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Antifungal activity of endophytic fungi extracts isolated from *Vitellaria paradoxa* against phytopathogenic fungi in cucumber crops

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ABSTRACT

This study assessed the antifungal activity of crude extracts from endophytic fungi isolated from *Vitellaria paradoxa* against *Fusarium oxysporum*, *F. solani*, *F. demerum*, *F. cladosporum*, *Aspergillus niger*, and *A. flavus* associated with cucumber plants. Morphological and molecular methods identified three suspected endophytes (MIK1, MIK2, and MIK3) as *A. niger*, *A. welwitschiae* and *Rhizopus arrhizus*. The endophyte culture broths were incubated on an orbital shaker for 14 days at 28 °C and 120 rpm and then extracted with organic solvents. The ethyl acetate yielded (4.57 g), methanol (4.18 g), and n-*hexane* (3.68 g) crude extracts. The fungal endophytes (MIK1, MIK2, and MIK3) were screened for their antagonistic activity against six pathogenic fungi using a dual culture method. The results show that all the endophytes strongly inhibit the growth of fungi, with inhibition rates of 92% and 99%. The antifungal activity of the crude extracts was tested using the poisoned food technique at concentrations of 0.25, 0.50, 1.0, 1.5, and 2.0 µg/mL. The results indicate that the ethyl acetate extract of *A. niger* exhibits potent inhibition against the growth of the same fungus, with inhibition rates of 78.18% and 79.12% at 2.0 µg/mL. All the fungal crude extracts possess antifungal activity and can be used to manage the six fungal species tested. Further field experiments should be conducted for practical applications against other plant pathogens to reduce reliance on synthetic agrochemicals.

KEYWORDS: Antagonistic activity, *Fusarium*, Inhibition rates

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INTRODUCTION

The annual production yield losses caused by agricultural pests, particularly phytopathogenic fungi and weeds, account for approximately 13%, estimated at $220 billion worldwide (Abdel-Fattah *et al.*, 2011; Alwathnani & Perveen, 2012). This is a serious concern in the region, where the people's economic livelihoods depend on agriculture. Cucumber (*Cucumis sativus*) is a widely cultivated crop affected by many fungal diseases, particularly *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum*. This soil pathogen invades the plant's vascular system, leading to severe reductions in both yield and quality (Wang *et al.*, 2016; Sun *et al.*, 2017).

Synthetic pesticides are a significant contributing factor to the highly productive modern intensive agriculture. Still, a heavy reliance on them has created certain problems, such as adverse effects on human and animal health, the environment, production costs, the long-term impact of pesticide residues in agricultural products, and the development of resistance in weeds and crop pathogens (Cullen *et al.*, 2019). To minimize these problems, cheap and environmentally friendly management strategies must be found.

Biological control is identified as one of the alternatives to reduce the problems and is also suitable for sustainable agricultural programs. Research in biocontrol of crop fungal diseases and weeds using endophytic fungi has increased and yielded positive results over the years (Ferrigo *et al.*, 2016).

Endophytic fungi are known for their mutualistic and antagonistic association with plants, where they grow within plant tissues for at least part of their life without causing any apparent symptoms of disease (Hyde *et al.*, 2019). They

colonize various plant tissues, including roots, stems, leaves, and seeds, providing many benefits to the host plant, such as growth promotion, resistance to pathogens and pests, and increased tolerance to environmental stresses. Beyond their ecological significance, endophytic fungi produce a diverse range of secondary metabolites, including terpenoids, flavonoids, alkaloids, quinines, phenolics, and hydrocarbons, which are essential for applications in agriculture, biotechnology, medicine, and environmental remediation (Naik & Krishnamurthy, 2010). These bioactive compounds play an important role in plant defense against different pathogens and as biological control agents (Joseph & Priya, 2011; Nair & Padmavathy, 2014).

Colletotrichum gloeosporioides isolated from *Psidium guajava* was found to produce *guajadial*, a sesquiterpene with remarkable antifungal activity against *Candida albicans* (Packiaraj *et al.*, 2016). Similarly, *Pestalotiopsis microspora* produces pestalotiopsin A, a polyketide derivative with inhibitory effects against various plant-pathogenic fungi, making it a potential biocontrol agent for sustainable agricultural practices (Jiang *et al.*, 2023). Moreover, the endophytic genus *Alternaria* is a ubiquitous group that grows in diverse ecosystems, producing a broad array of secondary metabolites, primarily including polyketides, nitrogen-containing compounds, quinones, and terpenes (Deshmukh *et al.*, 2022; Zhao *et al.*, 2023).

Endophytic fungi have also been found to exhibit antagonistic activity against phytopathogenic organisms by inducing physiological changes and modifying gene expression in plants, thereby increasing plant productivity through enhanced photosynthesis. Thus, utilizing extracts from these species could be a highly successful, environmentally friendly, and cost-effective management tool that enhances agricultural production (Sahoo *et al.*, 2017). Thus, the ecological roles played by endophytic fungi make them suitable for application in agriculture and the pharmaceutical industry; as such, they can be utilized for the development of bio-pesticides (Lata *et al.*, 2018).

It has been reported that endophytic fungi of the same species may be isolated from the same plant. However, only one of the endophytes can produce a highly physiologically active compound when grown in culture media (Mukherjee *et al.*, 2012; Arora & Ramawat, 2017; Debnath *et al.*, 2019). Mukherjee *et al.* (2012) reported that the fungus endophyte *Trichoderma* sp., found in various environments, is the source of more than 60% of biofungicides now licensed globally. Similarly, *Fusarium* sp., *Pythium* sp., *and Rhizoctonia* sp. have also been successfully controlled by the endophytes *Trichoderma* sp. (Park *et al.*, 2019). According to Talapatra *et al.* (2017), *Aspergillus* was the only fungus against which the endophytic fungi *T. viride* demonstrated antagonistic activity; however, their hyphae overgrew on the mycelium of the pathogen.

Endophytic fungi represent a rich source of specialized metabolites and other compounds with antibiotic activity (Mousa & Raizada, 2013; Lugtenberg *et al.*, 2016). They can produce a wide range of bioactive compounds, including alkaloids, flavonoids, peptides, phenols, quinones, steroids, terpenoids, polyketides, and volatile organic compounds (VOCs). Terpenoids and polyketides have been reported to have antibiotic activity (Lugtenberg *et al.*, 2016).

Many studies have reported the production of biodiesel, hydrocarbon and hydrocarbon derivatives with fuel potential by some of the fungal endophytes such as *Gliocladium sp.*, *Ascocoryne sarcodies*, *Phoma*, and *Hypoxylon sp.* (Singh & Singh, 2010; Tomsheck *et al.*, 2010; Ahamed & Ahring, 2011; Strobel *et al.*, 2011). Another study by Yuan *et al.* (2011) demonstrated that the endophytic fungus *Muscodor* sp. produces volatile, biologically active molecules that inhibit pathogenic microbes. Mends *et al.* (2012) reported the potential of an endophytic fungus, *Nodulisporium* sp., isolated from *Myroxylon balsamum*, against several pathogens, demonstrating inhibitory activity against these pathogens.

Medicinal plants produce novel bioactive compounds with different functional roles and pharmaceutically important effects (such as antibiotics, immunosuppressants, antioxidants, antiarthritic, antimicrobial, antidiabetic, anticancer, and anti-inflammatory activities) that could be exploited to meet the need for new therapeutic agents to treat human diseases (Dar *et al.*, 2023; Gell, 2024; Radha *et al.*, 2024). These compounds are typically extracted from natural sources or synthesized through microbial fermentation or transformation. Among these methods, biotransformation has been more widely used owing to its promising applications (Liu *et al.*, 2021). This particular application of endophytes has been receiving increased attention (Feng & Cui, 2025). Taxol ($C_{47}H_{51}NO_{14}$) is a diterpenoid produced by an endophyte, *Metarhizium anisopliae*, which colonizes within the bark of the *Taxus brevifolia* tree (Liu *et al.*, 2009). Taxol has garnered significant attention as a promising anticancer drug due to its unique mode of action, which prevents the depolymerization of tubulin molecules during cell division (Pandi *et al.*, 2013).

Vitellaria paradoxa is a medicinal plant rich in a plethora of phytochemicals that have been utilized in confectionery, cosmetics, and pharmaceutical industries due to their wide-ranging pharmacological properties, including anticancer, antibacterial, antidiabetic, antioxidant, anti-inflammatory, and antifungal properties (Ojo *et al.*, 2021). Furthermore, *V. paradoxa* is one of the most preferred tree species, with various functions among 79 other plants (Dimobe *et al.*, 2018). Among the several studies presented in the literature (Lokonon *et al.*, 2017; Tamou *et al.*, 2023; Ahoyo *et al.*, 2024), limited information is available on harnessing the potential of the endophytes of this plant. In this study, endophytes were isolated from *V. paradoxa*, and their extracts were tested against phytopathogenic fungi of cucumber crops.

