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Research Article

Antimicrobial Potential of Endophytic Fungi from *Capsicum annuum* and *Vigna Unguiculata* Against *Xanthomonas campestris* and *Fusarium oxysporum*

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ABSTRACT

This study investigated the isolation and characterization of endophytic fungi from *Capsicum annuum* and *Vigna unguiculata*, and evaluated their biocontrol potential against *Xanthomonas campestris* and *Fusarium oxysporum*. Endophytes were isolated from surface-sterilized plant tissues collected in Katsina State, Nigeria. Four fungi *Alternaria* sp., *Aspergillus niger*, *Penicillium* sp., and *Rhizopus stolonifer*—were identified based on morphological characteristics. Antimicrobial activity of fungal culture filtrates was assessed in vitro using the agar well diffusion method at concentrations ranging from 25 to 100 µg/mL. Secondary metabolites of active isolates were analyzed using Liquid Chromatography–Mass Spectrometry (LC-MS). Results showed a dose-dependent inhibition by *Penicillium* sp. and *A. niger*, while *Alternaria* sp. and *R. stolonifer* exhibited no activity. *Penicillium* sp. demonstrated the highest efficacy, with inhibition zones of 24.5 ± 2.2 mm against *X. campestris* and 16.6 ± 0.3 mm against *F. oxysporum* at 100 µg/mL. *Aspergillus niger* showed moderate inhibition with zones of 17.1 ± 1.5 mm and 11.3 ± 0.22 mm, respectively. LC-MS profiling revealed the production of penicillin G and griseofulvin by *Penicillium* sp., and 2-phenylethanol by *A. niger*. However, both fungi also produced mycotoxins such as ochratoxin A, patulin, and gliotoxin. The study highlights the strong biocontrol potential of *Penicillium* sp. and *A. niger*, alongside significant biosafety concerns, emphasizing the need for non-toxigenic strains for sustainable agricultural application in Nigeria.

Keywords: Antimicrobial Activity; Biocontrol; Endophytic Fungi; Mycotoxins; Secondary Metabolites

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INTRODUCTION

Endophytic fungi are microorganisms that reside symbiotically within healthy plant tissues without causing disease. They confer significant benefits to their hosts, including enhanced nutrient uptake, improved tolerance to abiotic stresses, and increased resistance to pathogens (Stone *et al*., 2000; Khan *et al*., 2015; Hamilton *et al*., 2012). This biocontrol potential is realized through mechanisms such as the production of antimicrobial secondary metabolites and the induction of systemic resistance (ISR) in the plant, priming its immune defenses (Kaul *et al*., 2012; Pieterse *et al*., 2014). Their efficacy across major crops and ecological versatility make them a cornerstone for sustainable agriculture, reducing reliance on synthetic chemicals (Rodriguez *et al*., 2009).

Globally, the imperative for sustainable agriculture is clear, given the need to support food security amid climate change. The overuse of chemical pesticides has led to a "pesticide treadmill" of pathogen resistance, environmental harm, and health hazards, necessitating a paradigm shift (Hawkins *et al*., 2019; Goulson *et al*., 2018). Endophytic fungi, present in virtually all plants, represent a promising alternative. They enhance host fitness through a multifaceted defense arsenal, including direct antimicrobial production, immune system priming, and competitive exclusion of pathogens (Kusari *et al*., 2019; Pieterse *et al*., 2014; Compant *et al*., 2019).

In Northern Nigeria, agricultural productivity is severely threatened by bacterial and fungal diseases. Key pathogens include *Xanthomonas campestris* (bacterial blight) and *Fusarium oxysporum* (wilt), which can cause significant yield losses in staple and high-value crops like cowpea (*Vigna unguiculata*) and pepper (*Capsicum annuum*) (Togola *et al*., 2023). Pepper, for instance, is severely affected by wilts, anthracnose, and bacterial spot (Saxena *et al*., 2022). Farmers' heavy reliance on chemical pesticides exacerbates these issues, creating an urgent need for sustainable biocontrol solutions (Brent & Hollomon, 2007).

Research demonstrates significant biocontrol potential against these pathogens from endophytes such as *Trichoderma* spp., *Beauveria bassiana*, and native *Aspergillus* and *Penicillium* isolates (Mendoza *et al*., 2023; Kaur *et al*., 2021). However, translating these potential faces major hurdles, including biosafety concerns over mycotoxin co-production by beneficial fungi and commercialization challenges related to formulation and field performance (Escrivá *et al*., 2022; Glare *et al*., 2023).

Therefore, this research was aimed at evaluating the biocontrol potential of native endophytic fungi from pepper and cowpea. The specific objectives were to: (1) isolate and identify associated endophytic fungi; (2) evaluate the *in vitro* inhibitory effects of fungal extracts on key pathogens (*X. campestris, F. oxysporum*); and (3) characterize the metabolites produced by active isolates using analytical chemistry.

