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Foliar Diseases of *Hibiscus Cannabinus* L (Kenaf) and the Identity of Associated Fungi in Three Agroecological Zones of Southwest Nigeria

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Abstract: *Hibiscus cannabinus L. (Kenaf), is affected by many field diseases. However, there is limited information on the fungal diseases of the crop and the causal pathogens in South West Nigeria. Disease assessment was conducted at the research stations of the Institute of Agricultural Research and Training located in different agroecological zones namely- Rainforest (Ikenne), Derived savanna (Ilora) and rainforest/savannah Transition (Ibadan). Fungal diseases of kenaf were identified and associated fungal species were isolated. Pathogenicity test was carried out in the screenhouse using Completely Randomized Design in three replicates. Identities of fungal pathogens were determined using cultural, morphological and molecular methods. Anthracnose, Curvularia leaf spot, Coniella leaf blight and Zonate leaf spot were the major fungal diseases associated with kenaf. Pathogen identification and subsequent pathogenicity tests revealed Colletotrichum gloeosporioides Curvularia lunata, Coniella diplodiella,and Corynespora cassicola, as the causal pathogens of the diseases respectively.*
Key words: Ecological zones, Foliar diseases, Identification, Kenaf, Pathogenicity.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is an important bast fibre crop belonging to the family Malvaceae (Hidayat *et al*., 2022). Owing to its versatility, it is regarded as one of the most important natural fibre crops cultivated worldwide (Alexopoulou and Monti, 2013; Akinrotimi and Okocha, 2018). In addition to fibre production, kenaf is cultivated as a leafy vegetable for human consumption, used as forage for livestock, and serves as a raw material for several industries, including paper, textiles, biocomposites, construction, and bioenergy (Falasca *et al*., 2014; Mahmood *et al*., 2018; Hassan et al., 2023; Kujoana *et al*., 2023).

According to FAOSTAT (2020), India was the world's leading producer of kenaf during the 2017–2018 production period, contributing approximately 12% of global production, followed by China. The global kenaf market is projected to exceed USD 854 million by 2025, reflecting the increasing demand for this renewable and environmentally friendly crop (Vayabari *et al*., 2023). The growing recognition of kenaf's economic and industrial value has encouraged its cultivation beyond traditional production areas, leading to increased adoption in several countries, (Danalatos and Archontoulis, 2010) including Nigeria.

In Nigeria, kenaf cultivation is concentrated predominantly in the South-Western states, particularly Ogun and Oyo States, which account for approximately 60% of the country's total production. The North-Central and North-West regions contribute about 16.4% and 15.5% of national production, respectively (LINKS, 2020). FAOSTAT (2020) estimated that Nigeria produced approximately 1,460 tonnes of kenaf fibre, ranking fourth among African producers and thirteenth globally.

Despite its economic importance, the production potential of kenaf is constrained by several field diseases, particularly those caused by fungal pathogens, which have been identified as major production constraints in many kenaf-growing regions (Singh *et al*., 2013; Singh *et al*., 2016). These diseases reduce plant growth, fibre yield, and quality, thereby limiting the profitability and sustainability of kenaf production. As the cultivation of kenaf continues to expand across Nigeria, there is an increasing need to document the symptoms, occurrence, and distribution of its major diseases to facilitate early diagnosis, accurate pathogen identification, and the development of effective integrated disease management strategies. Therefore, this study aimed to assess the fungal diseases of kenaf and identify the associated fungal pathogens across different agroecological zones in South-West Nigeria.

Theoretical underpinning

The Disease Triangle Theory explains why foliar diseases differ across agroecological zones through the interaction of host susceptibility, pathogen virulence, and environmental conditions. Koch's Postulates validate the causal role of the isolated fungi, while plant disease epidemiology and host–pathogen interaction theories explain disease occurrence, spread, and severity. Collectively, these frameworks provide a sound scientific basis for investigating the occurrence, identification, and pathogenicity of fungal pathogens associated with foliar diseases of kenaf.

MATERIALS AND METHODS

Disease assessment

The assessment of foliar diseases of kenaf was carried out in three different agroecological zones in south western Nigeria during the planting season of 2015 and 2016 at Ibadan, located at the rain-forest savannah transition zone (Longitude 03˚84′E and Latitude 07˚38′N), Ilora at derived savannah (Longitude 003˚82′ E and Latitude 07˚81′N) and Ikenne at Rainforest (Longitude 03˚70′E and Latitude 06˚85′N), located at the Research and Training Farms of IAR&T situated at these locations. Disease assessment was done by visual observation and description of symptomized leaves.

Isolation and Identification of Fungal Pathogens

Foliar samples of kenaf exhibiting disease symptoms were collected during both cropping seasons for the isolation of associated fungal pathogens. In the laboratory, small sections about 2 mm were excised from the advancing margins of lesions, including portions of adjacent healthy tissue. The pieces were surface-sterilized in 1% sodium hypochlorite (NaOCl) solution for 1 min, rinsed five times with sterile distilled water, blotted dry on sterile filter paper, and aseptically transferred onto Potato Dextrose Agar (PDA) medium amended with streptomycin (300 µg/l) in 90-mm Petri dishes. The plates were incubated at 27 ± 1°C for seven days.

Emerging fungal colonies were purified using the hyphal-tip technique (Sinclair and Dhingra, 2017). Subculturing was repeated until pure cultures of each isolate were obtained.

The purified isolates were characterized based on their cultural and microscopic characteristics. Colony morphology, including colour, texture, growth pattern, and margin characteristics, was recorded after incubation. For microscopic examination, a small portion of mycelium bearing reproductive structures was mounted on a clean glass slide in a drop of sterile distilled water and examined under a compound microscope (Leica Microsystems). Morphological features such as hyphal characteristics, conidial shape, size and septation were observed and compared with standard taxonomic descriptions. Identification of the fungal isolates was carried out using the keys and descriptions provided by Watanabe (2002), Dugan (2008), and Barnett and Hunter (2010).

Pathogenicity Test of Fungal Isolates

The pathogenicity of all fungal isolates obtained from diseased kenaf leaves was evaluated under greenhouse conditions. A conidial suspension of each isolate was prepared by flooding the surface of a 5-day-old pure culture grown on Potato Dextrose Agar (PDA) with sterile distilled water and gently scraping the colony surface to dislodge the spores. The resulting suspension was used as the inoculum.

Healthy four-week-old kenaf plants were inoculated with the respective fungal suspensions, while control plants sprayed with sterile distilled water only. Immediately after inoculation, each plant was covered with a transparent polyethylene bag for 24 h to maintain high relative humidity and promote infection. The plants were maintained in a greenhouse at 28 ± 2°C and monitored daily for symptom development.

