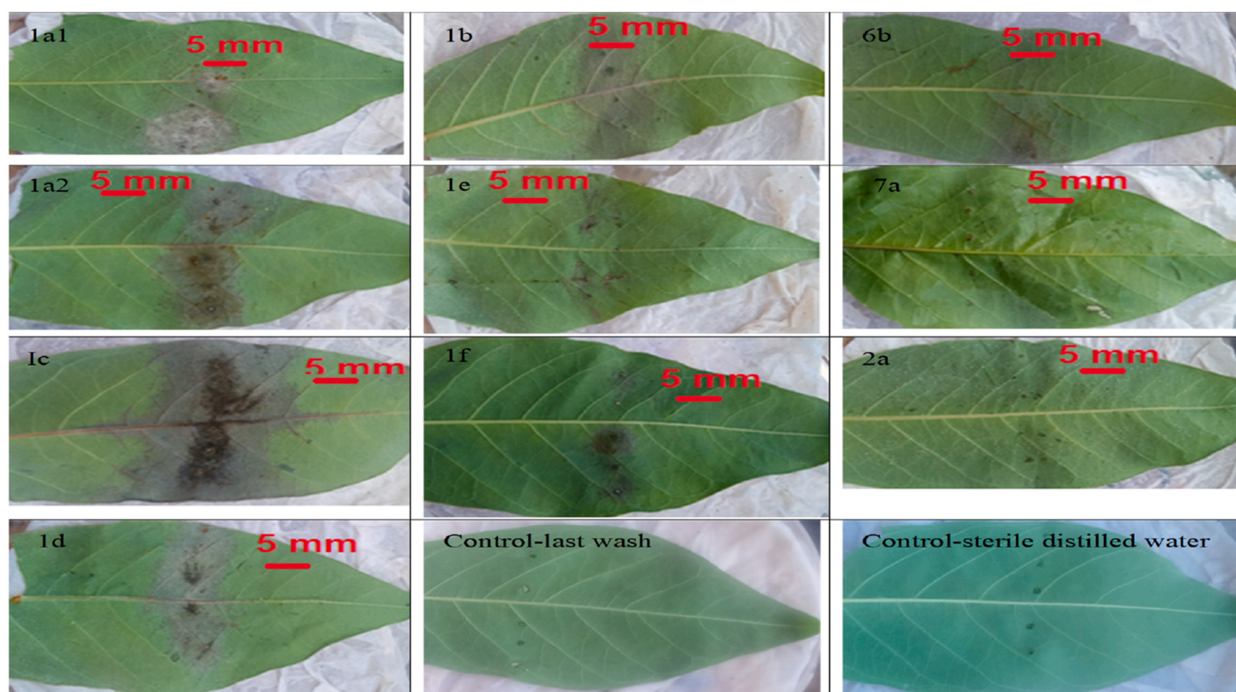


Fig. 9. Pathogenicity of the fungal isolates and controls using in vitro detached leaf assays. *Alternaria* spp.: 1a1, 1a2, 1c, 1d; *Cladosporium* spp.: 1b, 1e, 1f, *Epicoccum* spp.: 6b; *Preussia* spp.: 2a, 7a.

high content of carbohydrates in the roots and leaves used as a source of minerals, vitamins, and proteins. However, cassava leaf diseases remain a significant constraint in cassava production in Kenya. This study investigated the possible cause, effect, and severity of some morphological aberrations observed in the Kasukari cassava variety in experimental farms in Kitui County, Kenya.

Data from our study shows BLS as the disease responsible for the observed morphological aberrations in the Kasukari variety. Morphological characteristics identified by microscopy, pathogenicity tests, and genetic analysis indicated the presence of fungal pathogens belonging to *Alternaria* spp., *Cladosporium* spp., and *Epicoccum* spp.

The isolate identified as being from genus *Preussia* was a fungal endophyte since it did not produce any disease symptoms. *Preussia* sp. has been reported as an endophyte fungus [33]. However, further studies need to be conducted to determine the role of *Preussia* spp, when in synergy with pathogenic fungal pathogens isolated in the current study, potentially influencing disease development.