MATERIALS AND METHODS

Sample Collection and Source of Plant Materials

Healthy, mature plants of *Capsicum annuum* (pepper) and *Vigna unguiculata* (cowpea) were collected from the Ajiwa Irrigation site in Katsina State, Nigeria. Plant selection was based on the absence of visible disease symptoms. The samples were carefully placed in sterile polythene bags, labeled, and transported to the Plant Pathology Laboratory in the Department of Plant Science and Biotechnology, Federal University Dutsin-ma, for immediate processing.

Isolation and Morphological Identification of Endophytic Fungi

Endophytic fungi were isolated from surface-sterilized plant tissues following an established protocol (Schulz *et al*., 1993). Root, stem, and leaf segments were sequentially immersed in 70% ethanol for 1 minute, 2% sodium hypochlorite for 5 minutes, and then rinsed three times in sterile distilled water. The sterilized tissues were aseptically cut into 5 mm² pieces using a sterile scalpel and plated onto Potato Dextrose Agar (PDA) medium, prepared and sterilized according to standard methods (Cappuccino & Sherman, 2014). Surface-sterilized explants were plated on PDA and incubated at 25 ± 2°C for 7–14 days to allow endophytic growth. The efficacy of the surface sterilization process was concurrently verified by plating the final sterile rinse water or performing tissue imprints on agar to ensure all observed colonies originated from internal tissues.

Fungal colonies growing from the plant tissues were sub-cultured onto fresh PDA to obtain pure isolates. Preliminary identification was based on macroscopic characteristics (colony morphology, color, texture, and growth rate) (Barnett & Hunter, 1998). For microscopic identification, lactophenol cotton blue mounts were prepared and observed under a light microscope (Olympus CX23) at 40x and 100x magnification. Fungal structures, including hyphal morphology, conidiophores, and spore characteristics, were used for identification to the genus level with reference to standard mycological texts (Ellis, 1971; Pitt & Hocking, 2009). For isolates tentatively identified as *Aspergillus* sp. and *Fusarium* sp. based on morphology, genomic DNA was extracted using the [e.g., CTAB method or a commercial kit]. The Internal Transcribed Spacer (ITS) region of ribosomal DNA was amplified using primers ITS1 and ITS4, and for *Fusarium*, the translation elongation factor 1-alpha (TEF1-α) gene was also amplified. Purified PCR products were sequenced, and sequences were compared to the NCBI GenBank database using BLASTn for definitive species-level identification.

In Vitro Antagonistic Assay of Fungal Extracts

The target pathogens were isolated from symptomatic pepper and cowpea plants collected from farms in Katsina State, Nigeria. *Xanthomonas campestris* was isolated from leaf lesions on semi-selective mCS20ABN medium, while *Fusarium oxysporum* and *Pythium* spp. were isolated from vascular tissues and rotted roots, respectively, on

PDA. Pathogenicity of each purified isolate was confirmed via Koch's postulates on healthy seedlings of their respective host plants. These confirmed pathogenic isolates were then used as target strains in the antagonism assays.

Preparation of Fungal Crude Extracts: Each endophytic fungal isolate was inoculated into 250 mL Erlenmeyer flasks containing 100 mL of Potato Dextrose Broth (PDB). The flasks were incubated at 25°C on a rotary shaker (120 rpm) for 14 days to facilitate secondary metabolite production (Khan *et al*., 2015). The cultures were then filtered through Whatman No. 1 filter paper to separate the mycelial biomass from the culture filtrate, which served as the crude fungal extract.

Antimicrobial Assay: This standardized suspension (100 µL) was spread evenly onto the surface of solidified Mueller-Hinton Agar (for bacteria) or Potato Dextrose Agar (for fungi) plates using a sterile cotton swab. Wells (6 mm in diameter) were aseptically bored into the agar using a sterile Cork borer. Aliquots (100 µL) of the fungal culture filtrate (crude extract) were pipetted into the wells, with sterile Potato Dextrose Broth (PDB) used as a negative control. The plates were incubated at 28°C. The zone of inhibition (ZOI) around each well was measured in millimeters using a digital caliper after 24 hours (for bacteria) or 48–72 hours (for fungal pathogens), depending on the growth rate.

For the fungal pathogens, the percentage inhibition of mycelial growth was calculated using the formula:

$$\text{Percentage Inhibition} = \frac{C-T}{C} \times 100$$

Where *C* is the colony diameter in the control plate and *T* is the colony diameter in the treatment plate (Schulz *et al*., 1993). Assays were performed in triplicates.

Extraction and Characterization of Secondary Metabolites

Metabolite Extraction: Secondary metabolites were extracted from the PDB cultures of selected endophytic fungi with potent inhibitory activity. After 14 days of incubation, the broth cultures were filtered, and the cell-free filtrates were subjected to liquid-liquid extraction using an equal volume mixture of methanol and chloroform (1:1 v/v) in a separatory funnel (Strobel *et al*., 2004). The organic phase containing the metabolites was collected and concentrated using a rotary evaporator (Heidolph) at 40°C under reduced pressure. The resulting crude extract was stored at 4°C until required for analysis (Cappuccino & Sherman, 2014).