Following symptom expression, diseased tissues were collected from the inoculated plants, and the associated fungi were re-isolated using the same isolation procedure described previously. The cultural and microscopic characteristics of the re-isolated fungi were compared with those of the original isolates to confirm their identity in accordance with Koch's postulates. Pathogenicity tests were conducted for all fungal isolates recovered during the 2016 cropping season.

The fungal isolate confirmed as the causal pathogen through pathogenicity testing was subsequently subjected to molecular characterization to validate its taxonomic identity.

Molecular characterization of the pathogenic fungi

The molecular identification was carried out by extracting DNA from pure cultures of each pathogenic fungi using a Biospin Fungus Genomic DNA Extraction Kit (Bio- Flux®) following the instructions of the manufacturer.

Polymerase Chain Reaction (PCR) was carried out in order to amplify the internal regions in the ribosomal genes using the One Taq Quick Lad master mix with standard buffer. Each reaction mix contained 2.5 µL of buffer of 10X reaction, 1.25 µL of MgCl, 0.5 µL of dNTP's, 1.0 µL of ITS 1 and 4, 0.5 of Taq DNA polymerase, 2.0 µL of DNA and 16.25 µL of injectable water to obtain a final volume of 25 µL . The amplification was performed with initial denaturing temperature of 95 ºC for 5 minutes, followed by 30 cycles at 95 ºC for 45 seconds for the denaturing, 57 ºC for 45 seconds for the hybridization and 72 ºC for 1 minute for the extension and a final cycle of 72 ºC for 5 minutes for the final extension and a final refrigeration temperature of 10 ºC. The quality of the DNA was determined by loading 5 μl of the DNA template on 1% agarose gel at 45 V for 30 minutes. After agarose gel electrophoresis with a standard DNA ladder, the gels were viewed under ultra violet gel imager.

The amplified regions were sequenced and submitted for sequencing at Inqaba Africa Genomic platform in South Africa along with the primers used for amplification (ITS1 and ITS4). The sequences obtained were analyzed using Finch TV software and phylogenetic analyses was determined using Mega 7.0 (Kumar *et al*., 2016; Sidiq, 2016). The sequences obtained were submitted to the GenBank and accession number assigned. The fungal isolates were assigned species by comparing the sequences with those in NCBI database.

RESULTS

Symptoms of Fungal Diseases Associated with Kenaf

Four fungal diseases, namely Curvularia leaf spot, zonate leaf spot, Coniella leaf blight and anthracnose, were consistently observed on kenaf plants across the three study locations in South-West Nigeria during both the 2015 and 2016 cropping seasons.

Curvularia leaf spot was characterized by the development of small reddish-brown lesions with tan to greyish centres on infected leaves (Figure 1a). As the disease progressed, the necrotic tissue detached from the leaf lamina, producing characteristic shot-hole symptoms (Figure 1b).

Zonate leaf spot initially appeared as small, circular lesions approximately 3 mm in diameter (Figure 2 a). With disease progression, the lesions enlarged and developed distinct concentric rings, giving them a characteristic target-board or zonate appearance (Figure 2b).

Coniella leaf blight was characterized by the appearance of light brown, water-soaked lesions on the leaf surface (Figure 3a). These lesions gradually expanded, coalescing to cover large portions of the leaf blade. At advanced stages of infection, the affected tissues became tan to straw coloured, resembling bleached tissue (Figure 3b). Numerous black pycnidia subsequently developed within the necrotic lesions, indicating the reproductive structures of the pathogen.

Anthracnose symptoms were distinguished by irregularly shaped necrotic lesions scattered on the leaf surface (Figure 4a and 4b). The lesions possessed brittle, light brown to grey centres surrounded by distinct dark reddish-brown margins.

Phenotypic characteristics of the fungi associated with the disease symptom

The cultural characteristics of each fungus associated with the identified disease symptoms were given in figure 5. The figure showed the color, growth pattern and conidia shapes of each isolate with detailed description in Table 1.

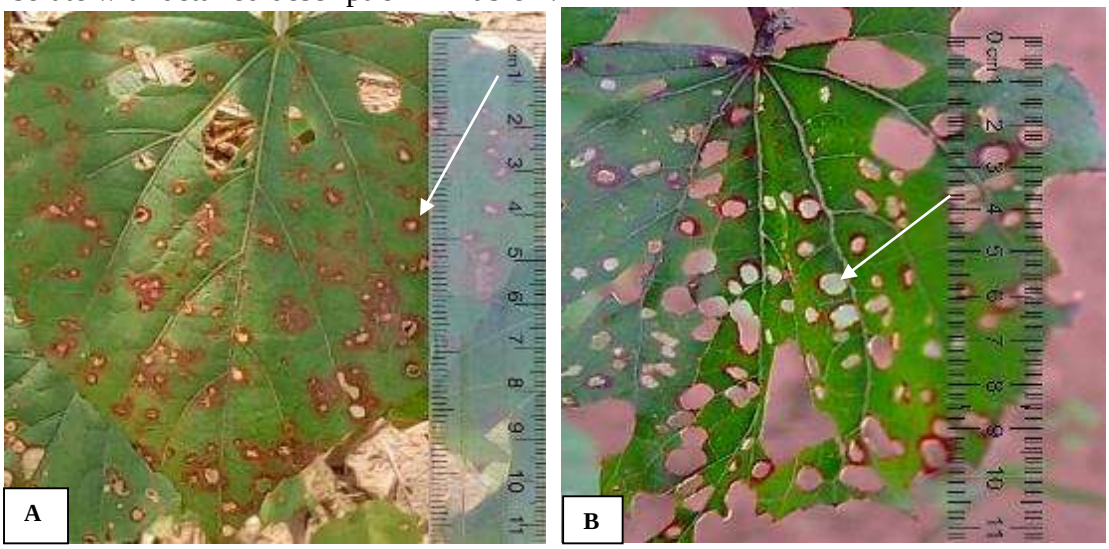

Figure 1: Curvularia leaf spot disease in kenaf showing brown and ash spots with red margin at early stage (arrowed) (A) and B showed the disease at the advanced stage with holes at the advanced stage (arrowed).