METHODOLOGY

Sample Collection and Preparation

Healthy fresh leaves, bark, and fruits were collected from the *Vitellaria paradoxa* plant at Bagaj district in Karu Local Government Area, Nasarawa State, Nigeria. The samples were

brought to the laboratory in separate clean paper envelopes for processing. The plant materials were further identified at the herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, Bayero University, Kano. A voucher specimen number (BUKHAN 0489) has been deposited in the herbarium for further reference. The plant materials were air-dried in the laboratory for 14 days and then blended to form a fine powder, which was sieved through a 2 mm mesh. The samples were stored separately in sterile Ziplock bags for further analysis.

Preparation of Cultural Media

The pathogenic and endophytic fungi were cultivated on Potato Dextrose Agar (PDA). About 40 g of medium was added to 1.5 L of distilled water and homogenized. The prepared medium was then autoclaved at 121 °C for 15 minutes at a pressure of 2 atm before use and subsequently poured into separate sterile Petri dishes under a laminar airflow hood. After cooling, the plates were maintained in an upright position for culturing fungi. Although PDA was chosen in this study, it is expensive because of its simple formulation and can support the sporulation of several fungi (Devi *et al.*, 2018).

Isolation and Culture of Endophytic Fungi

The isolation and identification of endophytic fungi were done as described previously. Endophytic fungi were isolated from dry powdered leaves, bark, and seeds of *V. paradoxa* before surface sterilization. Samples of each tissue were washed with tap water, immersed in 70% ethanol for 3 minutes, followed by 1% sodium hypochlorite for 3 minutes, and then rinsed in sterilized distilled water 2-3 times to remove disinfectants. Two (2.0 g) powdered samples were blotted dry with sterile Whatman No.1 filter paper and placed on 90 × 15 mm PD Petri plates. The inoculated plates were then incubated in an upright position at 28 °C for 7 days (Larran *et al.*, 2007). After the incubation period, the growth of the different fungal strains was observed. Sub-culturing was carried out to obtain pure isolates (MIK1, MIK2, and SB3), which were maintained separately on fresh PDA slants at -4 °C in the Forest Biotech Laboratory, Department of Forest Management, Faculty of Forestry, UPM, Serdang, Malaysia, for further use. The pure cultures of the suspected endophytic fungal strains were morphologically identified based on mycelial growth patterns and spore characteristics. Thin mycelia pieces of the pure culture were picked from the edge of the plate with a sterile needle and spread on the glass slide with a drop of distilled water added. The air-dried covered slip slides were examined under a light microscope at X40 and X100 magnifications at the Department of Forestry Management, University Putra Malaysia. Photographs of the fungal preparation were taken using a Nikon Eclipse E200 microscope and a Nikon Digital Sight DS-L1 camera (Nikon Corporation, Tokyo, Japan) (Rana *et al.*, 2019).

DNA Extraction

Fungal DNA isolation was achieved using the Favor PrepTM Fungi/Yeast Genomic DNA Extraction Mini Kit according to the manufacturer's protocol as described by Gul *et al.* (2016). About 10 g of genomic DNA was ground in liquid nitrogen using a mortar and pestle. Purity and concentration were determined using a Nano-Drop 2000 Spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). After extraction of total DNA, the fungi were identified by amplification of the ITS1 primer region (5'TCCGTAGGTGAACCTGCGG3') and the ITS4 primer region 5'TCCTCCGCTTATTGATGATGC3'.

Gel Electrophoresis

The DNA fragments were separated by electrophoresis in a 1% agarose gel using 1x TAE buffer (90 mM Tris-acetate and two mM EDTA, pH 8). They were stained with Fluorescein and analyzed using a FluorChemTM (Alpha Innotech, USA) to verify quality and purity. The DNA bands were visualized under ultraviolet (UV) light.

PCR Amplification

The isolated DNA was amplified by polymerase chain reaction (PCR) using MyCyclerTM (Bio-Rad, USA). The amplified DNA was further purified in a total volume of 25 µL using exTEN PCR Master Mix and 1 µL of each primer (10 µM). Optimal PCR efficacy was achieved with an initial denaturation at 95 °C for 15 minutes, followed by 35 cycles of 1 minute at 94 °C, 2 minutes at 72 °C, and a final extension at 72 °C for 10 minutes. The PCR-amplified products were sent to First Base Laboratory Sdn Bhd, Selangor, Malaysia, for sequencing. The DNA ITS sequences region and the base sequence data were compared using the BLAST database of the NCBI GenBank.

Sequence Alignment and Phylogenetic Analysis

The DNA ITS sequencing was performed on both strands of the submitted DNA fragments at First Base Laboratory Sdn Bhd, Selangor, Malaysia. The nucleotide sequences obtained of the endophytic fungal strains were compared with the sequences available in the NCBI databases using the BLAST homology search. A phylogenetic tree was constructed based on ITS sequence data for the fungal isolates and reference strains using the neighbor-joining method in MEGA 7.0 software (Kumar *et al.*, 2018). The consensus nucleotide sequence data for the selected isolates were deposited in the NCBI GenBank using the BLASTn tool.

Extraction of Fungal Crude Extracts

A 5 mm diameter mycelial disc for each endophytic fungus (MIK1, MIK2, and MIK3) was cultivated by inoculating the discs into 250 mL Erlenmeyer flasks containing 100 mL PDB medium (Yashavantha *et al.*, 2015). The flasks were incubated on an orbital shaker (SK-300) at 28 °C and 120 rpm for 14 days, until the carbon sources were depleted (Fawole & Yahaya, 2017). At the end of the incubation period, the fermented broth cultures were shaken thoroughly and kept overnight at the Fume Hood until two transparent, immiscible layers formed.

An equal volume (1:1) of ethyl acetate (EtOAc), methanol (MeOH), and *n-hexane* were added, and the mycelia were filtered with Whatman filter paper No. 1. The supernatants were then transferred into the separating funnel for separation of the organic layers containing the metabolites. They were washed with a 2 M brine solution to remove impurities, and the crude metabolites were concentrated by vacuum evaporation at 40 °C to 5 mL, followed by vacuum drying to obtain the organic crude extracts (Shah *et al.*, 2023). The extraction was repeated twice, and the final crude extracts obtained were weighed and stored in separate sterile vials at -4 °C in the Faculty of Forestry and Environment Laboratory, University Putra Malaysia, for GC-MS analysis.

Test Organisms

The six phytopathogenic fungi *F. solani*, *F. oxysporum*, *F. demerum*, *F. Cladosporium*, A. *niger*, and A. *flavus* used in this study were isolated from infected cucumber tissues (leaves and fruits) collected from Paringida drip - irrigated vegetable farm in Kura Local Government, Kano state, Nigeria (Figure 1). The samples were separately washed in running water and surface sterilized by immersion in 70% ethanol for 3 min, and 1% sodium hypochlorite (NaOCl) solution for 3 min, followed by washing (three times) in sterilized distilled water. The samples were cut into small pieces using sterilized scissors, dried in a sterile laminar flow hood, and then placed on Potato Dextrose Agar (PDA) in Petri dishes. The Petri dishes were incubated at 28 °C for 7 days. Pure cultures were obtained by subculturing the isolates, maintained on fresh PDA slants at -4 °C, and stored in the Molecular and Microbiology Laboratory at the Centre for Dry Land Agriculture, Bayero University, Kano, for further experiments.

Test for Antagonistic Activity

Preliminary screening of endophytic fungi for their antagonistic property was assessed against six plant pathogens using the dual culture method (Chagas *et al.*, 2013). A 5 mm mycelial disc (diameter) of the suspected endophytic fungi (MIK1, MIK2, and MIK3) and pathogens: *Fusarium. solani*, *F. oxysporum*, *F. demerum*, *F. Cladosporum*, A. *niger*, and A. *Flavus* cut from the edge of plates of each fungus were placed on the opposite sides of PDA plates and incubated at room temperature for 7 days. Three replications were used for each treatment. Control plates were set up similarly, but with test pathogens only. After 7 days of incubation, the zone of inhibition was measured with a plastic ruler, and the percentage growth inhibition was calculated using grid cell measurement as follows:

$$I\% = \frac{r1 - r2}{r1} X100$$

Where I = percentage inhibition, r1 = radial growth of the test pathogen in the control plates, and r2 = radial growth of the endophytes.