Chromatographic Analysis and Metabolite Identification: The chemical profiles of the crude extracts were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Separation was performed on an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) with helium as the carrier gas. The oven temperature was programmed from 50°C (hold 2 min) to 300°C at a rate of 10°C/min. Mass spectra were obtained in electron impact (EI) mode at 70 eV, scanning from m/z 50 to 600 (Hanson, 2005).

Metabolites were tentatively identified by comparing their mass spectra and retention indices with those of reference compounds in the National Institute of Standards and Technology (NIST) spectral database. Quantification of specific compound classes (e.g., alkaloids, terpenoids, phenolics) was performed by constructing calibration curves using external standards: caffeine for alkaloids, β-sitosterol for terpenoids, and gallic acid for phenolic compounds (Singleton *et al*., 1999).

Data Analysis

All data collected were subjected to appropriate statistical analysis

RESULTS

Endophytic Fungi Isolated from *Capsicum annuum* and *Vigna unguiculata*

A total of four distinct endophytic fungal isolates were obtained from *Capsicum annuum* and *Vigna unguiculata*. Isolates A, B, and D were recovered from *C. annuum*, whereas Isolates B and C were obtained from *V. unguiculata*.

Morphological characterization of Isolate A, preliminarily identified as *Aspergillus* section *Nigri*. (A) Macroscopic view of a 7-day-old colony on Potato Dextrose Agar (PDA) at 25°C, showing dark green conidia and a colorless to pale yellow margin. The insert panel shows the reverse side of the colony, highlighting the characteristic oil-drop-like appearance. (B) Photomicrograph (1000x magnification, scale bar = 10 µm) of a lactophenol cotton blue mount, displaying typical morphological structures including septate hyphae, unbranched conidiophores with smooth walls, and radiate conidial heads bearing chains of dark, rough-walled conidia.

Microscopic examination revealed morphological characteristics consistent with *Alternaria* spp., including pineapple-shaped, multicellular conidia with both horizontal and vertical septa, arranged in chains.

Isolate B was identified as *Aspergillus niger*, producing colonies that were initially white, later turning black due to abundant conidia formation. Under microscopic observation, vesicles were entirely covered with phialides, from which chains of black conidia originated.

Isolate C produced green colonies interspersed with white, fluid-like areas. Microscopy revealed the presence of sporangia containing spores, accompanied by well-developed rhizoids. The isolate is identified as *Penicillium sp*.

Isolate D (*Rhizopus stolonifer*) exhibited colonies that were initially white but turned gray to black upon maturation, attributed to sporangia production (Plate 4.1). Microscopic observations showed coenocytic (aseptate) hyphae, lacking septa and containing multiple nuclei (Plate 4.2).