The three pathogens from the genera *Alternaria*, *Colletotrichum*, and *Cladosporium* have been reported to cooperate to cause BLS on cassava leaves [8]. The identified pathogens in this study are thought to be working in synergy and via interaction to generate phytotoxins, which play a significant part in the colonization of the plant [20]. The advancing mycelia release toxins responsible for the yellow halo around the lesions. Shi et al. [34] describe *Epicoccum sorghinum* as an emerging and notorious fungal pathogen with a broad host spectrum that causes a reduction in crop quality and leads to devastating crop losses. *Epicoccum sorghinum* is pathogenic to a wide variety of crops, including sorghum [35], and also causes ring spot disease of sugarcane [36]. *Epicoccum* spp. has also been reported to be associated with brown leaf spot disease in tea in China [37,38] and in the United States [39]. Although our ITS-based analysis suggests *Epicoccum* spp is associated with BLS symptoms in cassava in Kenya, the lack of multi-locus sequencing and the limited pathogenicity assays make it a preliminary report. To the best of our knowledge, this study provides the first indication of *Epicoccum* spp., potentially playing a role in cassava BLS in Kenya, warranting further comprehensive investigation. Chang et al. [35] confirm that *Epicoccum sorghinum* leads to fungal contamination upon infection by producing mycotoxins such as tenuazonic acid (TeA) and polyketide epicorepoxydon B, chemicals that are harmful to animal and human health and result in economic losses.

Although only *Epicoccum* spp. was re-isolated, *Alternaria* spp. and *Cladosporium* spp. were also identified as having an association with symptomatic leaves. *Alternaria* spp. had the highest prevalence with four isolates, followed by *Cladosporium* spp. with three isolates. Orina et al. [40] demonstrated that *Alternaria* spp. prevails in many regions of the world in the grain microbiome. In Egypt, *Alternaria* spp. is pathogenic in various crops, including tomatoes and potatoes, where they cause severe early blight in leaves [41]. *Alternaria* also causes leaf spots on rapeseed [42], ornamental plants [43], and leaf blight on bananas [44]. *Alternaria tenuissima* was reported to be causing the Luobuma leaf spot in China [45]. The pathogenicity of Alternaria spp. relies on the susceptibility or resistance of the host plant and the large production of host-specific and non-specific toxins [46].

Cladosporium spp. is a causal agent of leaf spot disease in maize, peach, ornamental flowers, cucumber, oats, peanuts, and spinach. *Cladosporium* produces conidiophores in chains or singly and is usually tall and upright, with branching at the apex [47]. *Cladosporium* spp. is considered one of the heterogenous complexes and the largest hyphomycete genera that settle on lesions made by phytopathogenic fungi as secondary infections. Certain species of *Cladosporium* can cause lesions, leaf spots, or hyperparasite growth on other fungi [48]. These phenomena explain why *Alternaria* and *Cladosporium* spp. were prevalent in the current study.

Molecular identification through amplification of the ribosomal internal transcribed spacer DNA (ITS rDNA) region using primers ITS1/ITS4 was used to support morphological characterization. PCR amplification with ITS, a universal barcoding marker for identifying fungi [49], revealed that the sequences were homologous to *Epicoccum*, *Alternaria*, *Cladosporium*, and *Preussia* species. Harnelly et al. [50] confirmed the ITS gene as an accurate DNA barcode for identifying fungi and can clearly distinguish between species. In our study, ITS-based identification provides preliminary evidence on the presence of *Epicoccum* genera in symptomatic cassava tissues. However, the absence of multi-locus sequencing the resolution of species-level identification. In a study, Saleem and El-Shahir [51] used ITS rDNA to characterize isolates of *Alternaria* species molecularly.

There was no significant difference in BLS prevalence across the three Kasukari plots. This suggests that the source of the infection was the germplasm. Since the germplasm used was the same, if all were infected, the result would have been shown in all three Kasukari plots, which was observed in this study. This also concurs with the reports suggesting that cassava disease occurrence is primarily facilitated by infected cuttings [11]. The high incidence of the disease perpetuated through infected cuttings is dangerous since it can lead to increased severity, incidence, and decreased cassava yield if combined with the consistent use of diseased planting materials [52].