Preparation of Test Materials

Five milligrams (5 mg) of each solvent extract (ethyl acetate, methanol, and *n*-hexane) were separately dissolved in 10% (v/v) DMSO (dimethyl sulfoxide) to make a 500 µg/mL stock solution for each extract. The stock solutions were stored in separate sterilized labeled bijou bottles in a freezer at -4 °C and preserved at the Forest Biotech Laboratory, Department of Forest Management, Faculty of Forestry, University Putra

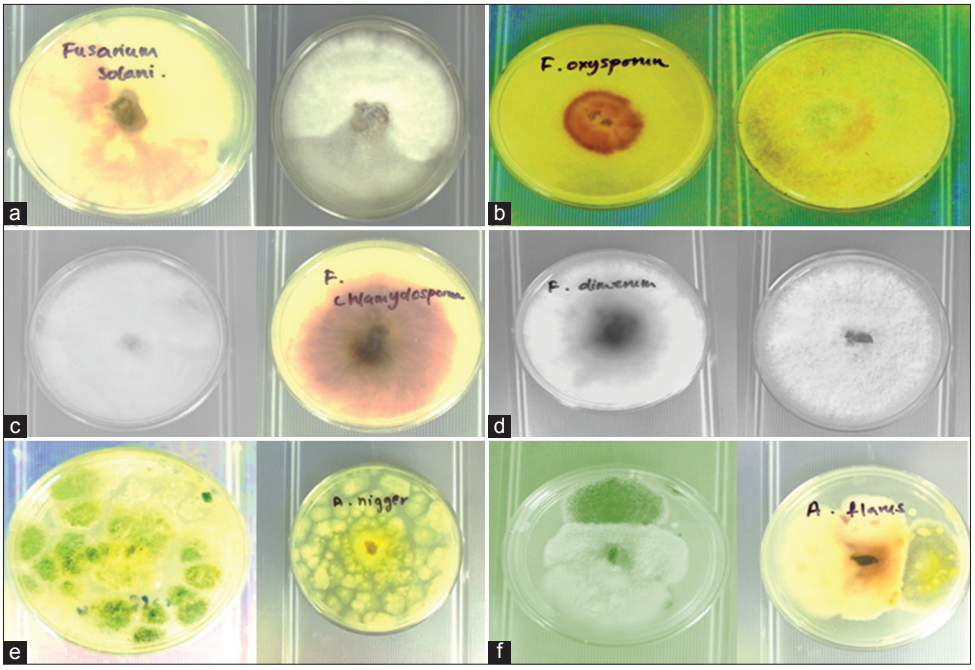

Figure 1: Morphological identification of isolated fungi (a-f) upper and reverse sides used in bioassays after 7 days on PDA at 28 °C

Malaysia (UPM). The different concentration levels of the test materials for bioactivity tests designed in this research were prepared using the serial dilution method.

Test for Antifungal Activity

The antifungal activity of the three endophytic crude extracts (ethyl acetate, methanol, and *n-hexane*) against test organisms was assessed against six plant pathogens (*F. solani*, *F. oxysporum*, *F. demerum*, *F. Cladosporium*, A. *niger* and A. *flavus*) using poisoned food method (Gulhane *et al*., 2018). The extracts were first dissolved in 10% DMSO to make different concentrations of 0.25. 0.50, 1.0, 1.5, and 2.0 µg/mL. Each treatment concentration was added to molten PDA plates using a micropipette and allowed to solidify. A 5 mm fungal disc picked from the edge of each pure pathogen culture with a sterilized needle was inoculated at the center of each PDA plate containing test material. Plates treated with DMSO only, without the extracts, served as controls. The sealed Petri plates were incubated at room temperature (28 °C) for 7 days. The experiment was performed thrice in triplicate. After the incubation period, the diameter of inhibition zones (DIZ) was measured using a plastic ruler, and the antifungal activity was assessed based on the percentage of mycelial growth inhibition, which was calculated using the formula:

$$GI\% = \frac{G1 - G2}{G1} X100$$

Where GI=the colony radius of the fungi in control, and G2=the colony radius of fungi in treatments (Cai *et al*., 2023).

RESULTS AND DISCUSSION

Isolation and Characterization of endophytic Fungi

The suspected fungal isolates from V. *paradoxa* organs were identified as *Fusarium solani* (Figure 1a), *F. oxysporum*, *F. cladosporioides*, *F. dematium*, *Aspergillus flavus*, and A. *niger*. Figure 1b, identified as *F. oxysporum*, presents a colony with a uniform, circular shape and a slight color gradient that may suggest different stages of growth or varying concentrations of spores. The coloration seems yellowish with possible signs of browning or darkening in the center, which could indicate spore production or aging of the colony. The colony has a fine texture, as is observed in filamentous fungi.

In Figure 1c, the colony identified as *F. cladosporum* has a circular growth pattern with a distinct dark center and a lighter, possibly reddish periphery. This gradient is a common characteristic in some *Fusarium* species. The central area looks denser, possibly indicating a higher concentration of fungal biomass or spore production.

Meanwhile, in Figure 1d, which was identified as *F. demerum*, the colony is predominantly white and appears fluffy or cottony, which is characteristic of many molds. There is a darker or greener area at the center. The texture appears dense and aerial.

In Figure 1e, the colonies have a characteristic greenish color, which is typically associated with mold species. They appear somewhat circular, as distinct colonies rather than a single continuous one, suggesting that this could be the result of multiple inoculation points or contamination of a single culture. The green color is typical of conidial heads in molds such as *Aspergillus* or *Penicillium*, indicating the formation of spores.

Figure 1f was identified as A. *flavus* with white colonies in the center and a distinct black color near the margin. On the reverse side, the colony shows a slight golden-yellow pigmentation, a distinctive characteristic. Typically has a granular or powdery texture, with a dense spore layer.

BLAST Results

The BLAST output displayed lists of matches or hits, along with descriptions of the organisms and their corresponding genetic sequences (e.g., genomic contig, partial cDNA) for the isolates. The top hit is for A. *niger contig An18c0160* with an E-value of 2e-127, which is highly significant. Most of the other sequences, such as those from A. *luchuensis*, A. *brasiliensis*, and A. *chevalieri*, also exhibit strong matches with low E-values. The query coverage rates were found to be within the range of 39% to 66%. Percent identities are quite high, with some near or at 100%.

The distance tree of the Sample MIK1 strain shows the relationships between various species or strains of fungi based on their translation elongation factor 1-alpha (tef1) gene sequences (Figure 2). The tree displays a well-defined clade comprising various *Calonectria* species and strains. These include species like *C. pseudonaviculata*, *C. naviculata*, *C. multiviculata*, *C. multiphialidica*, and others. Another major grouping includes ascomycete fungi, which are distantly related to the *Calonectria* strains.

Some strains, such as *C. pseudonaviculata* and *C. naviculata* appear multiple times, likely representing different isolates or strains that have been sequenced for the same gene (tef1). *C. chinensis* strains, including CBS 114827, appear in a separate sub-clade but are still within the broader grouping of *Calonectria*. There is a clear sub-grouping for *Aspergillus* species (A. *viridinutans*, A. *thermomutatus*, A. *fumigatus*), which are well-separated from *Calonectria*. There is a mention of "ascomycete fungi and unknown 65 leaves" toward the bottom, which may require further study or classification.

The lists of the matches or hits for the Sample MIK2 strain. All the first ten (10) sequences show the same maximum score of 911. Like the max score, the total score for all sequences is 911. The entire query sequence is covered by these alignments (100% query coverage). The E-values are all 0.0. All hits have 100% identity and 100% query coverage.

The distance tree result of the Sample MIK2 strain focuses on the evolutionary relationships between various isolates and strains of A. *niger* and A. *welwitschiae*, based on partial

sequences of the calmodulin (CaM) gene (Figure 3). The tree includes multiple strains of both *A. niger* and *A. welwitschiae*. These two species are closely related, but the tree reveals slight genetic divergence, as evidenced by their branching patterns. The genetic distance (0.002) between the sequences is small. The strains of *A. niger* (e.g., DTO 421-I3, CMXY1963) and *A. welwitschiae* (e.g., CN100E6, IHEM 21970).

The matched sequence of Sample MIK3 belongs to *Rhizopus arrhizus*, an isolate from a wood compost site. The maximum and total scores are similar, observed at 93.5. The query coverage is only 14%, and the E-value is 1e-13. The query sequence shows a significant match with *R. arrhizus*, specifically in the ITS1 and 5.8S ribosomal RNA regions.

The phylogenetic tree of Sample MIK3 is presented in Figure 4. It can be observed that *R. arrhizus* is positioned in a clade. The branching pattern of the tree suggests that *R. arrhizus* shares a common ancestor with the other species in the same field.