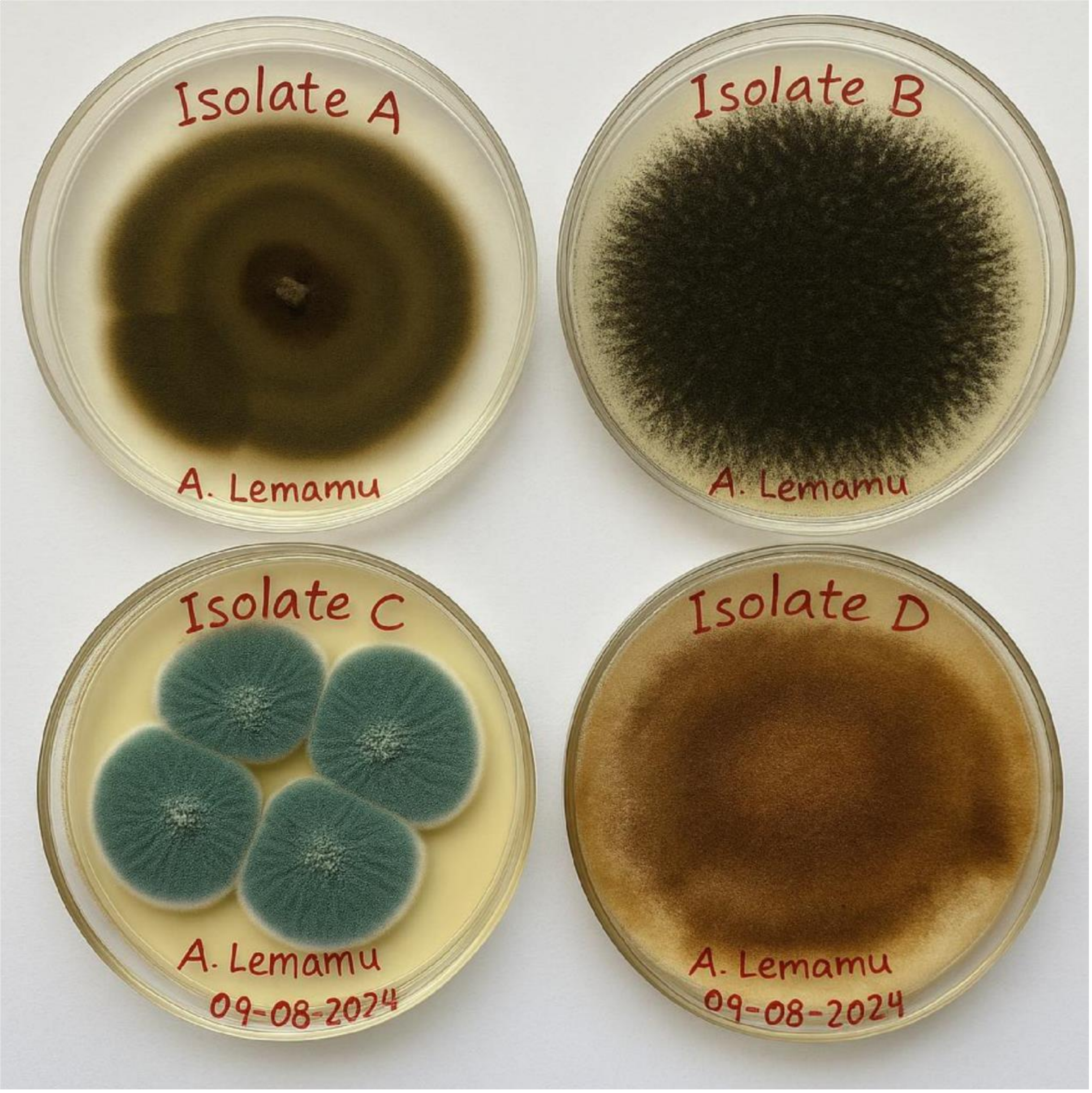
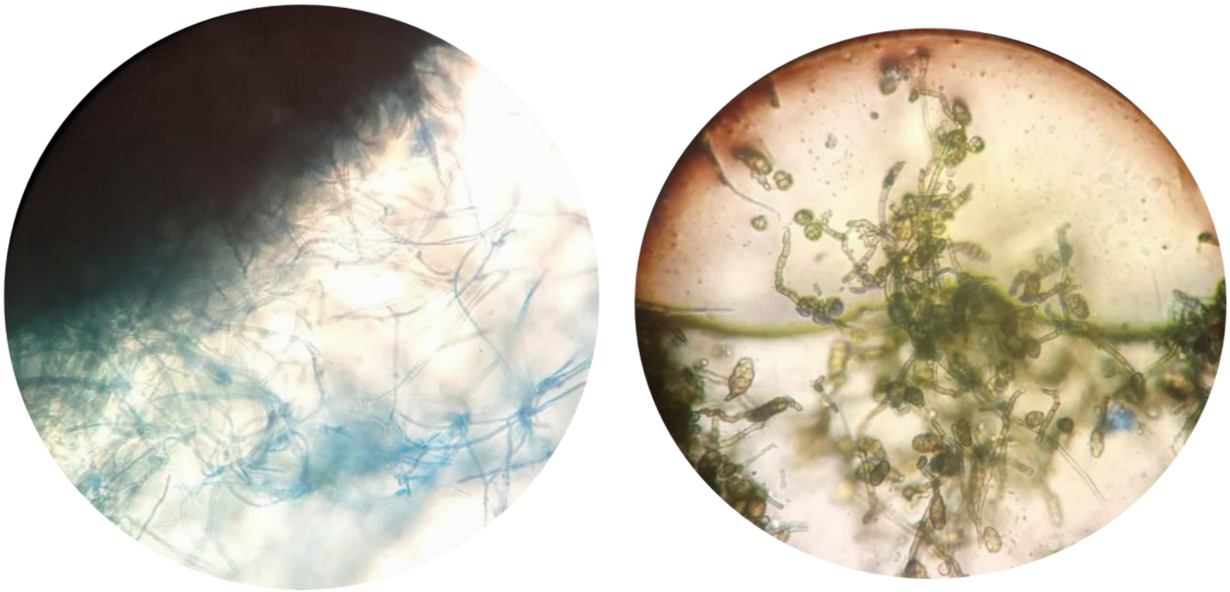
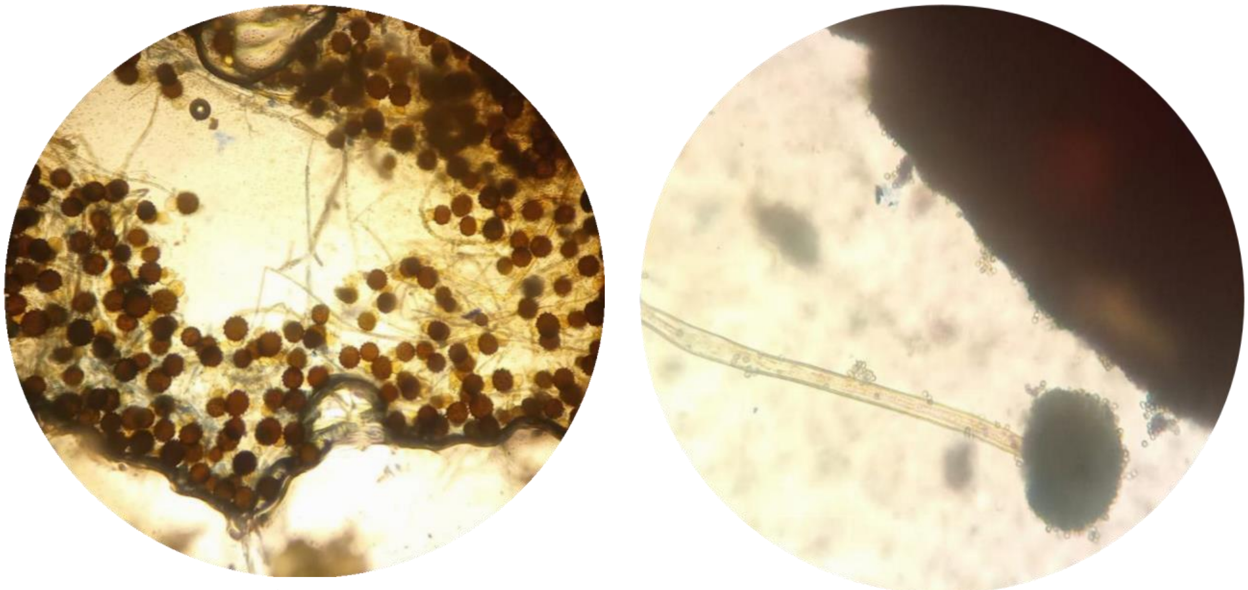