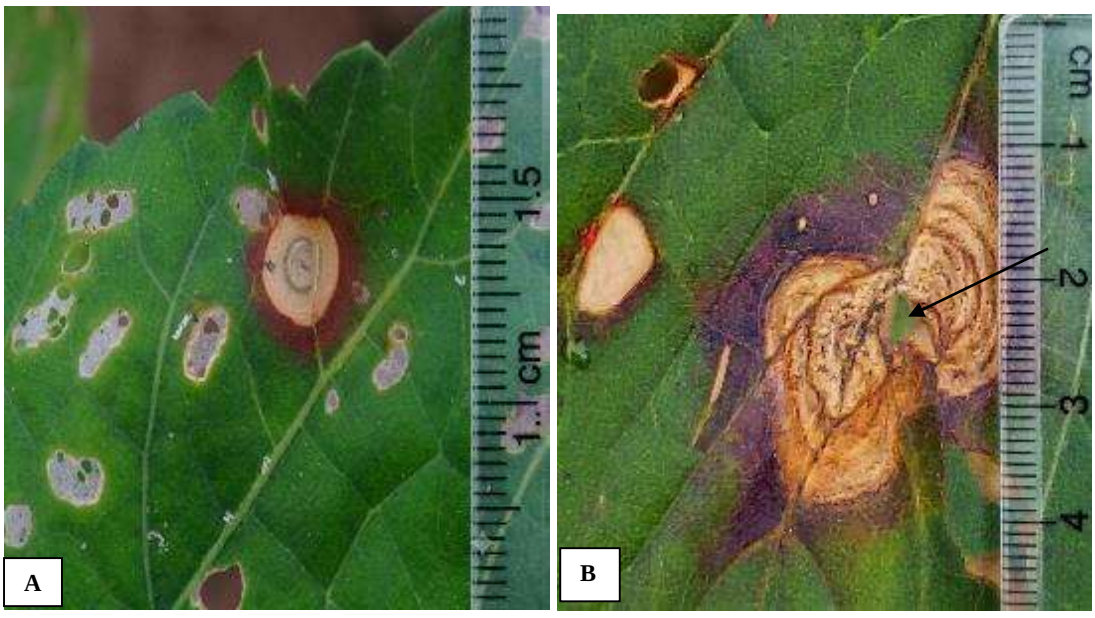

Figure 2. Zonate leaf spot disease of kenaf showing (a) ring spots with circular centre at the early stage (arrowed), (b) advanced stage of the disease symptom showing concentric necrotic areas and defoliation on the leaves (arrowed).

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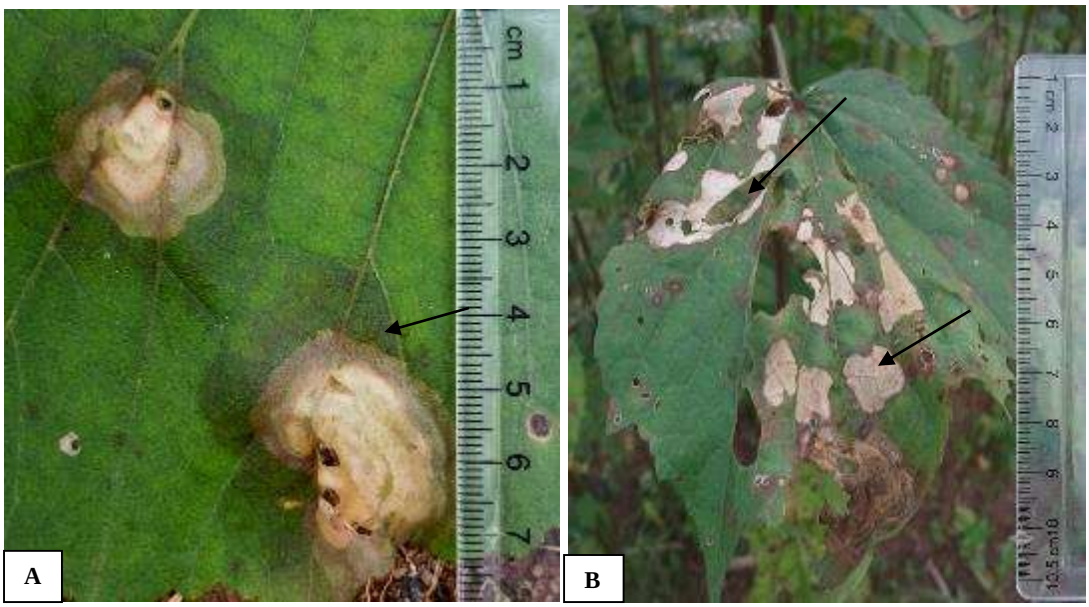

Figure 3. Coniella leaf blight disease in kenaf showing (a) light brown spot with borders appearing as though water-soaked at early stage (arrowed) and (b) whitish spots appearing as if bleached, and perforated areas at the advanced stage (arrowed).

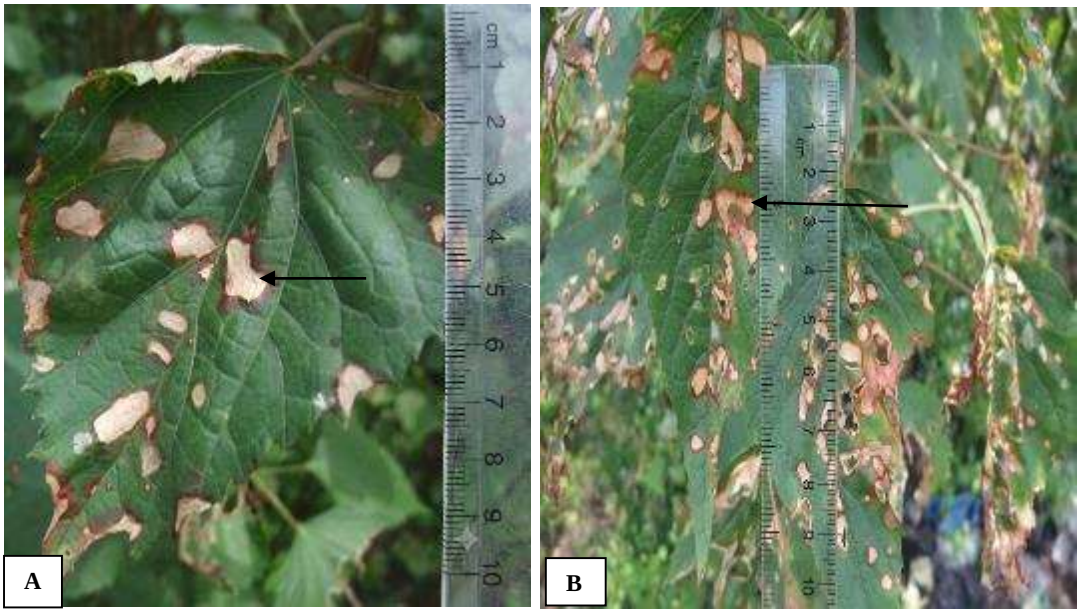

Plate 4: Anthracnose disease of kenaf showing (a) small and dark red spot, irregular in shape at early stage of the disease (arrowed) and (b) spots with ash to white centre and red borders at the advanced stage (arrowed).