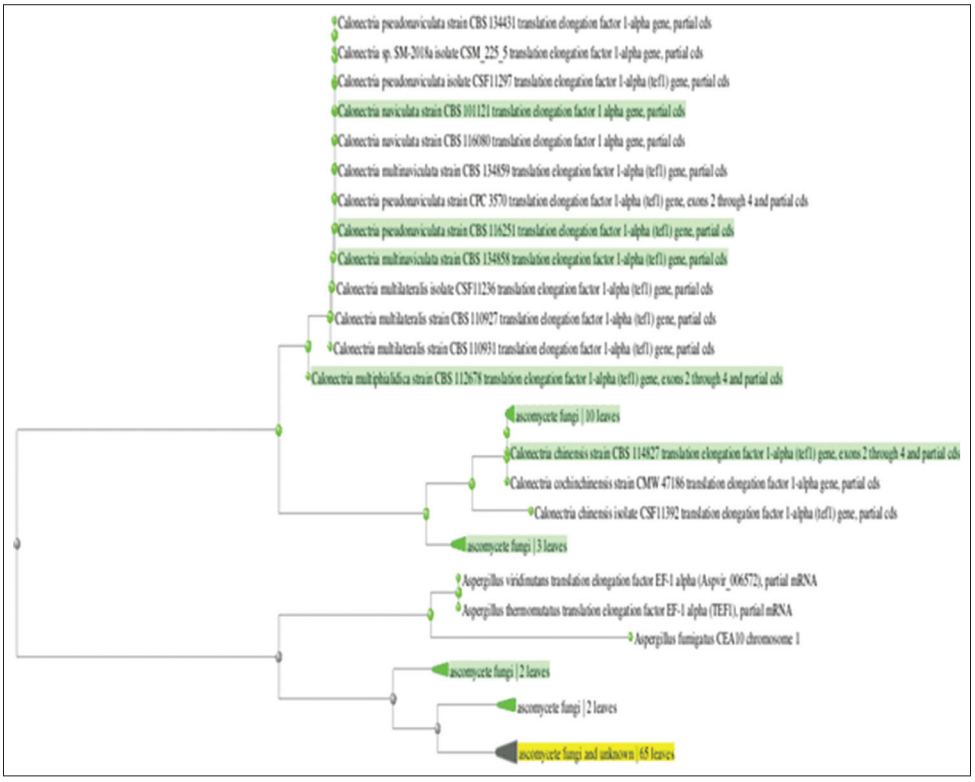

Figure 2: Phylogenetic tree for Sample MIK1strain

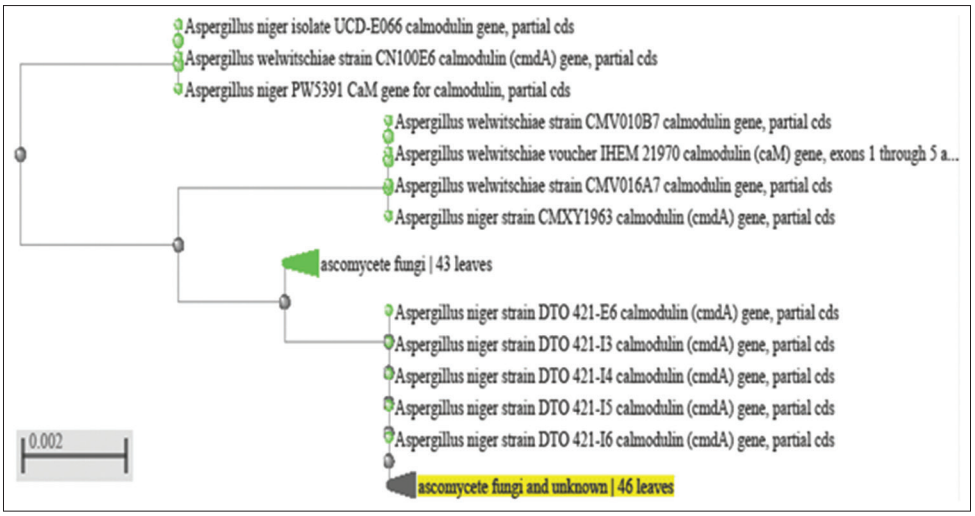

Figure 3: Phylogenetic tree of the Sample MIK2

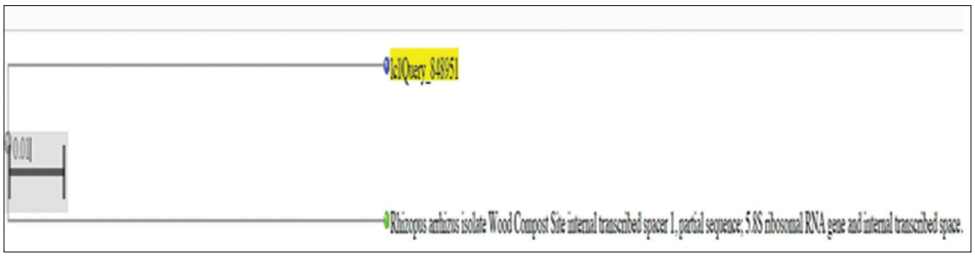

Figure 4: Phylogenetic tree of Sample MIK3

Yields of Extract

Table 1 shows the physical properties and yields of endophytic crude extracts in different solvents. The results showed that the highest yield was achieved for ethyl acetate extract (4.57 g) followed by methanol extract (4.18 g), and the lowest yield was obtained for *n-hexane* extract (3.63 g).

Antagonistic Activity Test

Three endophytic fungi (MIK1, MIK2, and MIK3) isolated from dried powder organs of Nigeria V. *paradoxa* were subjected to preliminary antagonistic screening against six plant pathogenic fungi, the causative agents of Cucumber rot diseases, using dual culture (Table 2 & Figure 5). The results show that all the endophytes exhibited varying degrees of antagonistic activity against the test plant pathogens by modification of their mycelial growth structures MIK1 exhibited 99% inhibition against *F. oxysporum*, *F. solani* by 98%, A. *niger* by 94%, and A. *flavus* by 99%, *F. cladosporum* by 97% but no activity against *F. demerum*. MIK2 strain inhibited *F. solani* by 99%, *F. cladosporum* by 95%, A. *niger* by 92% and the mycelial growth rate of A. *flavus* by 99% but did not affect *F. oxysporum*. MIK3 strain showed activity against *F. oxysporum* by 96%, *F. demerum* by 99%, A. *niger* by 92%, and A. *flavus* by 99% but did not inhibit the growth of *F. cladosporum*.

According to growth assessment indices given by Puig and Cumagun (2019), an inhibition zone of <50 indicates low activity, 60-65% shows high activity, and >75% indicates very strong activity. However, the endophytes exhibited strong antagonistic activity against the tested fungal strains but failed to inhibit *F. dimerum*, *F. cladosporum*, and *F. oxyporum*. The findings of the study showed that V. *paradoxa* harbors fungal endophyte species with antagonistic efficacy against the tested plant pathogens. The high inhibitory effects exhibited by the fungal endophytes may be due to the synthesis of biologically active secondary metabolites that inhibit the growth of pathogenic fungi or competition for nutrients in the culture medium. ANOVA test shows that growth inhibition caused by endophytic fungi against plant pathogens was significantly different ($p<0.005$).

Antifungal Activity Test

The antifungal activity of ethyl acetate extract from A. *niger* (AM270408.1) against the six plant pathogens was assessed at five different concentrations (Table 3). The results indicate

Table 1: Physical properties and yields of crude extracts from different endophytic fungi

Crude extracts	Fungal isolates	Color	Weight (g)	%Yield
Ethyl acetate	*Aspergillus niger*	light brown	4.57	18.02
methanol	*Aspergillus welwitschiae*	yellow bright	4.18	16.48
n-hexane	*Rhizopus arrhizus*	light pink	3.63	14.31

Table 2: Antagonistic activity of suspected endophytic fungi against plant pathogens in dual culture assay

	Test pathogens					
Endophytes	*F. oxyporum*	*F. solani*	*F. dimerum*	*F. cladosporum*	*A. niger*	*A. flavus*
	GI(%)	GI(%)	GI(%)	GI(%)	GI(%)	GI(%)
MIK1	99	98	-	97	94	99
MIK2	-	99	-	95	92	99
MIK3	96	97	99	-	92	99

GI(%)=growth inhibition; -= no activity

Table 3: Antifungal activity of various concentrations of *A. niger* extract against the growth of plant pathogens

Test fungi		Conc (µg/mL)				
		0.25	0.50	1.0	1.5	2.0
F. solani	Inhibition (%)	37.40	52.42	67.10	68.32	68.30
F. oxysporum		46.32	53.31	61.42	-	72.21
F. demerum		-	68.32	65.33	73.31	64.42
A. niger		63,45	67.43	69.42	75.12	78.18
F. cladosporium		60.12	68.31	75.22	-	79.11
A. flavus		34.12	-	53.21	53.10	62.41

$p<0.05$ (ANOVA); - = no inhibition

that the extract inhibited mycelial growth of *F. solani* at all concentrations, with an inhibition rate ranging between 37% and 72%. *F. oxysporum* showed inhibition rates between 46.32% and 77.21% but exhibited no activity at a 1.5 µg/mL concentration. Similarly, the crude extract showed activity against *F. demerum*, with an inhibition range of 65.33% to 73.12% at all concentrations but had no effect at 0.25 µg/mL. Furthermore, the crude extract inhibited the mycelial growth of A. *niger* at all concentrations, with inhibition rates of 63.45% to 78.18% respectively. *F. cladosporum* was inhibited at all concentrations, except at 1.5 µg/mL, with inhibition values of 60.12% to 79.11% respectively.