Plate 1: Showing Endophytic Isolates from *C. annuum* and *V. unguiculata* on PDA



Isolate C Isolate D



Isolate A Isolate B

Plate 2. Photomicrographs of endophytic fungal isolates from *Capsicum annuum* and *Vigna unguiculata*
(A-B) Isolate B (cf. *Penicillium* sp.). (A) Branching pattern showing biverticillate and terverticillate conidiophores. (B) Detail of phialides and chains of smooth-walled, globose to subglobose conidia. (C-D) Isolate C (cf. *Aspergillus* section *Nigri*). (C) Characteristic radiate conidial head with uniseriate phialides borne on a straight, smooth-walled stipe. (D) Detail of rough-walled, globose conidia forming chains. All images are from lactophenol cotton blue mounts. Scale bars = 10 µm

Table 1 presents the inhibitory effect of culture filtrates from four endophytic fungal isolates against the bacterium *Xanthomonas campestris* across a range of concentrations (25–100 µg/mL). The effect is measured as a zone of inhibition (ZOI) in millimeters, with data presented as Mean ± Standard Deviation. Different lowercase superscript letters (a, c, d, e, f, g, h) indicate statistically significant differences between means. The fungal isolates exhibited highly variable antibacterial activity. *Aspergillus niger* and *Penicillium* sp. showed a clear, concentration-dependent inhibitory effect, with the zone of inhibition increasing as the concentration of their culture filtrate increased. *Alternaria* sp. and *Rhizopus stolonifer* showed no activity against *X. campestris* at any tested concentration, with results identical to the negative control. *Penicillium* sp. was the most potent isolate. It produced the largest zones of inhibition at every concentration (24.5 ± 2.2 mm at 100 µg/mL). *Aspergillus niger* was inhibitory but less potent than *Penicillium* sp. at equivalent concentrations (17.1 ± 1.5 mm at 100 µg/mL). The control column (0 µg/mL) confirmed that no inhibition occurred in the absence of the fungal filtrates, validating that the observed effects were due to the treatments. The table demonstrates that the antibacterial effect was isolate-specific, not a universal property of all endophytic fungi tested. *Penicillium* sp. emerges as the most promising candidate for further investigation, given its strong, dose-dependent inhibition of *Xanthomonas campestris*.

Table 2 shows the inhibitory effect of culture filtrates from four endophytic fungal isolates on the growth of *Fusarium oxysporum*. The antifungal activity was tested across a concentration gradient (25–100 µg/mL), and the results are presented as the mean zone of inhibition (in mm) ± standard deviation. The lowercase superscript 'a' denotes values that are not statistically different from the negative control (0 µg/mL), indicating no significant inhibitory effect. Aspergillus niger and Penicillium spp. demonstrated clear, dose-dependent antifungal activity against *F. oxysporum*. The zone of inhibition increased progressively with higher concentrations of their culture filtrates. Alternaria spp. and Rhizopus stolonifer showed no inhibitory activity at any concentration tested, with results identical to the control. Penicillium spp. was the most effective antifungal agent. It produced the largest zones of inhibition at every corresponding concentration (e.g., 16.6 ± 0.3 mm at 100 µg/mL). Aspergillus niger was active but consistently less potent than *Penicillium* spp. (e.g., 11.3 ± 0.22 mm at 100 µg/mL).