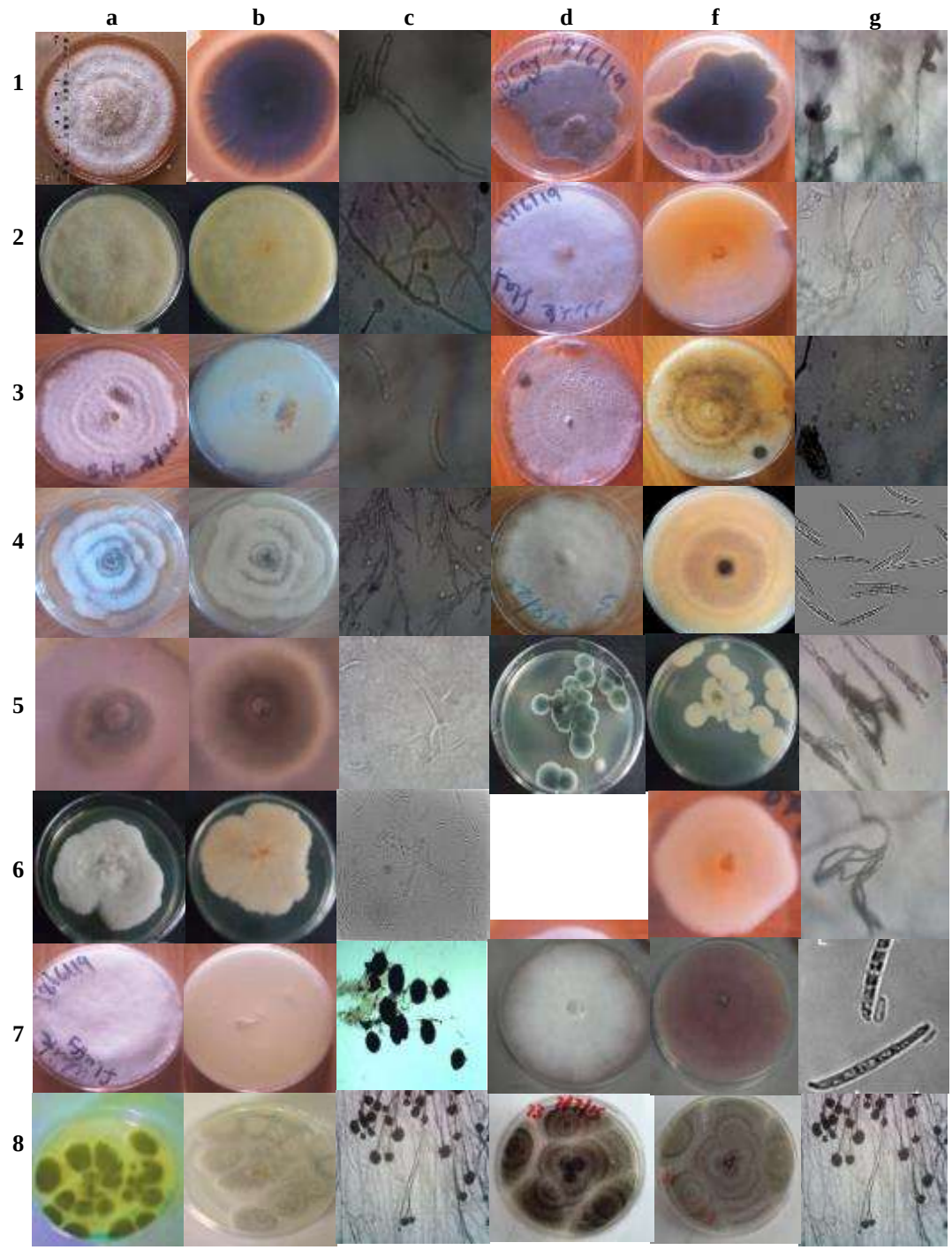

Figure 5: Cultural and morphological characteristics of the fungal isolates; *C. cassicola* (1a-surface, 1b- reverse,1c- conidia) *C. lunata* (1d-surface, 1e- reverse, 1f - conidia) *R. stolonifer* (2a-surface, 2b- reverse, 2c- sporangiophore), *C. gloeosporioides* (2d-surface, 2e- reverse, 2f- conidia) *F. chlamydosporium* (3a-surface, 3b- reverse, 3c- conidia) *C. diplodiella* (3d-surface, 3e- reverse, 3f- conidia) *Beltrania* sp (4a- surface, 4b- reverse, 4c- conidia) *F. sacchari* (4d-surface, 4e- reverse, 4f- conidia), *C. capscici* (5a-surface, 5b- reverse, 5c- conidia), *P. oxalicum* (5d-surface,5 e- reverse, 5f- conidia), *F. oxysporum*, (6a-surface, 6b- reverse, 6c- conidia), *P. lilacinus* (6d-surface, 6e- reverse, 6f- conidia), *N oryzae* (7a-surface, 7b- reverse, 7c- conidia), *Fusarium fujikuroi* (7d- surface, 7e- reverse, 7f- conidia), *A. flavus* (8a-surface, 8b- reverse, 8c- conidia), *A. niger* (8d- surface, 8e- reverse, 8f- conidiophore)

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Organism	**Colony characteristics**			**Morphological characteristics**	
	Surface colour	Reverse colour	Colony texture	Conidia shape	Conidia septation
Aspergillus niger	White colony with typical black spores	Cream	Velvety	Round to globose	None
`Rhizopus stolonifer	White to light gray	Off-white	Cottony	Globose to ovoid or ellipsoidal	Septate
Fusarium chlamydosporum	White zonated colony	Off-white	Thick wooly	Slightly curve	Septate
Penicilium oxalicum	Grayish green surrounded by white border	Beige	Suede	Ellipsoidal	No septation
Aspergillus flavus	Yellowish green	Cream	Powdery	Globose	No septation
Coniella diplodiella	White	Luteous	Flat and powdery	Fusiform, narrowly ellipsoidal	
Beltrania sp	Umber at the centre and dirty white at the outer region. The surface is folded with smooth edges.	Dirty white	Floccose	Biconic	No septation
Fusarium sacchari	Whitish to off white	Lilac	Dense cottony	Slender, slightly curved	Septate
Fusarium oxysporum	Pinkish white	Light pink	Fluffy	Cylindrical to boat shaped	Septate
Paecelomyces lilacinus	Pink to light red	Off-white	Wooly	Ovoid to fusoid	Non septate
Corynespora cassiicola	Light gray	Black	Thick powdery	Long ellipsoidal to cylindrica	Septate
Fusarium fujikuroi	Whitish to light purple colony	Off-white	powdery	Macroconidia slender and straight	Septate

Curvularia lunata	Shinning black with rough edges	Black	Suade	Curved slightly with expanded third cell from the pole end	Transversely Septate
Colletotrichum gloeosporioides	White raised circular colony	Light orange	Slightly fluffy	Oblong	None septate
Nigrospora oryzae	White thick	Off-white	Thick powdery	Globose	None septate
Colletotrichum capsici	Gray to black colony	Off-white	Powdery	Slightly curve fusiform	Septate

