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Inhibitory effects of kenaf (*Hibiscus cannabinus* L.) derivatives on the growth of causal pathogen of leaf blight disease of sunflower (*Helianthus annuus* L.)

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Abstract

Sunflower (*Helianthus annuus* L.) is an important oilseed crop, but its productivity is significantly constrained by fungal diseases, particularly leaf blight caused by *Alternaria* spp. This study investigated the isolation, identification, and pathogenicity of fungi associated with leaf blight symptoms in sunflower, and evaluated the inhibitory effects of extracts derived from kenaf (*Hibiscus cannabinus* L.) biochar, core, and leaf on the growth of the pathogen. Diseased sunflower leaves were collected, surface sterilized, and cultured on Potato Dextrose Agar (PDA) for fungal isolation. The isolates were identified based on cultural and morphological characteristics. Pathogenicity tests were conducted to confirm the causal pathogen of leaf blight disease. The causal pathogen was subjected to molecular evaluation to ascertain its identity. The antifungal activity of kenaf-derived extracts was assessed at 1% and 5% concentrations using the poisoned food technique. Radial mycelial growth of *Alternaria tenuissima* was measured and compared with an untreated control. Results showed that all extracts significantly ($p \leq 0.01$) reduced fungal growth compared to the control. Among the treatments, kenaf core extract at 5% concentration exhibited the highest inhibitory effect having colony diameter of 3.2 mm followed by leaf and biochar extracts, with 3.30 mm and 3.35 mm colony diameter respectively. The findings demonstrate that kenaf-derived materials possess bioactive compounds with antifungal properties capable of suppressing *A. tenuissima* growth. This suggests their potential as eco-friendly alternatives to synthetic fungicides. Further studies are recommended to isolate and characterize the active compounds and to validate their effectiveness under field conditions.

Introduction

Sunflower (*Helianthus annuus*) is an annual herbaceous plant in the family of *Asteraceae* mostly grown for its seed that contains edible oil (FAO, 2021; USDA, 2022). It is one of the most important industrial crops in the world because of its edible vegetable oil, which ranks after soybean and rapeseed oil in production volume (Adeleke and Babalola, 2020). The oils contain saturated fatty acids (Omega 3 and Omega 6), Vitamins A, D, and E which are preferred by Cardiologist because of its health benefit (Jilo *et al*., 2025). However, its productivity is significantly constrained by various fungal diseases. Among these, leaf blight, caused by *Alternaria* sp, It is one of the most widespread and damaging disease of sunflower (Chattopadhyay, 1999; Wang *et al*., 2014; Kgatle, 2019; Wang *et al*., 2019). The disease manifests as large necrotic spots which coalesce on leaves, reducing photosynthetic efficiency and leading to premature defoliation, thus resulting to economic losses and food insecurity. Controlling plant diseases is crucial for maintaining environmental sustainability, ensuring food safety and preventing future outbreak (Mwangi *et al*., 2023; FAO, 2024).

The widespread use of synthetic fungicides has raised concerns regarding environmental pollution, pathogen resistance, and human health risks (Ahmad *et al*., 2024; Kumar and Donthi, 2024; Okagu *et al*., 2023). Consequently, there is growing interest in

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sustainable alternatives derived from plant materials and organic residues. Botanicals have been widely reported to possess antimicrobial properties due to the presence of bioactive compounds such as phenolics, flavonoids, and terpenoids (Gupta *et al*., 2022; Elshafie, *et al*., 2023).

Kenaf (*Hibiscus cannabinus*), a multipurpose fibre crop, has gained attention not only for its industrial applications but also for its bioactive potential (Norhisham *et al*., 2023). Different parts of the plant, including leaves, core, and biochar derived from its biomass, have been shown to exhibit antimicrobial and antifungal activities (Ryu *et al*., 2017; Arulrajah *et al*., 2021).

Despite these promising attributes, limited information exists on the comparative antifungal efficacy of kenaf-derived extracts against major sunflower pathogens. Therefore, this study is aimed to isolate, identify, carry out pathogenicity of fungi associated with leaf blight disease of sunflower and investigate the efficacy of kenaf biochar, core and leaf aqueous extracts on the causal pathogen.

Materials and Methods

Collection and Isolation of Fungi

Diseased sunflower leaves showing typical Alternaria symptoms (brown necrotic lesions with concentric rings) were collected from the research field of Institute of Agricultural Research and Training during the 2025 growing season. Samples were stored in sterile bags and transported to the laboratory for analysis.