A strong positive correlation between filtrate concentration and inhibitory effect is evident for the two active fungi, confirming that the antifungal compounds are effective in a dose-dependent manner. The antifungal effect against *Fusarium oxysporum* is specific to certain endophytic fungi. *Penicillium* spp. exhibits the strongest potential as a source of antifungal metabolites, while *Alternaria* spp. and *Rhizopus stolonifer* appear inactive against this particular pathogen under the tested conditions.

LC–MS Analysis of Secondary Metabolites Produced by *Aspergillus niger*

Table 3 presents the secondary metabolites detected in the culture filtrate of the endophytic fungus *Aspergillus niger* using Liquid Chromatography–Mass Spectrometry (LC–MS). Compound identification was achieved by comparing the obtained mass spectra with entries in the National Institute of Standards and Technology (NIST) spectral database. The table summarizes the peak number, retention time, compound name, molecular formula, and molecular weight of the identified metabolites. LC–MS analysis revealed the presence of several bioactive secondary metabolites. These included gliotoxin, an epipolythiodioxopiperazine (ETP) mycotoxin, detected at a retention time of 14.97 min with a molecular weight of 326.39 g/mol. Ochratoxin A, a chlorinated pentaketide mycotoxin, was detected at 10.90 min with a molecular weight of 403.82 g/mol. Patulin, a polyketide-derived mycotoxin, was identified at 9.70 min with a molecular weight of 250.25 g/mol. In addition, 2-phenylethanol, an aromatic alcohol, was detected at 13.80 min with a molecular weight of 122.17 g/mol. The LC–MS chromatogram (Fig. 1) exhibited multiple peaks distributed across the retention time range, indicating the production of a chemically diverse set of metabolites by *A. niger*. An early eluting peak was observed at approximately 2.5 min, followed by a cluster of prominent peaks between 6 and 10 min, with the highest signal intensity occurring near 9 min. Additional peaks were detected between 12 and 15 min. These findings confirm that *Aspergillus niger* produces a diverse profile of secondary metabolites, including both antimicrobial compounds and known mycotoxins, as identified by LC–MS analysis.

Table 1: Showing the Antimicrobial Effect of Endophytic Fungi on *Xanthomonas campestris*

Fungal Isolate	Control	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
***Alternaria* sp.**	0.00±0.00^{a}	0 .00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Aspergillus niger	0.00±0.00^{a}	9.2±0.8^{c}	12.5±1.1^{d}	14.8±1.3^{e}	17.1±1.5^{f}
***Penicillium* sp.**	0.00±0.00^{a}	13.4±1.2^{d}	19.3±1.7^{f}	22.7±2.0^{g}	24.5±2.2^{h}
Rhizopus stolonifer	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Key: Values are Means ±Standard Deviations; means followed by the same superscripts within the same column are not significantly different ($P \geqslant 0.05$).

Table 2: Inhibitory effect of endophytic fungal culture filtrates on the mycelial growth of *Fusarium oxysporum*

Fungal Isolate	Control	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
***Alternaria* sp.**	0 .00±0.00^{a}	0 .00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Aspergillus niger	0 .00±0.00^{a}	4.7 ± 0.64^{d}	7.0 ± 0.22^{c}	9.7 ± 0.07^{b}	11.3 ± 0.22^{a}
***Penicillium* sp.**	0 .00±0.00^{a}	6.3 ± 0.3^{d}	9.3 ± 0.2^{c}	13.3 ± 0.2^{b}	16.6 ± 0.3^{a}
Rhizopus stolonifer	0 .00±0.00^{a}	0 .00±0.00^{a}	0 .00±0.00^{a}	0 .00±0.00^{a}	0 .00±0.00^{a}

Values represent the zone of inhibition (mm, Mean ± Standard Deviation). Means within a row followed by different superscript letters are significantly different ($p < 0.05$) according to [e.g., Tukey's HSD test].

Table 3: Metabolites Produced by *Aspergillus niger* Identified Using LC-MS Chromatogram and the NIST Spectral Database

Peak	Retention Time (Mins)	Compound/Metabolites	Molecular Formula	Molecular Weight
263	14.97	Gliotoxin	C13H14N2O4S2	326.39
239	10.90	Ochratoxin A	C20H18ClNO6	403.82
191	09.70	Citrinin	C13H14O5	250.25
177	13.80	2-Phenylethanol	$C_8H_{10}O$	122.17