Table 1: Phenotypic characteristics of the fungal isolate from Curvularia leaf spot, Zonated leaf spot, Coniella leaf blight, Anthracnose and Leaf blight diseases and their identification

Pathogenicity test of fungi isolated from the diseased symptoms

Table 2 presents the fungal isolates recovered from the different disease symptoms observed on kenaf and the corresponding pathogens identified as their causal agents. Pathogenicity tests and subsequent re-isolation confirmed that the symptoms produced by *Curvularia lunata* were identical to those observed under field conditions, establishing it as the causal agent of Curvularia leaf spot. Likewise, *Coniella diplodiella* was confirmed as the causal pathogen of Coniella leaf blight, *Colletotrichum gloeosporioides* was identified as the causal agent of anthracnose, and *Corynespora cassiicola* was confirmed as the pathogen responsible for zonate leaf spot. The characteristic symptoms produced following artificial inoculation were consistent with those observed in naturally infected plants, thereby fulfilling Koch's postulates and confirming the pathogenicity of the respective fungal isolates. Incubation period after inoculation and subsequent disease progression of each pathogenic fungi is presented in Table 3.

Genotypic characterization of the pathogenic fungi

Representative fungal isolates initially identified morphologically as *Curvularia lunata*, *Corynespora cassiicola*, *Coniella diplodiella*, and *Colletotrichum gloeosporioides* were further subjected to molecular characterization to confirm their identities. The resulting DNA sequences were deposited in the GenBank database and assigned the accession numbers **OK345525**, **OK345523**, **OK345522**, and **OK345521** for *C. lunata*, *C. cassiicola*, *C. diplodiella*, and *C. gloeosporioides*, respectively. BLAST analysis against the National Center for Biotechnology Information (NCBI) database showed more than 99% sequence similarity with authenticated reference sequences, confirming the identities of the isolates and validating the initial morphological identification (Table 4).

Fungi	*Curvularia* leaf spot	Zonate leaf spot	Coniella leaf blight	Anthracnose
Aspergillus flavus	+	-	-	-
Aspergillus niger	+	-	-	-
Beltrania sp	-	+	-	-
Colletotrichum capsici	+	-	-	-
Colletotrichum gloeosporioides	-	-	-	+
Coniella diplodiella	-	-	+	-
Corynespora cassiicola	-	+	-	+
Curvularia lunata	+	-	-	-
Fusarium chlamydosporium	+	-	+	-
Fusarium fujikuroi	-	-	-	-
Fusarium sacchari	+	-	-	+
Fusarium oxysporum	-	-	-	+
Nigrospora oryzae	+	-	-	-
Paecelomyces lilacinus	+	-	-	-
Penicillium oxalicum	-	+	+	+
Rhizopus stolonifer	-	-	-	-

Table 2: Fungi isolated from disease symptoms of kenaf in Ikenne, Ibadan and Ilora (+ present, - absent)

Days after inoculation	***Curvularia lunata***	***Colletotrichum gloeosporioides***	***Corynespora cassiicola***	***Coniella diplodiella***
0	Plants inoculated	Plants inoculated	Plants inoculated	Plants inoculated
4	Small brown lesions with tan centres appeared	Small dark reddish-brown lesions appeared	Small brown circular lesions appeared	No visible symptoms
5	Lesions increased slightly in number and size	Lesions increased in number and became irregular in shape	Lesions enlarged with faint concentric rings	Small light brown, water-soaked lesions appeared
7	rapid increase in lesion number with coalescence of adjacent lesions	lesions enlarged with extensive irregular necrosis	lesions enlarged with distinct concentric rings and yellow halos	water-soaked lesions enlarged and spread over the leaf surface
10	Lesions enlarged, resulting in partial leaf blighting and initial defoliation	Severe necrosis developed with extensive lesion coalescence	Moderate to severe leaf blight developed	Lesions expanded to cover approximately 25% of the leaf area
14	Extensive leaf blight accompanied by marked defoliation	Severe necrosis with extensive defoliation	Severe blight with extensive defoliation	Lesions became pale whitish, dry and brittle
21	severe defoliation with extensive leaf necrosis	complete leaf blight and severe defoliation	extensive defoliation and severe blighting	numerous black pycnidia developed on the necrotic lesions

Table 3. Incubation period and disease progression of *C. lunata.*, *C. gloeosporioides*, *C. cassiicola*, and *C. diplodiella* following artificial inoculation of Kenaf plant under controlled conditions

Isolate (accession number)	Query length (bp)	Similarity (%)	Organisms with highest identity in NCBI (Accession number)
OK345525	530	100	*Curvularia lunata* (KX347474, KT283672), *Curvularia verruculosa* (MN215715, MF568070, MM215714)
OK345523	525	100	*Corynespora cassiicola* (MK429872, MN733135, MK571393, KF493657)
OK345522	580	99.82	*Coniella diplodiella* (MK532913)
OK345521	539	100	*Colletotrichum gloeosporioides* (KC342045)

Table 4: Closest organism of isolated fungi and maximum similarity percentage based on a BLAST search analyses of ITS gene sequence
BLAST (Basic Local Alignment Search Tool)
ITS (Internal Transcribe Spacer)
NCBI (National Centre for Biotechnology Information)

Phylogenetic trees were constructed using ITS nucleotide sequences retrieved from the NCBI GenBank database, with a sequence similarity cutoff of ≥ 95% and 1,000 bootstrap replicates. Figure 6 presents the phylogenetic tree of *Curvularia lunata*. The isolate clustered closely with reference isolates of *Curvularia lunata*, including accession KP901305, the unidentified *Curvularia* sp. (MT497417), and *Exserohilum rostratum* (MN599624), indicating a close evolutionary relationship within the family. Figure 7 shows the phylogenetic tree of *Corynespora cassiicola*, where the study isolate grouped closely with *C. cassiicola* reference strains MW300948 and MG334433.1, confirming its species identity. At the 95% similarity cutoff, the *Coniella diplodiella* isolate formed a distinct clade with *Coniella vitis* (MG764053) and *Melanconiella hyperopta* (MH866004), as shown in Figure 8. Similarly, the *Colletotrichum gloeosporioides* isolate clustered within the *C. gloeosporioides* clade alongside reference strain MT729918 (Y40), forming a well-supported monophyletic group (Figure 9).