The high prevalence of BLS infection in Kasukari can cause significant losses to farmers in arid and semi-arid lands like Kitui. This is because reduced production rates due to diseases and low rainfall can lead to food insecurity in ASALs. For instance, in Kitui County, Kenya, crop failure affects household income and availability of food at least once every five years [53]. Since Kasukari is a drought-resistant cassava, farming with adequate control of diseases can help increase both the income and food supply within ASALs, as the farmers sell their roots to generate income and consume them. Generally, the highest severity scores for BLS disease were 2 (mild/light) and 3 (moderate). A few Kasukari plants had traces of BLS leaf symptoms. This clearly shows that the increased occurrence of diseased cassava plants may limit the number of asymptomatic plants selected for planting. On the same note, severely infected plants cannot provide healthy planting materials for propagation. [52]. Brown leaf spot significantly affected the growth of the Kasukari variety, mainly affecting leaves. The infection affected leaves, causing distortion and defoliation. Moreover, distortion of leaves has been shown to negatively affect yield due to too much defoliation [10]. Depending on the surface area of the leaf affected, farmers can harvest small roots if the microbial infection is severe. Robson et al. [54] showed a positive correlation between a decrease in storage roots and the severity of the symptom.

Furthermore, the occurrence of the brown leaf spot disease in the Kasukari variety in Kitui is a phenomenon that can result in high yield losses in cassava. For example, BLS is responsible for enormous annual losses of cassava in the tropics and subtropics [5]. Additionally, the infection caused by BLS may lead to mycotoxicity, and affected cassava, upon consumption, can be poisonous. This

can threaten Kitui's economy since significant funding is directed toward the control of the disease.

5. Study limitation

Pathogenicity was assessed using a limited number of isolates and was conducted on only one cassava variety (Kasukari), which may not fully represent the broader cassava population or variability across other varieties. Future studies including multiple varieties and a larger number of isolates are recommended to generalize these findings. Additionally, the lack of multi-locus sequencing limited the accuracy of species-level identification; therefore, further molecular characterization is needed to better support the generalization of the findings.

6. Conclusions

Our study suggests that the morphological aberrations observed in the Kasukari variety resulted from brown leaf spot infection, with *Epicoccum* spp. among the fungal genera potentially contributing to the symptoms. ITS sequences and phylogenetic analysis showed that the fungal isolates causing BLS on cassava were identified as belonging to the genera *Alternaria*, *Epicoccum*, *Preussia*, and *Cladosporium*. However, due to limitations inherent in single-locus ITS identification, further molecular characterization of the identified *Epicoccum* spp. using multi-gene sequence data is necessary. The findings from the study confirmed the high prevalence and severity of BLS in cassava in lower eastern Kenya. Therefore, the study recommends that most farmers in the region practice field sanitation practices, such as burning unhealthy plant parts and disinfecting farm equipment, to minimize the spread of the disease. Improved farming practices and effective disease monitoring can help reduce the impact of the disease on the plant. As a control mechanism, most farmers need to scout for early disease indicators on their farms regularly. Thus, a need to accurately inform farmers on the best farming and management practices and create control and eradication programs to enhance cassava yield in Kenya. However, the research was conducted when the rains were scanty, and the cassava had not been established well. Thus, the effects of climate change variations might have facilitated the occurrence of the observed diseases.

CRediT authorship contribution statement

Ephine Awuor Onyango: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sarah Naulikha Kituyi:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Carol Wangui Hunja:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Josphert Ngui Kimatu:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Evans Nyaega Nyaboga:** Writing – review & editing, Resources, Methodology.

Data availability statement

Data included in article/supplementary material are readily available for sharing upon request. The nucleotide sequences of the ITS region for ten isolates obtained in this study were deposited in GenBank through an online submission portal and were assigned the following accession numbers: PP471615 - PP471624.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Josphert Ngui Kimatu reports financial support was provided by National Research Fund, Kenya. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2026.e45093.