Furthermore, the extract demonstrated strong activity against A. *flavus* at all concentrations, except 0.50 µg/mL, with an inhibition rate ranging from 34.12% to 62.41%. The study's findings show that growth inhibition increased as

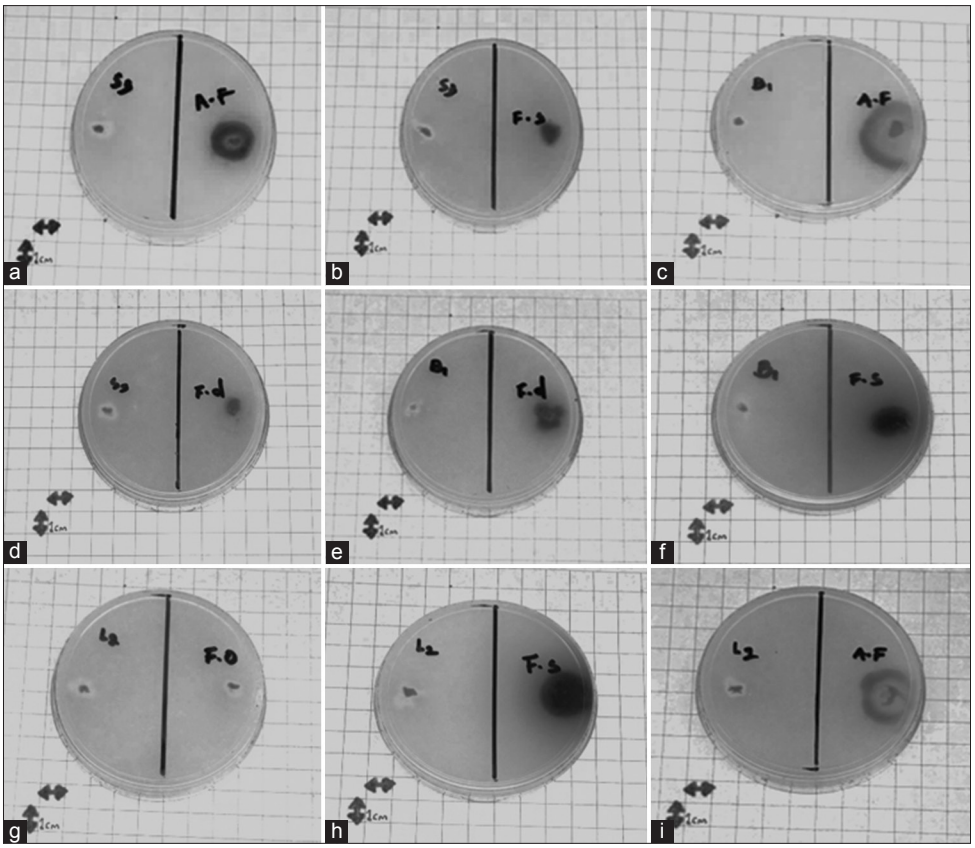

Figure 5: Dual culture between endophytic fungi (MIK1, MIK2, and MIK3). a) *A. flavus*, b) *F. solani*, c) *A. flavus*, d) *F. demerum*, e, f) *F. demerum*, g) *F. oxysporum*, h) *F. solani* and i) *A. flavus*. A Mycelial disc on the left side of the plate is the endophytic fungus, while on the right is the pathogen: Scale bars=1 cm

the concentration increased, and the extract demonstrated moderate to very high activity against all the tested pathogens. Amongst all the tested pathogens, A. *niger* and *F. cladosporium* were more susceptible to all the concentrations. The highest activity was observed at 78.18% (2.0 µg/mL), and the lowest at 37.40% (0.25 µg/mL), respectively. The results further show that A. *niger* crude extract, at a concentration of 1 µg/mL, inhibited all tested fungi. The ANOVA test indicates that the growth inhibition of the tested extracts was significantly different from that of the control ($p<0.05$).

DISCUSSION

The use of microbially-derived compounds in biological control against plant diseases is gaining research attention for sustainable agriculture as the chemical pesticides cause undesirable effects on other organisms in the environment, human health through the food chain, and the development of resistance, resulting in significant losses in crop yield (Takim *et al*., 2023). Their use as natural biocontrol agents is considered a better alternative for producing safe foods (Zanna *et al*., 2021). Multiple-resistant pests, particularly phytopathogens and weeds, continue to pose a challenge in agriculture worldwide, primarily due to the overuse of synthetic agrochemicals. Therefore, effective management is necessary for productive agriculture in the twenty-first century. The bioactive compounds from endophytic fungi have been the major source and inspiration for the successful management of weed infestations and fungal diseases. Thus, the current study was designed to assess the antifungal and herbicidal activity of crude extracts from endophytic fungi against six pathogenic fungal species and three weed species in laboratory bioassays. The potential herbicidal properties of the fungal extracts, based on their efficacy in the laboratory bioassay, were further assessed under screen house conditions on both fresh and dry biomass of the same weeds.

In this study, three endophytic fungi A. *niger* (MIK1strain), A. *welwitschiae* (MIK2 strain) and *R. arrhizus* (MIK3 strain) were isolated from surface-sterilized dry powdered tissues (leaves, bark, and seeds) of Nigerian *V paradoxa* in the laboratory. To our knowledge, this is the first study to screen for antifungal activity of crude extracts from endophytic fungi of Nigeria *V. paradoxa* against phytopathogenic fungi and weeds of agricultural importance, as there is currently no published literature describing research in this area.

BLAST results for MIK1 revealed the top hit for *Aspergillus niger contig An18c0160* with an E-value of 2e-127, which is highly significant. The query covers (e.g., 99%, 97%) indicate that most of the sequence was matched to the reference sequences, showing high similarity. Percent identities are quite high, with some near or at 100%, indicating very close genetic relationships between the query and these sequences. Whereas

the BLAST results for MIK2 indicate a perfect match between the query sequence and multiple *Aspergillus* species sequences, particularly *A. welwitschiae* and *A. niger*. This suggests that the query sequence belongs to or is very closely related to these species. All hits have 100% identity, 100% query coverage, and E-values of 0.0, which signifies that these sequences are almost identical at the nucleotide level. In MIK3, the BLAST of the query sequence shows a significant match with *R. arrhizus*, specifically in the ITS1 and 5.8S ribosomal RNA regions. The match is significant with a moderately low E-value, but the query coverage is relatively low at 14%, meaning that the alignment covers only a portion of the query sequence. The relatively high percentage identity of nearly 79% suggests that the sequences are similar in the aligned region, though not identical.

The phylogenetic analysis conducted on the different fungal strains provides significant insights into their evolutionary relationships, particularly about *Calonectria*, *Aspergillus*, and other ascomycete fungi. The phylogenetic trees constructed using different gene markers, such as *translation elongation factor 1-alpha (tef1)* and *calmodulin (CaM)* genes, help in understanding the genetic divergence and speciation events among these organisms.

The phylogenetic tree, as seen in the analysis of *Calonectria* species, demonstrates clear clustering patterns that highlight the evolutionary relationships among various strains such as *C. pseudonaviculata*, *C. naviculata*, and *C. multiviculata*. These strains are closely grouped, indicating that they share a recent common ancestor and belong to the same clade. The grouping based on the *tef1* gene confirms earlier findings that have used similar genetic markers for differentiating species within the *Calonectria* genus (Figure 2). For example, Lombard *et al.* (2010) used *tef1* sequences to distinguish different species of *Calonectria* and proposed their effectiveness as a genetic marker for taxonomic studies within this genus.