The infected leaf tissues were surface sterilized with 1% sodium hypochlorite for 2 minutes, rinsed in 5 changes of sterile distilled water, blotted dry and cut into small pieces. Sterilized tissue fragments were plated on Potato Dextrose Agar (PDA) augmented with streptomycin sulphate ($50mgL^{-1}$) to prevent bacterial growth. the plates were later incubated at 25°C for 5 days. Emerging fungal colonies were sub-cultured using the hyphal-tip method to obtain pure cultures.

Morphological Identification of fungal isolates

Fungal isolates were identified based on colony morphology (color, texture, growth rate) and microscopic features (shape and septation of conidia). Following identification guides by Barnet and Hunter (2010), Watanabe, (2010) and Petrini and Petrini (2013).

Pathogenicity Test of the fungal Isolates

Pathogenicity test was carried out in the screen house of the institute (IAR&T). Surface sterilized sunflower seeds were planted at 2 seeds in 10 kg sterilized soil in a 10 L plastic. At 4 weeks after planting the healthy sunflower plants were inoculated with spore suspensions (1×10^6 spores/mL) prepared from 7-day-old fungal cultures grown on PDA by spraying spore suspensions on leaves. Controls were treated with sterile water. Each pot was covered immediately with transparent polythene bags for 42 hrs to maintain high humidity withi the plant region for quick and effective fungal establishment according to Oduwaye and Enikuomehin (2013). Fungi were re-isolated from inoculated plants to confirm their role as the causal agent. Visual assessment of symptoms was done 14 days after inoculation.

Molecular identification of the pathogenic fungi

The pathogenic isolate, preliminarily identified as *Alternaria* sp. based on morphological characteristics, was further analyzed using molecular techniques to confirm its species-level identity. Genomic DNA was extracted from pure cultures of Alternaria sp using a Biospin Fungus Genomic DNA Extraction Kit (Bio-Flux®) according to the instructions of the manufacturer (Beijing BioMays Biotechnology Co., Ltd).

Polymerase Chain Reaction (PCR) amplification was performed following the protocol of White *et al* (1990). Agarose gel electrophoresis was later carried out. A 1.5% agarose gel was prepared using 1 x TBE-buffer and stained with 5 μl of SYBR Safe and poured into casting tray having a comb to solidify. The first well of the solid gel was loaded with 1.5 μl 1 Kb marker, followed by 2 μl of each amplified product and a control at the end. The gel was connected to electric voltage of 100 V for 45 minutes to allow migration of amplified PCR products. The DNA bands formed were visualized under UV light and images photographed using a camera connected to a computer.

The amplified products of the target fragments obtained above were cleaned using the Qiagen PCR cleaning kit according to the manufacturer instructions.

The cleaned fragments were submitted for Sanger sequencing at Inqaba Africa Genomic platform in South Africa together with the primers used for amplification (ITS1 and ITS4). Alignment of sequences of the isolates and phylogenetic analyses were determined using Mega 7.0 (Kuimar *et al*., 2016).

Assessment of fungicidal effect of the extract of kenaf biochar, core and leaf on the mycelial growth of Alternaria sp.

Preparation of extracts from Kenaf biochar, core and leaf

Kenaf materials were collected from Kenaf and Jute Improvement program of IAR&T. The leaves and core were washed, air-dried, and ground into powder. The biochar was produced by pyrolyzing kenaf biomass. The grinded kenaf leaf and core weighing 1 g, and 5 g was soaked in 100 ml of sterile distilled water separately, while 0.2 g of biochar was soaked in 20ml and 4ml of sterile distilled water to make 1% and 5% respectively. This was soaked for 24 hrs and sieved using sterilized muslin cloth. The extracts were sterilized under UV-light for 2 hrs at 254 nm wavelength.

Assessment of fungicidal effect of the extract on the mycelial growth of Alternaria sp.

One millilitre of each extract was dispensed into 90 mm diameter Petri dishes and sterilized molten PDA was added to each Petri dish and swirled to allow uniform distribution of the extracts. Actively growing margin of the fungal colonies were picked with a 2 mm diameter cork borer and gently placed at the centre of the plates. The control plates were inoculated with each of the fungus but no extract was added. Each test was replicated three times and the plates were incubated at room temperature for 7 days. Percentage inhibition of mycelial growth was calculated using the Equation:

$$\text{Growth inhibition (\%)} = \frac{dc - dt}{dc} \times 100$$

where: *dc* is diameter of fungal colony in control treatment and *dt* is diameter of fungal colony with treatment.