References

[1] J.H. Cock, D.J. Connor, Cassava, in: Crop Physiology Case Histories for Major Crops, Elsevier, 2021, pp. 588–633.
[2] A.A.C. Alves, Cassava botany and physiology, Cassava Biol Prod Util. 1 (2002) 67–89.
[3] S.S. Nyawira, P. Bolo, W. Ntinyari, L. Orero, K. Onyango, A. Manjella, et al., Kenya Context Assessment Report: a desk-top Review of the Context of Agroecological Principles of Kiambu and Makueni Counties, Alliance of Bioversity International and CIAT, 2023.
[4] W.G. Adebayo, Cassava production in Africa: a panel analysis of the drivers and trends, Heliyon 9 (9) (2023) e19939.
[5] R.R. Prasad, M.R.U. Dean, B. Alungo, V.V. Chand, Prevalence and incidence of cassava (Manihot esculenta) brown leaf spot disease caused by Cercospora heningsii in Macuata Province, Vanua Levu, Fiji, J. Agric. Sci. 13 (8) (2021) 91.
[6] Y.L. Pei, T. Shi, C.P. Li, X.B. Liu, J.M. Cai, G.X. Huang, Distribution and pathogen identification of cassava brown leaf spot in China, Genet. Mol. Res. 13 (2) (2014) 3461–3473.
[7] J.C. Lozano, R.H. Booth, Diseases of cassava (Manihot esculenta Crantz), PANS Pest Artic. News Summ. 20 (1) (1974) 30–54.
[8] D.O. Muraina, V.O. Dania, V.O. Azuh, Detection and characterisation of Curvularia lunata causing brown leaf spot disease of cassava (Manihot esculenta Crantz) in Southwest Nigeria, Arch Phytopathol Plant Prot 58 (20) (2025) 1124–1138.
[9] E.J. McCallum, R.B. Anjanappa, W. Gruissem, Tackling agriculturally relevant diseases in the staple crop cassava (Manihot esculenta), Curr. Opin. Plant Biol. 38 (2017) 50–58.
[10] E.C. Julião, M.D. Santana, R. do L. Freitas-Lopes, A. dos P. Vieira, J.S.B. de Carvalho, U.P. Lopes, Reduction of brown leaf spot and changes in the chlorophyll a content induced by fungicides in cassava plants, Eur. J. Plant Pathol. 157 (2) (2020).
[11] I.N. Koima, C.O. Orek, S.N. Nguluu, Distribution of cassava mosaic and cassava brown streak diseases in agro-ecological zones of lower eastern Kenya, Int J Innov Sci Res Technol 3 (2018) 391–399.
[12] F. Doohan, Fungal pathogens of plants, Fungi Biol Appl (2011) 313–344.
[13] J.H.B. Nascimento, LRB de Andrade, SAS de Oliveira, EJ de Oliveira, Phenotypic variability in resistance to anthracnose, white, brown, and blight leaf spot in cassava germplasm, Plants 13 (9) (2024) 1187.
[14] T.J. Onyeka, A.G.O. Dixon, E.J.A. Ekpo, Field evaluation of root rot disease and relationship between disease severity and yield in cassava, Exp. Agric. 41 (3) (2005) 357–363.
[15] E. Moses, Development of appropriate strategies to control cassava diseases in Ghana, Role Plant Pathol Food Saf Food Secur (2010) 11–24.
[16] A. Ramcharan, K. Baranowski, P. McCloskey, B. Ahmed, J. Legg, D.P. Hughes, Deep learning for image-based cassava disease detection, Front. Plant Sci. 8 (October) (2017) 1–7.
[17] N. Kagimbi, T. Losenge, E. Majiwa, C. Obiero, M. Kigomo, M.K. Boitt, et al., Land suitability assessment for mango production in Kitui County, Kenya, J. Agric. Sci. Technol. 23 (2) (2024) 114–146.
[18] A.A. Amboka, C. Gachene, L. Olaka, Evaluation of hydrogels in improving soil-water retention, plant survival and climate adaptation strategies in Kitui County, Kenya, J. Appl. Sci. Environ. Manag. 29 (2) (2025) 425–435.
[19] E.J. Mutunga, C.K. Ndungu, M. Mwangi, P.C. Kariuki, Socioeconomic determinants of farmers' vulnerability to climate variability and extreme events in Kitui County, Kenya, Am J Clim Chang 13 (4) (2024) 647–663.
[20] P.W. Ng'ang', D.W. Miano, J.M. Wagacha, P. Kuria, Identification and characterization of causative agents of brown leaf spot disease of cassava in Kenya, J. Appl. Biol. Biotechnol. 7 (6) (2019) 1–7.
[21] M.A. Hussain, T. Mukhtar, M.Z. Kayani, M.N. Aslam, M.I. Haque, A survey of okra (Abelmoschus esculentus) in the Punjab province of Pakistan for the determination of prevalence, incidence and severity of root-knot disease caused by Meloidogyne spp, Pakistan J. Bot. 44 (6) (2012) 2071–2075.
[22] W.C. James, An Illustrated Series of Assessment Keys for Plant Diseases, 1971.