Additionally, the *C. chinensis* strain occupies a more genetically distinct position, reflecting its divergence from other *Calonectria* species. This aligns with previous studies that noted the genetic uniqueness of *C. chinensis*, especially in terms of its pathogenicity on plants (Figure 4).

The tree focusing on the *A. niger* and *A. welwitschiae* species, based on the *CaM* gene, reveals a close evolutionary relationship. These species form distinct but closely related clades, suggesting that they share a recent common ancestor. This finding corroborates previous work by (Varga *et al.*, 2011), which discussed the evolutionary split between *A. niger* and *A. welwitschiae* based on calmodulin and other gene markers (Figure 4).

The short branch lengths between strains within each clade suggest minimal genetic distance, typical for highly conserved genes like calmodulin. This supports the notion that *CaM* is an effective marker for studying relationships among closely related species but may not provide sufficient resolution for distant evolutionary splits. Susca *et al.* (2020) identified *calmodulin* as a conserved gene whose slight variation can still be informative for species-level differentiation in *Aspergillus* (Figures 3 & 4).

The distinction between the *Calonectria* and *Aspergillus* genera underscores the significant evolutionary divergence between these ascomycete fungi. While both are members of the *Ascomycota phylum*, their genetic lineages have diverged considerably, as indicated by their separation on the phylogenetic tree. Studies such as those by Tekpinar and Kalmer (2019) highlight how divergence in key genetic markers, like *tef1* and *CaM*, has played a role in the speciation of diverse fungal taxa within Ascomycota (Figure 4).

Another interesting observation is the presence of large clusters labeled as "ascomycete fungi and unknown" in both trees. This suggests that these sequences either represent uncharacterized fungi or species that have not yet been described in the literature. As fungal diversity remains largely unexplored, especially in environmental samples, this finding highlights the need for further investigation and classification. Research (Hawksworth & Lücking, 2017) estimates that a vast number of fungal species remain undiscovered, and phylogenetic analyses like this are key to uncovering and understanding this hidden diversity (Figure 4).

The results indicated that the ethyl acetate extract (*A. niger*) yielded the highest amount of extract (18.02%), followed by the methanol extract (*A. welwitschiae*) (16.8%) and the *n-hexane* extract (*R. arhizus*) (14.31%), respectively. The differences in extract yields could be due to the presence of a wide range of diverse bioactive compounds among the extracts and the differences in solvent polarity. Thus, the use of an appropriate solvent is crucial for achieving higher extract yields and bioactive compounds from the sample. The chemical components, extraction technique, extraction time, temperature, pH, and solvent polarity can all affect extraction yields.

A preliminary antagonistic activity test against six pathogenic fungi was conducted in a dual culture assay. The results show that *A. niger* (MIK1 strain) inhibits the mycelial growth of *F. oxysporum* by 99%, 98% *F. solani*, 97% *F. cladosporum*, 94% *A. niger* and 99% *A. flavus*. Similarly, *A. welwitschiae* (MIK2 strain) shows growth inhibition against *F. solani* by 99%, 95% *F. cladosporum*, 92% *A. niger*, 99% and 99% *A. flavus*. In addition, *R. arrhizus* (MIK3 strain) exhibited antagonistic activity against *F. oxysporum* by 96%, *F. solani* by 97%, 99% *F. dimerum*, *A. niger* by 92%, and *A. flavus* by 99%. The antagonistic efficiency of the endophytes may be due to mycoparasitism or competition for nutrients (Beneduzi *et al.*, 2012). Thus, interactions between antagonistic microorganisms and plant pathogens equally occur in nature as a mechanism to control or reduce the incidence of pest attacks against the host plant.

The antifungal activity of the extracts against the same plant pathogens was evaluated in a concentration-dependent manner using the poisoned food method. The results indicate that all treatment concentrations inhibit the fungal species tested, with varying levels of effectiveness. The ethyl acetate (*A. niger*) extract had shown significant inhibition of 68.32% at 1.5 µg/

mL against growth *F. solani*, 72.21% against *F.oxysporum* at 2.0 µg/mL, 73.31% against *F. demerum* at 1.5 µg/mL, 78.18% against A. *niger* at 2.0µg/mL, 78.11% against *F. demerum* at 2.0%, 62.41% at 2.0 µg/mL but did not inhibit the growth of *F. demerum* at 0.25 µg/mL, *F. oxysporum* at 1.5 µg/mL, *F. cladosporum* at 1.5 µg/mL and A. *flavus* at 0.5 µg/mL, Thus, the percentage inhibition was determined by measuring the zone of inhibition around the discs on PDA medium and the extracts had demonstrated varying degrees of inhibitions as the concentration increases. The results agree with several studies that employed various concentrations and observed higher extract concentrations correlating with increased inhibition of fungal growth (Rosas-Burgos *et al.*, 2009a, b; Singh *et al.*, 2024).

CONCLUSION

This study assessed the antagonistic and antifungal activity of dry powdered tissues of *V. paradoxa*. The results indicate that all the suspected endophytic fungal strains (MIK1, MIK2, and MIK3) displayed varying degrees of antagonistic activity against the test pathogenic fungi, with an inhibition range of 92% and 99%, respectively. The ethyl acetate extract from A. *niger* exhibited strong antifungal activity against the same pathogenic test fungi, with inhibition rates of 78.18% and 79.11%. All the fungal crude extracts possess antifungal compounds and can be used to manage the six fungal species and weeds tested. Further field experiments should be conducted for practical applications against other plant pathogens and weeds to reduce reliance on synthetic agrochemicals. The diversity of endophytic microorganisms in various plant species and environments, to identify novel strains with potent antifungal activities, should be explored. This can contribute to expanding the repertoire of natural alternatives to synthetic agrochemicals. Applying endophytic fungi as biocontrol agents or biofertilizers, either alone or as part of an integrated pest management strategy, may help reduce over-reliance on agrochemicals.

REFERENCES

Ahamed, A., & Ahring, B. K. (2011). Production of hydrocarbon compounds by endophytic fungi *Gliocladium* species grown on cellulose. *Bioresource Technology, 102*(20), 9718-9722. https://doi.org/10.1016/J.BIORTECH.2011.07.073

Ahoyo, C. C., Houéhanou, T. D., Yaoitcha, A. S., Akpi, B. P., Natta, A., & Houinato, M. R. B. (2024). How do plant demographic and ecological traits combined with social dynamics and human traits affect woody plant selection for medicinal uses in Benin (West Africa)? *Journal of Ethnobiology and Ethnomedicine, 20*(1), 55. https://doi.org/10.1186/s13002-024-00655-2

Alwathnani, H. A., & Perveen, K. (2012). Biological control of *fusarium* wilt of tomato by antagonist fungi and cyanobacteria. *African Journal of Biotechnology, 11*(5), 1100-1105. https://doi.org/10.5897/AJB11.3361

Arora, J., & Ramawat, K. G. (2017). An introduction to endophytes. In D. K. Maheshwari (Ed.), *Endophytes: Biology and Biotechnology* (Vol. 1, pp. 1-23). Cham, Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-319-66541-2_1

Beneduzi, A., Ambrosini, A., & Passaglia, L. M. P. (2012). Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology, 35*(4S1), 1044-1051. https://doi.org/10.1590/S1415-47572012000600020

Cai, L., Zhang, H., Deng, Y., Tian, W., Fan, G., & Sun, X. (2023). Antifungal activity of *Streptomyces hygroscopicus* JY-22 against *Alternaria alternata* and its potential application as a biopesticide to control tobacco brown spot. *Agronomy, 13*(7), 1944. https://doi.org/10.3390/AGRONOMY13071944

Chagas, F. O., Dias, L. G., & Pupo, M. T. (2013). A mixed culture of endophytic fungi increases production of antifungal polyketides. *Journal of Chemical Ecology, 39*(10), 1335-1342. https://doi.org/10.1007/S10886-013-0351-7

Cullen, M. G., Thompson, L. J., Carolan, J. C., Stout, J. C., & Stanley, D. A. (2019). Fungicides, herbicides and bees: A systematic review of existing research and methods. *Plos One, 14*(12), e0225743. https://doi.org/10.1371/JOURNAL.PONE.0225743

Dar, R. A., Shahnawaz, M., Ahanger, M. A., & ul Majid, I. (2023). Exploring the diverse bioactive compounds from medicinal plants: A review. *The Journal of Phytopharmacology, 12*(3), 189-195. https://doi.org/10.31254/PHYTO.2023.12307