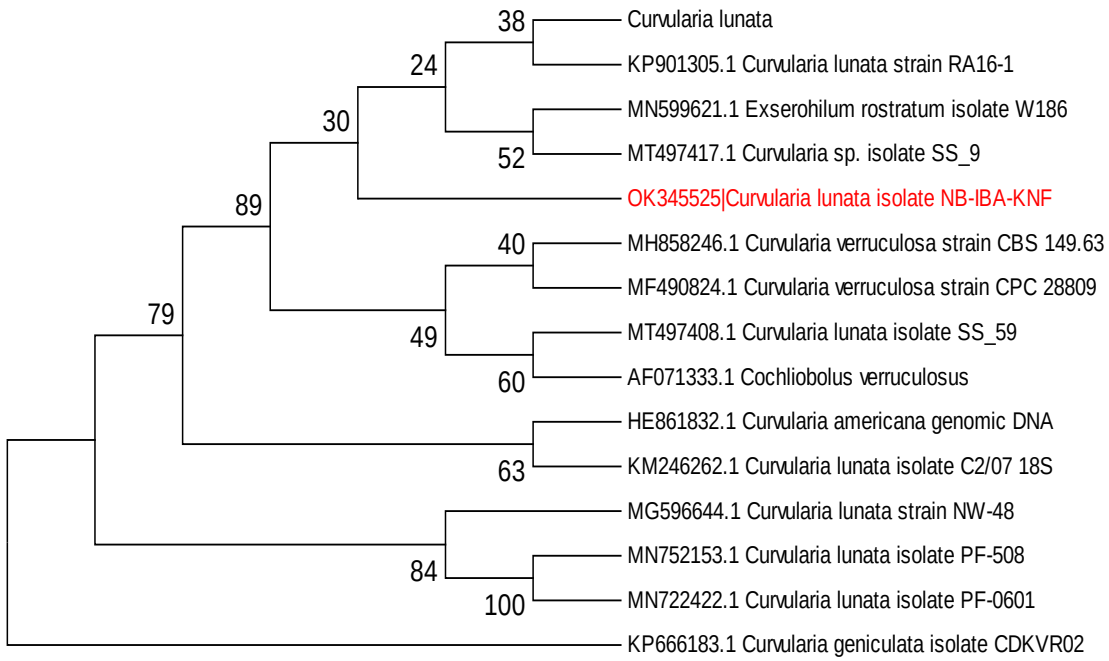

Figure 6: Neighbor-joining tree showing the phylogenetic position of *Curvularia lunata* (in red) obtained from curvularia leaf spot disease of kenaf

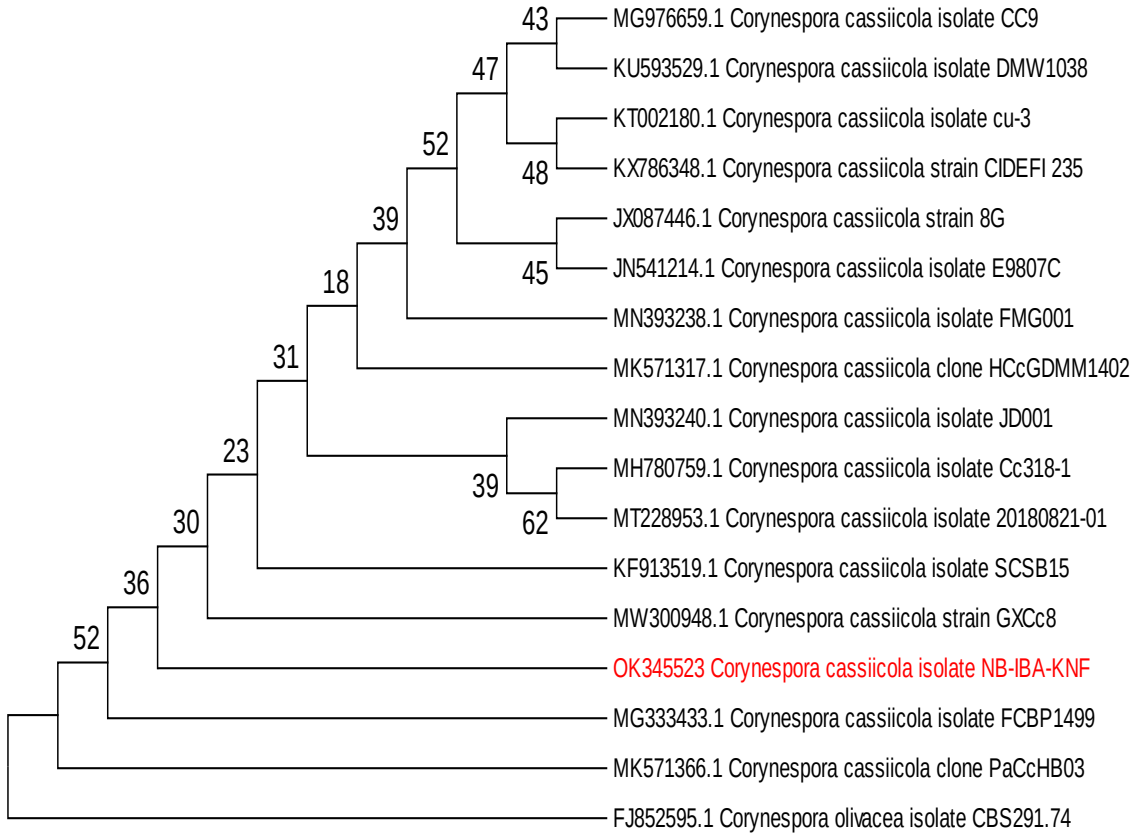

Figure 7: Neighbor-joining tree showing the phylogenetic position of *Corynespora cassiicola* (in red), obtained from zonate leaf spot disease of kenaf.