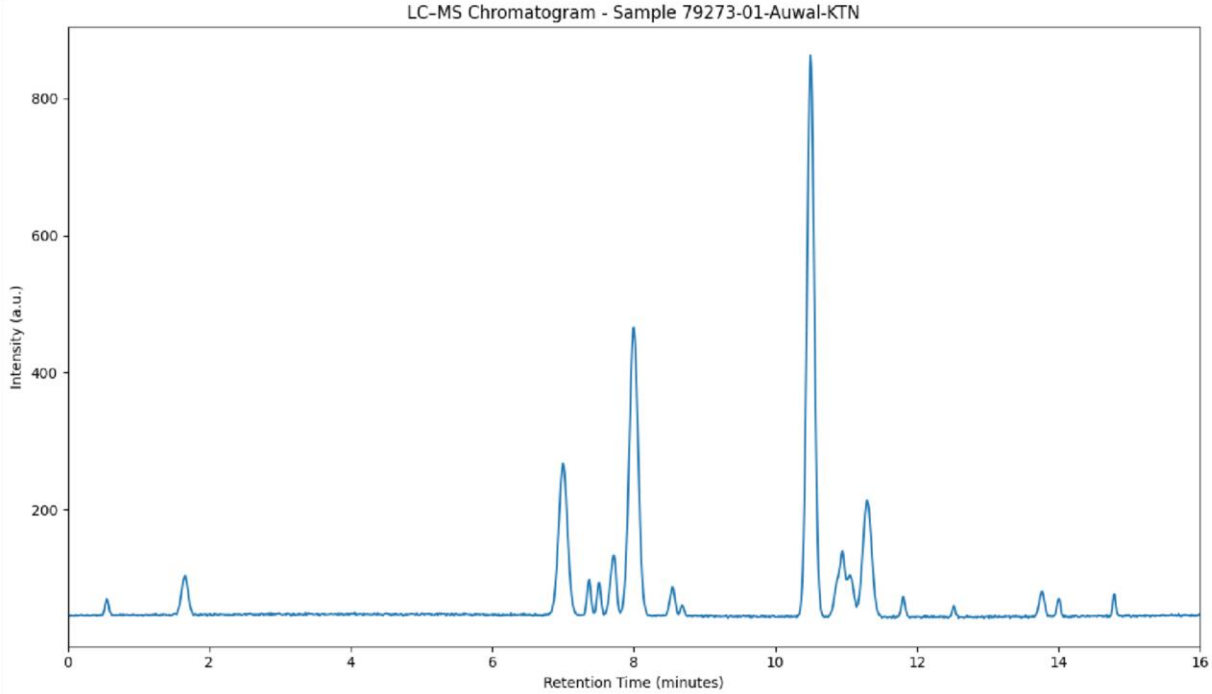


Figure 1: LC-MS chromatogram of the *Aspergillus niger* culture filtrate

LC–MS Analysis of Secondary Metabolites Produced by *Penicillium* spp.

Table 4 summarizes the secondary metabolites detected in the culture filtrate of *Penicillium* spp. using Liquid Chromatography–Mass Spectrometry (LC–MS). Compound identification was carried out by matching mass spectral data with the National Institute of Standards and Technology (NIST) database. The table presents the chromatographic peak number, retention time, compound name, molecular formula, and molecular weight of the identified metabolites. Seven secondary metabolites were identified from the culture filtrate. These included Penicillin G (MW: 334.39 g/mol), Griseofulvin (MW: 352.77 g/mol), Patulin (MW: 154.12 g/mol), Ochratoxin A (MW: 403.82 g/mol), Asterric acid (MW: 348.60 g/mol), Sulochrin (MW: 332.30 g/mol), and Cyclopenin (MW: 294.30 g/mol). The detected compounds comprised antibiotics, antifungal agents, and mycotoxins. The LC–MS chromatogram (Fig. 2) displayed multiple peaks distributed throughout the analytical run, indicating the presence of a complex mixture of secondary metabolites. A prominent early-eluting peak was observed at approximately 2.5 min. Several partially resolved peaks occurred between 10 and 20 min, while a distinct peak was detected at approximately 28.5 min, corresponding to a late-eluting compound. Overall, the LC–MS profile confirms that *Penicillium* spp. produces a diverse range of secondary metabolites, as evidenced by the broad distribution of chromatographic peaks across the run time.

Table 4: Some Metabolites Produced by *Penicillium spp*. Identified Using LC-MS Chromatogram and the NIST Spectral Database

Peak	Retention Time (Mins)	Compound/Metabolites	Molecular Formula	Molecular Weight
347	22.02	Asterric acid	C17H16O8	348.60
153	02.54	Patulin	C7H6O4	154.12
331	14.02	Sulochrin	C17H16O7	332.30
335	12.01	Penicillin G	C16H18N2O4S	334.39
404	28.63	Ochratoxin A	C20H18ClNO6	403.82
354	21.32	Griseofulvin	C17H17ClO6	352.77
295	**14.09**	**Cyclopenin**	$\mathbf{C_{17}H_{14}N_2O_3}$	**294.30**