[23] F.A.S. Lima Filho, Leite Íchl, A.S. Capucho, S.J. Michereff, R.L. Freitas-Lopes, U.P. Lopes, Accuracy and efficiency of assessments of cassava brown leaf spot aided by standard area diagram sets based on whole compound leaves or single central leaflets, Eur. J. Plant Pathol. 153 (2) (2019) 627–638.
[24] H.L. Barnett, B.B. Hunter, Illustrated Genera of Imperfect Fungi, 1972.
[25] R.A. Humber, Fungi: identification, in: Manual of Techniques in Insect Pathology, Elsevier, 1997, pp. 153–185.
[26] F.M. Dugan, The Identification of Fungi: an Illustrated Introduction with Keys, Glossary, and Guide to Literature, 2006.
[27] G. Osena, E.N. Nyaboga, N.O. Amugune, Rapid and efficient isolation of high quality DNA from cassava (Manihot esculenta crantz) suitable for PCR based downstream applications, Annu Res Rev Biol 12 (2) (2017).
[28] N. Valizadeh, H.A. Holasou, S.A. Mohammadi, K.M. Khawar, A comparison of genomic DNA extraction protocols in Artemisia annua L. for large scale genetic analyses studies, Iran J Sci Technol Trans A Sci. 45 (5) (2021) 1587–1595.
[29] R. Sangpueak, P. Phansak, N. Buensanteai, Morphological and molecular identification of Colletotrichum species associated with cassava anthracnose in Thailand, J. Phytopathol. 166 (2) (2018) 129–142.
[30] A. Aberkane, M. Cuenca-Estrella, A. Gomez-Lopez, E. Petrikkou, E. Mellado, A. Monzon, et al., Comparative evaluation of two different methods of inoculum preparation for antifungal susceptibility testing of filamentous fungi, J. Antimicrob. Chemother. 50 (5) (2002) 719–722.
[31] S.M. Imathiu, R.V. Ray, M. Back, M.C. Hare, S.G. Edwards, Fusarium langsethiae pathogenicity and aggressiveness towards oats and wheat in wounded and unwounded in vitro detached leaf assays, Eur. J. Plant Pathol. 124 (2009) 117–126.
[32] M.A. El-Sharkawy, Cassava biology and physiology, Plant Mol. Biol. 56 (4) (2004) 481–501.
[33] R.R. Mapperson, M. Kotiw, R.A. Davis, J.D.W. Dearnaley, The diversity and antimicrobial activity of Preussia sp. endophytes isolated from Australian dry rainforests, Curr. Microbiol. 68 (1) (2014) 30–37.
[34] X. Shi, S. Zhang, Y. Yang, L. Jia, D.D. Herrera-Balandrano, S. Wang, et al., Occurrence and management of the emerging pathogen Epicoccum sorghinum, Plant Dis. 109 (3) (2025) 520–531.
[35] C.-C. Chang, C.-Y. Li, Y.-H. Tsai, M. El-Shazly, C.-K. Wei, Z.-J. Yang, et al., Bioactive polyketides from the pathogenic fungus of Epicoccum sorghinum, Planta 253 (6) (2021) 116.
[36] N.R. Laurel, R.L. De Torres, J.-V.S. Mendoza, M.A.O. Balendres, F.M. Dela Cueva, Identification of Epicoccum sorghinum and its effect on stalk sugar yield, Sugar Tech 23 (6) (2021) 1383–1392.
[37] X.T. Bao, D.S.P. Dharmasena, D.X. Li, X. Wang, S.L. Jiang, Y.F. Ren, et al., First report of Epicoccum sorghinum causing leaf spot on tea in China, Plant Dis. 103 (12) (2019) 3282.
[38] Q.X. Yin, S.L. Jiang, D.X. Li, H.L. Huang, Y. Wang, D.L. Wang, et al., First report of Epicoccum nigrum causing brown leaf spot in tea in Guizhou Province, China, Plant Dis. 106 (1) (2022) 321.
[39] M. Imran, S. Khanal, X.-G. Zhou, S. Antony-Babu, M. Atiq, First report of leaf spot of rice caused by Epicoccum sorghinum in the United States, Plant Dis. 106 (10) (2022) 2758.
[40] A.S. Orina, O.P. Gavrilova, N.N. Gogina, P.B. Gannibal, T.Y. Gagkaeva, Natural occurrence of Alternaria fungi and associated mycotoxins in small-grain cereals from the Urals and West Siberia regions of Russia, Toxins 13 (10) (2021) 681.
[41] S.M. El-Ganainy, S.E. El-Abeid, Y. Ahmed, Z. Iqbal, Morphological and molecular characterization of large-spored Alternaria species associated with potato and tomato early blight in Egypt, Int. J. Agric. Biol. 25 (2021) 1101–1110.
[42] H.F.D. Al-lami, M.P. You, A.E. Mohammed, M.J. Barbetti, Virulence variability across the Alternaria spp. population determines incidence and severity of Alternaria leaf spot on rapeseed, Plant Pathol. 69 (3) (2020) 506–517.
[43] S. Matić, G. Tabone, A. Garibaldi, M.L. Gullino, Alternaria leaf spot caused by Alternaria species: an emerging problem on ornamental plants in Italy, Plant Dis. 104 (8) (2020) 2275–2287.
[44] B. Wang, Y. Zhang, J. Liu, O. Sheng, F. Liu, D. Qiu, et al., A new leaf blight disease caused by Alternaria jacinthicola on banana in China, Horticulturae 8 (1) (2021) 12.