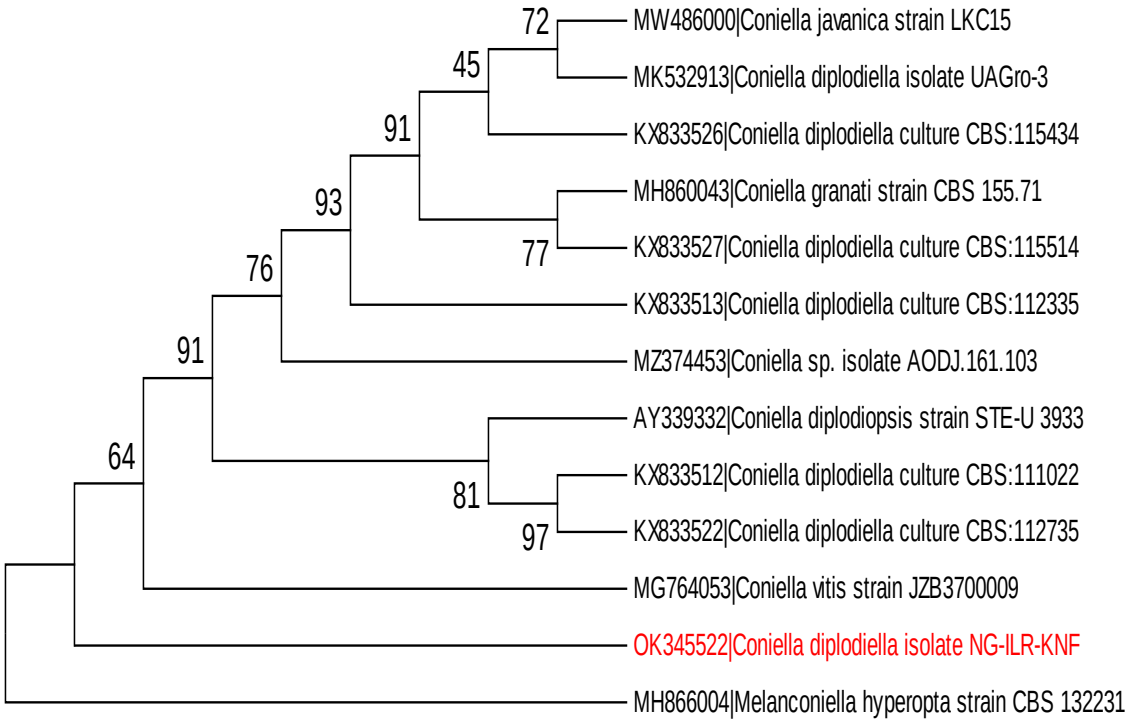

Figure 8: Neighbor-joining tree showing the phylogenetic position of *Coniella diplodiella* (in red), obtained from Coniella leaf blight disease of kenaf.

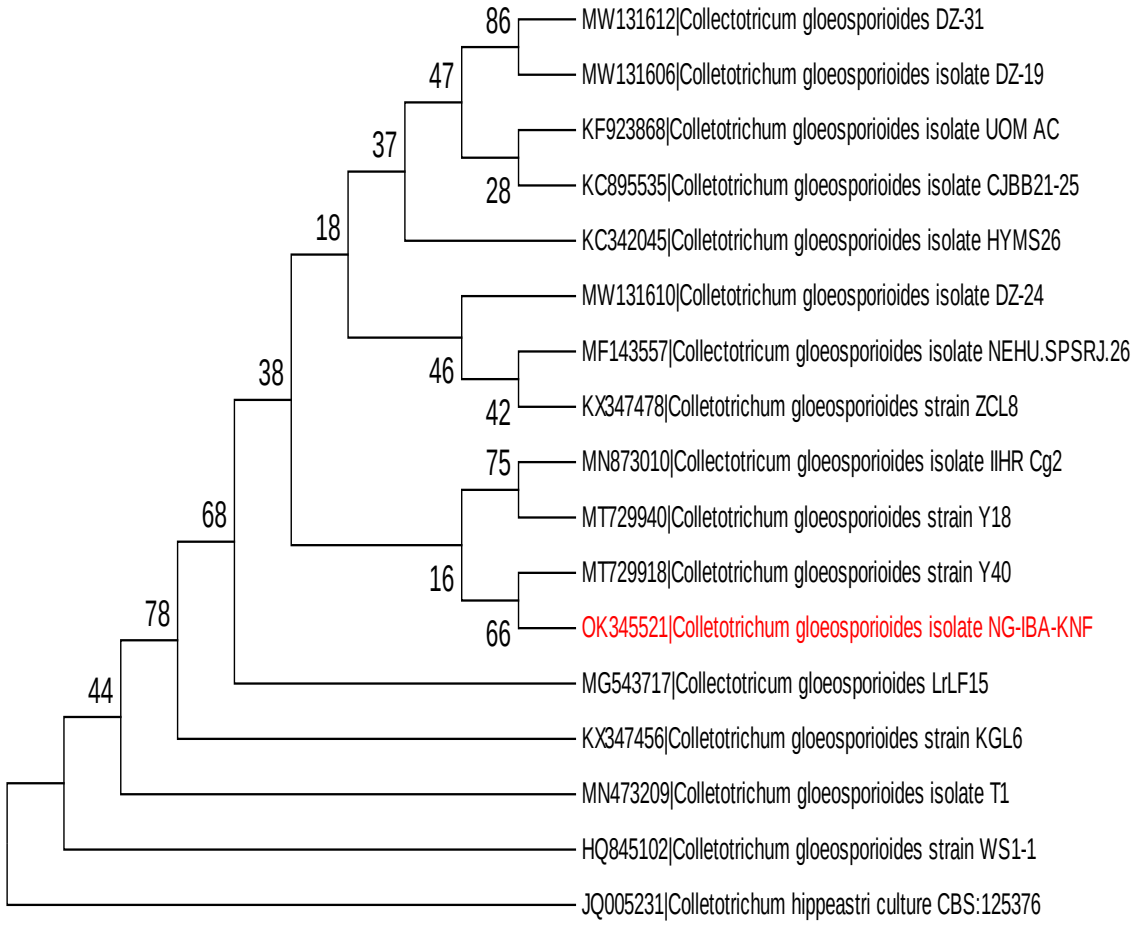

Figure 9: Neighbor-joining tree showing the phylogenetic position of *Colletotrichum gloeosporioides* (in red), obtained from anthracnose disease of kenaf.

DISCUSSION

The present study revealed the occurrence of Curvularia leaf spot, zonate leaf spot, anthracnose, and Coniella leaf blight on kenaf in southwestern Nigeria. These foliar diseases have previously been reported in association with kenaf in other production regions (Cho *et al*., 2012; Singh *et al*., 2013). For example, Mullin *et al*. (1993) reported Curvularia leaf spot on kenaf in Texas and identified the causal organism as *Curvularia senegalensis*. Species of *Curvularia* have also been implicated in leaf spot diseases of several economically important crops, including *Digitaria exilis* in Benin (Zinsou *et al*., 2020), hemp (*Cannabis sativa*), a close relative of kenaf (McPartland and Cubeta, 1997), and tomato (*Solanum lycopersicum*) in Egypt (Ismail *et al*., 2016). In the present study, morphological characteristics and ITS rDNA sequence analysis identified the pathogen associated with kenaf leaf spot as *Curvularia lunata*. To the best of our knowledge, this is the **f**irst report of *Curvularia lunata* causing leaf spot disease of kenaf in Nigeria, thereby expanding the known host-pathogen association and geographical distribution of this pathogen and providing important baseline information for disease diagnosis and the development of effective disease management strategies.

The identity of the causal pathogen was further confirmed through molecular characterization using the internal transcribed spacer (ITS) region amplified with the universal ITS1 and ITS4 primers, which are widely employed for fungal identification (Slippers *et al*., 2004; Begoude *et al*., 2010). Phylogenetic analysis of the ITS sequences showed that the isolate clustered within a well-supported clade containing reference isolates of *Curvularia lunata*, thereby confirming its taxonomic identity. The combined morphological and molecular evidence provided reliable identification of *C. lunata* as the causal agent of Curvularia leaf spot of kenaf.