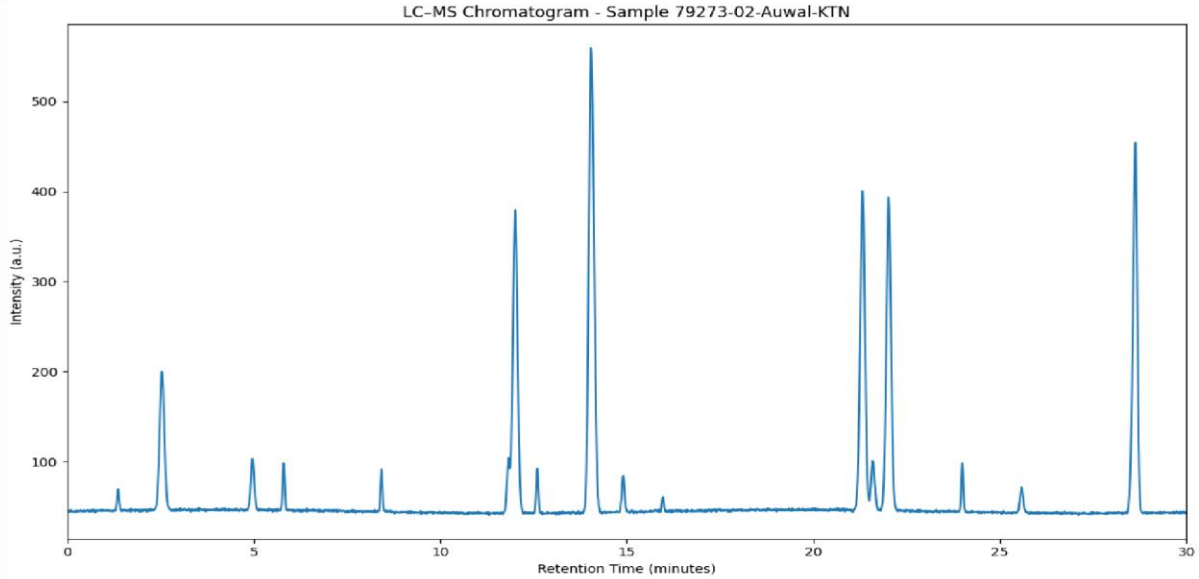


Figure 2. LC-MS Chromatographic Profile of Secondary Metabolites from an Endophytic *Penicillium* sp.

DISCUSSION

Our study successfully isolated four common endophytic fungi *Alternaria* sp., *Aspergillus niger*, *Penicillium* sp., and *Rhizopus stolonifer* from pepper and cowpea, which aligns with reports of these genera as frequent components of crop endophytic communities (Verma *et al*., 2022). The real interest, however, lay in testing their functional potential against major pathogens.

The *in vitro* bioassays revealed a clear split in activity. *Penicillium* sp. was a standout performer, showing strong, dose-dependent inhibition against both *Xanthomonas campestris* and *Fusarium oxysporum*. This potency is logically explained by its chemistry; our LC-MS analysis identified it as a producer of the classic antibiotic penicillin G and the antifungal drug griseofulvin, compounds long associated with the genus's antimicrobial prowess (Hawksworth & Lücking, 2023; González-Menéndez *et al*., 2019). *Aspergillus niger* also showed consistent, though more moderate, activity, which we can link to its production of the known antimicrobial compound 2-phenylethanol (Morales-Valenzuela *et al*., 2022). In contrast, *Alternaria* sp. and *Rhizopus stolonifer* showed no activity, underscoring that presence within a plant does not guarantee biocontrol utility and highlighting the need for functional screening (Ali *et al*., 2021).

The most critical and sobering finding emerged from our metabolite profiling. While the active fungi were producing beneficial antimicrobials, they were simultaneously synthesizing hazardous mycotoxins including ochratoxin A and patulin. This dual chemical nature presents a serious dilemma (Escrivá *et al.*, 2022). We have identified a highly promising biocontrol candidate in *Penicillium* sp., yet its associated toxigenic potential is a major biosafety concern, particularly for food crops (Rodríguez-Carrasco et al., 2021). This finding necessitates a cautious interpretation of biocontrol studies that report activity without concurrent toxin screening.

Looking ahead, our initial agar well diffusion assays, while useful for screening, have inherent limitations, such as the inability to determine minimum inhibitory concentrations (CLSI, 2020). The logical next steps involve more precise quantitative assays and, crucially, efforts to separate the beneficial antimicrobial compounds from the toxic ones through bioassay-guided fractionation (Keller, 2019). Ultimately, the true test lies beyond the Petri dish. Future work must investigate whether an isolate like *Penicillium* sp. can colonize seedlings and protect them *in planta*, and whether protection stems from direct antibiosis, the induction of systemic resistance, or both a mechanism well-documented for other endophytes (Pieterse *et al*., 2014; Bamisile *et al*., 2021).

CONCLUSION

In conclusion, our work underscores the dual promise and challenge of using native endophytes. We identified a potent fungal candidate but also unveiled a significant toxicity hurdle that must be addressed. The path forward for sustainable agriculture in Nigeria involves refining this potential, perhaps by seeking non-toxigenic strains or purifying the beneficial metabolites, to safely harness the power of these hidden fungal symbionts.

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