[45] Y. Lan, Z. Yan, T. Duan, Luobuma leaf spot disease caused by Alternaria tenuissima in China, J. Fungi 9 (11) (2023) 1062.
[46] M. Meena, S.K. Gupta, P. Swapnil, A. Zehra, M.K. Dubey, R.S. Upadhyay, Alternaria toxins: potential virulence factors and genes related to pathogenesis, Front. Microbiol. 8 (2017) 281961.
[47] C.R. Gnanam, Introduction to Mycology, MJP Publisher, 2019.
[48] K. Bensch, U. Braun, J.Z. Groenewald, P.W. Crous, The genus cladosporium, Stud. Mycol. 72 (2012) 1–401.
[49] C.L. Schoch, K.A. Seifert, S. Huhndorf, V. Robert, J.L. Spouge, C.A. Levesque, et al., Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi, Proc. Natl. Acad. Sci. 109 (16) (2012) 6241–6246.
[50] E. Harnelly, Hi Kusuma, Z. Thomy, S. Samingan, Internal Transcribed Spacer (ITS) gene as an accurate DNA barcode for identification of macroscopic fungus in Aceh, Biodiversitas J Biol Divers. 23 (5) (2022).
[51] A. Saleem, A.A. El-Shahir, Morphological and molecular characterization of some Alternaria species isolated from tomato fruits concerning mycotoxin production and polyketide synthase genes, Plants 11 (9) (2022) 1168.
[52] S.K. Torkpo, Y. Gafni, E.Y. Danquah, S.K. Offei, Incidence and severity of cassava mosaic disease in farmer's fields in Ghana, Ghana J. Agric. Sci. 53 (2018) 61–71.
[53] N.N. Omoyo, J. Wakhungu, S. Oteng'i, Effects of climate variability on maize yield in the arid and semi arid lands of lower eastern Kenya, Agric. Food Secur. 4 (1) (2015) 1–13.
[54] F. Robson, D.L. Hird, E. Boa, Cassava brown streak: a deadly virus on the move, Plant Pathol. 73 (2) (2024) 221–241.