Results and Discussion

A total of six fungal pathogens were isolated from the diseased symptoms and were identified morphologically as *Rhizopus* sp, *Botrytis cinerea*, *Cladosporium* sp, *Fusarium* sp., *Cuvularia* sp and *Alternaria* sp. (Table 1). *Alternaria* sp. developed dark brown to black colonies characterized by septate hyphae and muriform conidia arranged in chains, which is consistent with standard descriptions of the genus (Dong *et al* 2024; Sudarshan *et al*., 2025). Similarly, the morphological characteristics observed in this study are in close agreement with previous reports, Wang *et al*. (2019) described *Alternaria* sp. associated with sunflower leaf blight as forming zonated dark brown to black colonies. Alternaria had also been found to infect seeds of sunflower (Oduwaye *et al*., 2025) which is one of the main causes of sunflower plant infection.

Cladosporium sp. produced olive-green to dark colonies with branched conidiophores bearing chains of conidia, a diagnostic feature widely reported in earlier studies (Bensch *et al*., 2018: Amirmijani, 2019). Colonies of *Curvularia* sp. were fast-growing and greyish to black, with distinctive curved, multicell conidia possessing enlarged central cells, in agreement with established morphological keys (Manamgoda *et al*., 2014; Marin-Felix *et al*., 2020). *Botrytis cinerea* exhibited grey, fluffy mycelial growth with profuse conidia typical of grey mould pathogens, as previously described by Williamson *et al*. (2007). *Rhizopus* sp. demonstrated rapid growth, initially forming cottony white colonies that later turned grey to black due to sporangia development, which aligns with standard taxonomic descriptions (Watanabe, 2010; Sanap and Chavan, 2024). These consistencies validate the reliability of the morphological identification approach used in this study.

The pathogenicity test confirmed *Alternaria* sp as the causal pathogen of Alternaria leaf blight disease of sunflower. *Alternaria* sp produced necrotic lesions with concentric rings on the leaf (Plate 1). The symptoms produced by *Fusarium* sp, *Botrytis* cinerea, *Curvularia* sp, *Rhizopus* sp and *Cladosporium* sp were different from the symptoms produced by *Alternaria* sp. This finding is consistent with earlier reports that identified *Alternaria alternata*, *Alternaria tenuissima*

and *Alternaria helianthi* as the most destructive foliar pathogens of sunflower worldwide causing leaf blight disease of sunflower (Gulya *et al*., 2013, Wang *et al*., 2019). *Alternaria* species are known for their ability to produce host-specific toxins and cell wall-degrading enzymes, which facilitate rapid colonization and lesion expansion. The occurrence of *Cladosporium* sp. and *Curvularia* sp. suggests their involvement either as weak pathogens or secondary invaders. These fungi are commonly reported as foliar pathogens in several crops and may contribute to disease severity by enlarging lesions and accelerating tissue necrosis (Chaiwan *et al*., 2025). Their presence alongside *Alternaria* sp. may indicate synergistic interactions that enhance disease progression (Sikora *et al*., 2024).

Botrytis cinerea is pathogen that is typically associated with grey mould disease under high humidity conditions (Petrasch *et al*., 2019; Bi *et al*., 2023). Its presence in this study likely reflects opportunistic colonization of already infected or senescing tissues. Similarly, *Rhizopus* sp. is generally considered a saprophytic fungus but may act as a secondary colonizer, contributing to rapid tissue maceration in advanced stages of infection. (Lui *et al*., 2024; Mesquida-Pesci *et al*., 2025)

Molecular analysis further confirmed the identity of *Alternaria* sp. The *Alternaria* isolate clustered closely with reference sequences of *Alternaria tenuissima*, (Figure 1) with registered accession number PQ319781 having 100% similarity, thus, confirming its specie identity. The short branch lengths observed indicate high genetic similarity between the isolates and their respective reference sequences.

Molecular identification based on ITS sequencing and phylogenetic analysis is widely recognized as a reliable method for accurate fungal classification (Jeon *et al*., 2023). the clustering of the isolates with reference sequences confirms it identity as *Alternaria tenuissima*. Similar clustering patterns have been reported in recent studies, where closely related fungal isolates grouped within the same clades, reflect high genetic similarity (Ribaaoui *et al* 2022; Aung *et al*., 2024).