Debnath, S., Rawat, D., Mukherjee, A. K., Adhikary, S., & Kundu, R. (2019). Applications and constraints of plant beneficial microorganisms in agriculture. In S. M. Mirmajlessi & R. Radhakrishnan (Eds.), *Biostimulants in Plant Science* London, UK: IntechOpen Limited. https://doi.org/10.5772/INTECHOPEN.89190

Deshmukh, S. K., Dufossé, L., Chhipa, H., Saxena, S., Mahajan, G. B., & Gupta, M. K. (2022). Fungal endophytes: A Potential source of antibacterial compounds. *Journal of Fungi, 8*(2), 164. https://doi.org/10.3390/JOF8020164

Devi, K. S., Misra, D. K., Saha, J., Devi, Ph. S., & Sinha, B. (2018). Screening of suitable culture media for growth, cultural and morphological characters of pycnidia forming fungi. *International Journal of Current Microbiology and Applied Sciences, 7*(8), 4207-4214. https://doi.org/10.20546/ijcmas.2018.708.440

Dimobe, K., Tondoh, J. E., Weber, J. C., Bayala, J., Ouédraogo, K., & Greenough, K. (2018). Farmers' preferred tree species and their potential carbon stocks in southern Burkina Faso: Implications for biocarbon initiatives. *Plos One, 13*(12), e0199488. https://doi.org/10.1371/JOURNAL.PONE.0199488

Fattah, A. G. M., Haddad, E. S. A., Hafez, E. E., & Rashad, Y. M. (2011). Induction of defense responses in common bean plants by *Arbuscular mycorrhizal* fungi. *Microbiological Research, 166*(4), 268-281. https://doi.org/10.1016/j.micres.2010.04.004

Fawole, O., & Yahaya, J. (2017). Biocontrol potentials of crude extracts of soil fungi on *Amaranthus hybridus* and *Phyllanthus amarus*. *Journal of Agricultural Sciences, 62*(1), 41-49. https://doi.org/10.2298/jas1701041f

Feng, D.-H., & Cui, J.-L. (2025). The effect of endophytic fungus CA3-A with biotransformation or catalysis activity on the metabolite formation of traditional Chinese medicinal *Astragalus Membranaceus var. Mongholicus* (Bunge) P. K. Hsiao. *Plant Foods for Human Nutrition, 80*(1), 69. https://doi.org/10.1007/S11130-025-01317-8

Ferrigo, D., Raiola, A., & Causin, R. (2016). Fusarium toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules, 21*(5), 627. https://doi.org/10.3390/MOLECULES21050627

Gell, S. (2024). Pharmacognostic Analysis of Medicinal Plants: A Review. *Alternative and Integrative Medicine, 13*(5), 536.

Gul, H. I., Tugrak, M., Sakagami, H., Taslimi, P., Gulcin, I., & Supuran, C. T. (2016). Synthesis and bioactivity studies on new 4-(3-(4-Substitutedphenyl)-3a,4-dihydro-3H-indeno[1,2-c] pyrazol-2-yl) benzenesulfonamides. *Journal of Enzyme Inhibition and Medicinal Chemistry, 31*(6), 1619-1624. https://doi.org/10.3109/14756366.2016.1160077

Gulhane, A. R., Giri, G. K., & Khambalkar, S. V. (2018). Antifungal activity of aroma chemicals against graminicolous seed borne fungi by poisoned food method. *International Journal of Current Microbiology and Applied Sciences, 7*(7), 477. https://doi.org/10.20546/ijcmas.2018.707.477

Hawksworth, D. L., & Lücking, R. (2017). Fungal Diversity Revisited: 2.2 to 3.8 million Species. *Microbiology Spectrum, 5*(4), FUNK-0052-2016. https://doi.org/10.1128/microbiolspec.funk-0052-2016

Hyde, K. D., Xu, J., Rapior, S., Jeewon, R., Lumyong, S., Niego, A. G. T., Abeywickrama, P. D., Aluthmuhandiram, J. V. S., Brahamanage, R. S., Brooks, S., Chaiyasen, A., Chethana, K. W. T., Chomnunti, P., Chepkirui, C., Chuankid, B., de Silva, N. I., Doilom, M., Faulds, C., Gentekaki, E.,… Stadler, M. (2019). The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity, 97*(1), 1-136. https://doi.org/10.1007/s13225-019-00430-9

Jiang, P., Fu, X., Niu, H., Chen, S., Liu, F., Luo, Y., Zhang, D., & Lei, H. (2023). Recent advances on *Pestalotiopsis* genus: Chemistry, biological activities, structure–activity relationship, and biosynthesis. *Archives*

of Pharmacal Research, 46(6), 449-499. https://doi.org/10.1007/S12272-023-01453-2

Joseph, B., & Priya, M. R. (2011). Bioactive compounds from endophytes and their potential in pharmaceutical effect: A review. *American Journal of Biochemistry and Molecular Biology, 1*, 291-309. https://doi.org/10.3923/AJBMB.2011.291.309

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution, 35*(6), 1547-1549. https://doi.org/10.1093/MOLBEV/MSY096

Larran, S., Perelló, A., Simón, M. R., & Moreno, V. (2007). The endophytic fungi from wheat (*Triticum aestivum* L.). *World Journal of Microbiology & Biotechnology, 23*(4), 565-572. https://doi.org/10.1007/S11274-006-9266-6

Lata, R., Chowdhury, S., Gond, S. K., & White, J. F. (2018). Induction of abiotic stress tolerance in plants by endophytic microbes. *Letters in Applied Microbiology, 66*(4), 268-276. https://doi.org/10.1111/LAM.12855

Liu, K., Ding, X., Deng, B., & Chen, W. (2009). Isolation and characterization of endophytic taxol-producing fungi from *Taxus chinensis*. *Journal of Industrial Microbiology and Biotechnology, 36*(9), 1171. https://doi.org/10.1007/S10295-009-0598-8

Liu, X., Zhou, Z. Y., Cui, J. L., Wang, M. L., & Wang, J. H. (2021). Biotransformation ability of endophytic fungi: From species evolution to industrial applications. *Applied Microbiology and Biotechnology, 105*(19), 7095-7113. https://doi.org/10.1007/S00253-021-11554-X

Lokonon, B. E., Mangamana, E. T., Kakaï, R. G., & Sinsin, B. (2017). Assessing use, diversity and local conservation priorities of woody species within agroforestry systems along Ouémé catchment in Benin (West Africa). *Ethnobiology and Conservation, 6*(4), 1-19. https://doi.org/10.15451/ec2017-05-6.4-1-19

Lombard, L., Crous, P. W., Wingfield, B. D., & Wingfield, M. J. (2010). Phylogeny and systematics of the genus *Calonectria*. *Studies in Mycology, 66*, 31-69. https://doi.org/10.3114/SIM.2010.66.03

Lugtenberg, B. J. J., Caradus, J. R., & Johnson, L. J. (2016). Fungal endophytes for sustainable crop production. *FEMS Microbiology Ecology, 92*(12), fiw194. https://doi.org/10.1093/FEMSEC/FIW194

Mends, M. T., Yu, E., Strobel, G. A., Riyaz-Ul-Hassan, S., Booth, E., Geary, B., Sears, S., Taatjes, C. A., & Hadi, M. Z. (2012). An endophytic *Nodulisporium* sp. producing volatile organic compounds having bioactivity and fuel potential. *Journal of Petroleum & Environmental Biotechnology, 3*, 3. https://doi.org/10.4172/2157-7463.1000117

Mousa, W. K., & Raizada, M. N. (2013). The diversity of anti-microbial secondary metabolites produced by fungal endophytes: An interdisciplinary perspective. *Frontiers in Microbiology, 4*, 65. https://doi.org/10.3389/FMICB.2013.00065

Mukherjee, M., Mukherjee, P. K., Horwitz, B. A., Zachow, C., Berg, G., & Zeilinger, S. (2012). *Trichoderma–plant–pathogen* Interactions: Advances in Genetics of Biological Control. *Indian Journal of Microbiology, 52*(4), 522-529. https://doi.org/10.1007/S12088-012-0308-5

Naik, B. S., & Krishnamurthy Y. L. (2010). Endophytes: The real untapped high energy biofuel resource. *Current Science, 98*(7), 883.