The occurrence of anthracnose disease on kenaf observed in this study corroborates previous reports from different parts of the world (Douglas, 2011; Singh *et al*., 2013; Kwon *et al*., 2015; Cheng *et al*., 2020). Anthracnose has also been reported on roselle (*Hibiscus sabdariffa*), another economically important member of the family Malvaceae, in Nigeria (Apeyuan *et al*., 2017), as well as on white jute (*Corchorus capsularis*) (Niu et al., 2016) and ramie (*Boehmeria nivea*) (Wang *et al*., 2010) in China. These reports demonstrate the broad host range and economic importance of anthracnose-causing fungi among fibre and related crops.
In the present study, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. was identified as the causal agent of anthracnose on kenaf based on cultural and morphological characteristics, and its identity was subsequently confirmed through ITS sequence analysis. The colony morphology observed was consistent with previous descriptions of *C. gloeosporioides* isolated from fruits and vegetables (Sharma and Kulshrestha, 2015; Ma *et al*., 2014; Akgül *et al*., 2016). The molecular identification was further supported by phylogenetic analysis, in which the kenaf isolate clustered closely with authenticated *C. gloeosporioides* reference sequences from GenBank.

The pathogenic role of *C. gloeosporioides* observed in this study is consistent with previous reports describing the species as an important causal agent of anthracnose on numerous crops. Together with other *Colletotrichum* species, including *C. acutatum, C. boninense, C. karstii*, and *C. godetiae, C. gloeosporioides* has been reported to cause anthracnose of avocado (Onyeani and Amusa, 2015; Kimaru *et al*., 2018; Valencia *et al*., 2011). In addition, *C. gloeosporioides* has been identified as the primary causal agent of anthracnose in pepper (Li *et al*., 2021) and banana (Riera *et al*., 2019). The findings of the present study therefore extend the known host range of *C. gloeosporioides* and provide evidence that it is an important pathogen associated with anthracnose of kenaf in southwestern Nigeria.

Zonate leaf spot was also observed on kenaf in the present study and was confirmed through morphological and molecular characterization to be caused by *Corynespora cassiicola* (Berk. & Curt.) C.T. Wei. While the occurrence of zonate leaf spot on kenaf agrees with previous reports by Colyer *et al*. (1992) and Cho *et al*. (2012), the causal pathogen identified in those studies was *Cristulariella moricola*, which differs from the findings of the present study. This variation may be attributed to differences in geographical location, environmental conditions or pathogen diversity. Cho *et al*. (2012) reported *C. moricola* as the causal agent of zonate leaf spot in China, whereas Colyer *et al*. (1992) documented the disease in Louisiana, USA. In contrast, the present study identified *C. cassiicola* as the pathogen associated with zonate leaf spot of kenaf in southwestern Nigeria.

Corynespora cassiicola is a cosmopolitan fungal pathogen that is particularly prevalent in tropical and subtropical regions (Puia *et al*., 2021). The fungus has an exceptionally broad host range, infecting more than 530 plant species across numerous botanical families (Farr *et al*., 2019). Its wide distribution, broad host spectrum, and adaptability to diverse environmental conditions have established it as one of the most economically important foliar pathogens in tropical agriculture. The phylogenetic analysis performed in this study further confirmed the identity of the kenaf isolate, as it clustered closely with authenticated *C. cassiicola* reference sequences retrieved from GenBank, thereby providing robust molecular evidence for its identification.

The occurrence of Coniella leaf blight disease on kenaf had been previously reported by Adeoti and Emechebe (1996) on kenaf in the Northern part of Nigeria. The description of the disease given by the authors is similar to the observations in this study, Nejo,*m*. (2007) and Apeyuan, (2016) reported the same disease on *Hibiscus sabdariffa* in Abeokuta and Makurdi respectively. Pathogenicity test carried out in the study confirmed *Coniella diplodiella* (Spegazzini) Petrak & Sydow as the causal pathogen of the disease. However, contrary to the report of this study, *Coniella musaiensis* was reported as the causal pathogen of Coniella leaf blight on Kenaf (*Hibiscus cannabinus*) in Northern Nigeria by Adeoti and Emechebe (1996). Apeyuan *et al*. (2016) and Nejo *et al*. (2007) gave similar report on roselle (*Hibiscus sabdarifa*), stating *Coniella musaiensis* as the causal pathogen of Coniella leaf blight on roselle. The occurrence of *C. diplodiella* causing Coniella leaf blight on kenaf is therefore the first report of such occurrence. Furthermore, the genus *Coniella* had been reported to consist of several plant pathogens causing diseases of important agricultural crops (Apeyuan *et al*. 2016; Alvarez *et al*., 2016; Liu *et al*., 2020).

Implication

This research study provides the first comprehensive understanding of the major foliar fungal diseases affecting kenaf across three agroecological zones of Southwest Nigeria and confirms the pathogenic status of the associated fungi. The findings also provide a scientific basis for developing effective disease management strategies and breeding resistant varieties.

CONCLUSION

This study provides a comprehensive assessment of the major foliar fungal diseases affecting kenaf (*Hibiscus cannabinus* L.) in southwestern Nigeria. Field surveys revealed the occurrence of Curvularia leaf spot, zonate leaf spot, anthracnose, and Coniella leaf blight as the predominant foliar diseases of kenaf in the study area. The causal pathogens were isolated,

characterized using cultural and morphological features, and their pathogenicity was confirmed by fulfilling Koch's postulates.

Molecular characterization based on ITS rDNA sequencing and phylogenetic analysis further confirmed the identities of the pathogens as *Curvularia lunata, Corynespora cassiicola, Colletotrichum gloeosporioides,* and *Coniella diplodiella*. The phylogenetic analyses demonstrated that the isolates clustered closely with authenticated reference sequences retrieved from GenBank, providing strong molecular support for their identification. The integration of morphological, pathogenicity, and molecular evidence ensured accurate identification of the pathogens associated with the observed diseases.

The study also documents important new host-pathogen associations in Nigeria. To the best of our knowledge, this is the first molecularly confirmed report of *Curvularia lunata* causing Curvularia leaf spot, *Corynespora cassiicola* causing zonate leaf spot, and *Coniella diplodiella* causing Coniella leaf blight of kenaf in Nigeria. These findings expand the known geographical distribution and host range of these pathogens and provide baseline information for future epidemiological studies.

Overall, the results of this study contribute significantly to the understanding of the etiology and diversity of fungal pathogens associated with kenaf foliar diseases in Nigeria. The information generated will serve as a valuable resource for disease diagnosis, resistance screening, breeding programmes, and the development of integrated disease management strategies.

Future Research

Future research could focus on epidemiology of the identified fungal pathogens, including their sources of inoculum, modes of dissemination, seasonal dynamics, and survival between cropping seasons. Study can be carried out of effect of climatic factors on the incidence and severity of each disease over a period of time for the purpose of disease forecast. Screening of different kenaf genotypes for resistance to the foliar diseases and the development of integrated disease management strategies.

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