Table 1. Morphological characteristics of the fungal isolates associated with Alternaria leaf spot disease of sunflower

Organism	Colony characteristics			Morphological characteristics	
	Surface colour	Reverse colour	Colony texture	Conidia shape	Conidia septation
Rhizopus sp	White to light gray	Off white	Cottony	Ovoid to ellipsoidal	None
Botrytis cinerea	Dirty white	Dark gray	cottony	Ellipsoidal	No septation
Cladosporium spp	Brown-black	Cream	Tufted	Cylindrical	No septation
Curvularia lunata	Shinning black with rough edges	Black	Suade	Curved slightly with expanded third cell from the pole end	Transversely Septate
Fusarium sp	Whitish to pink colony	Off-white		Macroconidia slender and straight	Septate
Alternaria sp	Dark gray to black	Dark	Thick velvety	Club or Obclavate, sometimes ovoid or ellipsoidal shaped with black scars at the beaked end	Septate

Plate 1. Sunflower leaf showing symptoms of leaf blight at the early stage of development

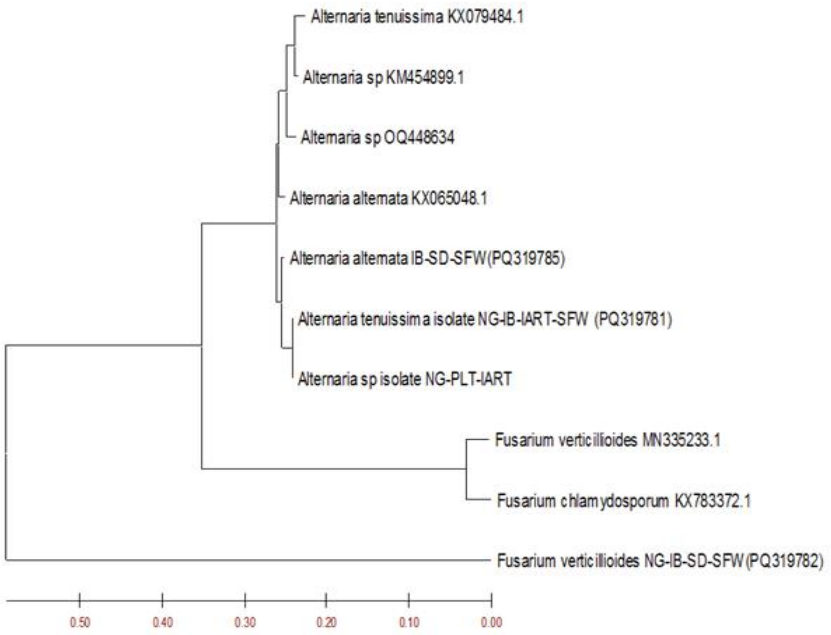

Figure 1. Neighbor-joining tree based on ITS-rDNA gene sequence similarity showing the phylogenetic position of *Alternaria* sp. (NG-PLT-IART) underlined in red

All treatments had significant and varying effect on the radial growth of *Alternaria tenuissima* colony with respect to the control ($P \leq 0.01$) (Table 2). These findings demonstrate that extracts from Kenaf core, biochar and leaf possess significant antifungal activity against *A. tenuissima*., the causal pathogen of Alternaria leaf blight of sunflower. The consistent reduction in radial growth across all treatments compared to the control confirms the inhibitory potential of these kenaf-derived products. This agrees with earlier reports that plant-based extracts contain bioactive compounds capable of suppressing fungal pathogens (El-Shahir *et al*, 2022; Cenobio-Galindo *et al*., 2024; Merino-Ramirez and Salvador-Reyes, 2026). Kenaf extracts have been reported to possess broad-spectrum antimicrobial activity, (Adnan 2020; Norhisham, *et al*., 2023). Furthermore, Oduwaye *et al*., (2024) reported the efficacy of extracts from kenaf as effective against leaf spot disease pathogen of kenaf (*Cecospora* sp), Kobaisy *et al*, (2001), also reported that kenaf leaf essential oil exhibit direct fungitoxic activity against *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, and *Colletotrichum acutatum*.