Nair, D. N., & Padmavathy, S. (2014). Impact of endophytic microorganisms on plants, environment and humans. *The Scientific World Journal, 2014*(1), 250693. https://doi.org/10.1155/2014/250693

Ojo, O., Kengne, M. H. K., Fotsing, M. C., Mmutlane, E. M., & Ndinteh, D. T. (2021). Traditional uses, phytochemistry, pharmacology and other potential applications of *Vitellaria paradoxa* Gaertn. (Sapotaceae): A review. *Arabian Journal of Chemistry, 14*(1), 103213. https://doi.org/10.1016/j.arabjc.2021.103213

Packiaraj, R., Jeyakumar, S., Ayyappan, N., Adhirajan, N., Premkumar, G., Rajarathinam, K., & Muthuramkumar, S. (2016). Antimicrobial and cytotoxic activities of endophytic fungus *Colletotrichum gloeosporioides* isolated from endemic tree *Cinnamomum malabatrum*. *Studies in Fungi, 1*(1), 104-113. https://doi.org/10.5943/sif/1/1/10

Pandi, M., Rajapriya, P., & Manoharan, P. T. (2013). Extraction and Characterization of Taxol: An anticancer drug from an endophytic and pathogenic fungi. In V. K. Gupta, & M. G. Tuohy (Eds.), *Laboratory Protocols in Fungal Biology* (pp. 523-527) New York, US: Springer. https://doi.org/10.1007/978-1-4614-2356-0_51

Park, Y. H., Chandra Mishra, R., Yoon, S., Kim, H., Park, C., Seo, S. T., & Bae, H. (2019). Endophytic *Trichoderma citrinoviride* isolated from mountain-cultivated ginseng (*Panax ginseng*) has great potential as a biocontrol agent against ginseng pathogens. *Journal of Ginseng Research, 43*(3), 408-420. https://doi.org/10.1016/J.JGR.2018.03.002

Radha, Kumari, N., Prakash, S., Sharma, N., Puri, S., Thakur, M., Singh, J., & Kumar, M. (2024). Medicinal and aromatic plants as potential sources of bioactives along with health-promoting activities. *Current Food Science and Technology Reports, 2*(4), 359-376. https://doi.org/10.1007/S43555-024-00042-8

Rana, K. L., Kour, D., Sheikh, I., Yadav, N., Yadav, A. N., Kumar, V., Singh, B. P., Dhaliwal, H. S., & Saxena, A. K. (2019). Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In B. P. Singh (Ed.), *Advances in Endophytic Fungal Research* (pp. 105-144) Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-030-03589-1_6

Rosas-Burgos, E. C., Cortez-Rocha, M. O., Cinco-Moroyoqui, F. J., Robles-Zepeda, R. E., López-Cervantes, J., Sánchez-Machado, D. I., & Lares-Villa, F. (2009). Antifungal activity in vitro of *Baccharis glutinosa* and *Ambrosia confertiflora* extracts on *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides*. *World Journal of Microbiology and Biotechnology, 25*(12), 2257-2261. https://doi.org/10.1007/S11274-009-0116-1/METRICS

Sahoo, S., Sarangi, S., & Kerry, R. G. (2017). Bioprospecting of Endophytes for Agricultural and Environmental Sustainability. In J. K. Patra, C. N. Vishnuprasad & G. Das (Eds.), *Microbial Biotechnology* (Vol. 1, pp. 429-458) Singapore: Springer. https://doi.org/10.1007/978-981-10-6847-8_19

Shah, S. K., Dey, Y. N., Madhavan, Y., & Maity, A. (2023). Fungal Endophytes: A storehouse of bioactive compounds. *Mini Reviews in Medicinal Chemistry, 23*(9), 978-991. https://doi.org/10.2174/1389557522999220422133020

Singh, N. K., Pandey, R. R., & Singh, M. S. (2024). Biological activities and GC-MS analysis of crude extract of an endophytic fungus *Fusarium* sp. F1C1. *Vegetos, 37*, 1720-1732. https://doi.org/10.1007/s42535-024-00817-3

Singh, S. P., & Singh, D. (2010). Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review. *Renewable and Sustainable Energy Reviews, 14*(1), 200-216. https://doi.org/10.1016/J.RSER.2009.07.017

Strobel, G., Singh, S. K., Riyaz-Ul-Hassan, S., Mitchell, A. M., Geary, B., & Sears, J. (2011). An endophytic/pathogenic *Phoma* sp. from creosote bush producing biologically active volatile compounds having fuel potential. *FEMS Microbiology Letters, 320*(2), 87-94. https://doi.org/10.1111/J.1574-6968.2011.02297.X

Sun, Y., Wang, M., Li, Y., Gu, Z., Ling, N., Shen, Q., & Guo, S. (2017). Wilted cucumber plants infected by *Fusarium oxysporum* f. sp. *cucumerinum* do not suffer from water shortage. *Annals of Botany, 120*(3), 427-436. https://doi.org/10.1093/AOB/MCX065

Susca, A., Villani, A., Moretti, A., Stea, G., & Logrieco, A. (2020). Identification of toxigenic fungal species associated with maize ear rot: Calmodulin as single informative gene. *International Journal of Food Microbiology, 319*, 108491. https://doi.org/10.1016/J.IJFOODMICRO.2019.108491

Takim, F. O., Awolade, V., Ajisope, T. A., & Lawal, M. B. (2023). Evaluation of two new herbicide mixtures for weed control in maize (*Zea Mays* L.). *Journal of Environmental Issues and Agriculture in Developing Countries, 4*(1), 71-78.

Talapatra, K., Das, A. R., Saha, A. K., & Das, P. (2017). In vitro antagonistic activity of a root endophytic fungus towards plant pathogenic fungi. *Journal of Applied Biology & Biotechnology, 5*(2), 68-71. https://doi.org/10.7324/JABB.2017.50210

Tamou, B. C. K., Mechling, J. M., Yabi, C. P., Godonou, G. E. F., Adjovi, E. C., Gibigaye, M., Lecomte, A., & Brosse, N. (2023). Use of additive based on non-timber forest products for the ecological stabilization of raw earth: Case of the *Parkia Biglobosa* nut and *Vitellaria Paradoxa*. *Journal of Renewable Materials, 11*(12), 4143-4160. https://doi.org/10.32604/jrm.2023.030509

Tekpinar, A. D., & Kalmer, A. (2019). Utility of various molecular markers in fungal identification and phylogeny. *Nova Hedwigia, 109*(1), 187-224. https://doi.org/10.1127/nova_hedwigia/2019/0528

Tomsheck, A. R., Strobel, G. A., Booth, E., Geary, B., Spakowicz, D., Knighton, B., Floerchinger, C., Sears, J., Liarzi, O., & Ezra, D. (2010). Hypoxylon sp., an endophyte of *Persea indica*, producing 1,8-cineole and other bioactive volatiles with fuel potential. *Microbial Ecology, 60*(4), 903-914. https://doi.org/10.1007/S00248-010-9759-6

Varga, J., Frisvad, J. C., Kocsubé, S., Brankovics, B., Tóth, B., Szigeti, G., & Samson, R. A. (2011). New and revisited species in *Aspergillus* section *Nigri*. *Studies in Mycology, 69*(1), 1-17. https://doi.org/10.3114/SIM.2011.69.01

Wang, M., Sun, Y., Gu, Z., Wang, R., Sun, G., Zhu, C., Guo, S., & Shen, Q. (2016). Nitrate protects cucumber plants Against *Fusarium oxysporum* by regulating citrate exudation. *Plant and Cell Physiology, 57*(9), 2001-2012. https://doi.org/10.1093/PCP/PCW124

Yashavanth R. H. C., Santosh, P., Rakshith, D., & Satish, S. (2015). Molecular characterization of an endophytic *Phomopsis liquidambaris* CBR-15 from *Cryptolepis buchanani* Roem. and impact of culture media on biosynthesis of antimicrobial metabolites. *3 Biotech, 5*(2), 165-173. https://doi.org/10.1007/s13205-014-0204-2

Yuan, Z., Su, Z., Mao, L., Peng, Y., Yang, G., Lin, F., & Zhang, C. (2011). Distinctive endophytic fungal assemblage in stems of wild rice (*Oryza granulata*) in China with special reference to two species of *Muscodor* (Xylariaceae). *Journal of Microbiology, 49*(1), 15-23. https://doi.org/10.1007/S12275-011-0213-3

Zanna, H., Tijani, Y., Abubakar, S., Modu, B., Damasak, A. A., & Uzairu, S. M. (2021). Fungicidal potential of selected plant extracts against human pathogenic fungi. *Scientific African, 13*(1), e00864. https://doi.org/10.1016/J.SCIAF.2021.E00864

Zhao, S., Li, J., Liu, J., Xiao, S., Yang, S., Mei, J., Ren, M., Wu, S., Zhang, H., & Yang, X. (2023). Secondary metabolites of *Alternaria*: A comprehensive review of chemical diversity and pharmacological properties. *Frontiers in Microbiology, 13,* 1085666. https://doi.org/10.3389/FMICB.2022.1085666