The most effective treatment is the Core extract at 5% having a radial diameter of 3.20 mm with respect to the control which have 3.90 mm diameter. Similarly, Biochar at 5% and Leaf at 5% also show moderate inhibition on the mycelial growth of Alternaria. The observed higher antifungal activity of the woody core extract may be attributed to differences in phytochemical composition among plant tissues. In many plants, structural tissues such as stems and cores are known to contain lignin-associated phenolic compounds and other secondary metabolites that contribute to antimicrobial activity (Abdul Khalil *et al*., 2023). In kenaf, variations in the distribution of bioactive compounds between leaves, stems, and other

plant parts have been reported, with certain fractions of the stem biomass exhibiting significant biological activity (Adnan *et al*., 2020; Sim & Nyam, 2021). This suggests that the woody core of kenaf may harbor higher concentrations of antifungal compounds compared to the leaves or biochar.

Table 2. Variation in the fungitoxic ability of kenaf biochar, core and leaf extract on *Alternaria tenuissima*

Treatment	Mean Radial Growth (mm)	Percent Inhibition
Biochar 1%	3.40 bc	12.82 ab
Biochar 5%	3.35 bc	14.10 ab
Core 1%	3.65 ab	6.41 b
Core 5%	3.20 c	17.95 a
Leaf 1%	3.40 bc	12.82 ab
Leaf 5%	3.30 bc	15.38 ab
Control	3.90 a	

Means with the same letter along the column are not significantly different at 1% probability according to Duncan's Multiple Range Test

Figure 2 showed the pooled effect of the two concentrations used. The 5% concentration showed a significantly lower mycelia radial growth. The observed dose-dependent response, where 5% concentrations were more effective than 1%, further supports the role of concentration in enhancing antifungal efficacy. Increased extract concentration likely corresponds to a higher availability of active compounds, thereby intensifying their inhibitory effects. This trend is consistent with previous studies on plant extracts, which demonstrate that antifungal activity increases with concentration due to greater interaction between bioactive compounds and fungal cells (Gupta and Sharma, 2021).

Table 3 shows the relationship between the radial growth and percent inhibition. An inverse relationship was observed between the radial growth of *A. tenuissima* and percent inhibition. this inverse relationship is consistent with recent studies in which the radial growth corresponds with increased antifungal efficacy of plant extracts (Abbass *et al*. 2022,

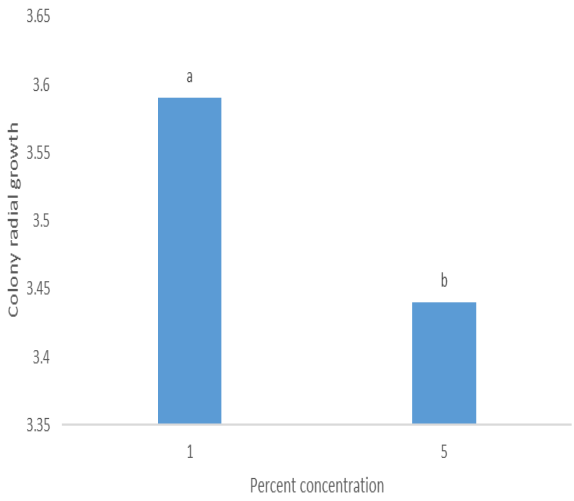

Figure 2. Effect of concentration on colony diameter of radial growth of *A. tenuissima*

Table 3. Relationship between radial growth of *A. tenuissima* and percent inhibition

Treatment	*A. tenuissima* _radial growth	*A. tenuissima* _inhibition
A. tenuissima _radial growth	1.00	-1.00**
A. tenuissima inhibition		1.00

Conclusion and Recommendations

The study revealed that sunflower leaf blight is associated with a complex of fungal pathogens, with *Alternaria tenuissima* identified as the primary causal organism. Other fungi, including C*ladosporium* sp., *Curvularia* sp., *Botrytis cinerea*, *Fusarium* sp and *Rhizopus* sp., play secondary roles that may increase disease severity.

Kenaf (*Hibiscus cannabinus*) derivatives were discovered to be effective in inhibiting fungal pathogens of sunflower with core extract at 5% concentration showing the highest antifungal activity. Kenaf-based extracts therefore represent promising, environmentally friendly alternatives to synthetic fungicides.

Further studies should be carried out to determine the active antifungal compounds in kenaf core. Also, the extracts should be evaluated under greenhouse and

field conditions for their efficacy in controlling leaf blight disease of sunflower.

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