CONFERENCE PROCEEDINGS, JANUARY 2025
Published by the Faculty of Science (FSC), FULafia
Print ISSN: 2354–3388 DOI: https://doi.org/10.62050/fscp2024.490

Morphological Identification of Endophytic Fungi of Tomato Plants (*Solanum lycopersicum* L.) in Lafia

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Abstract: Endophytic fungi are a diverse group of microorganisms that inhabit the internal tissues of plants without causing immediate harm. A study was carried out to isolate, identify and determine the distribution of endophytic fungi in tissues of tomato plants in Lafia. Tissue segments of tomato plant samples about 2 cm^2 in size were surface sterilized in 70% ethanol for 2 min, followed 1% sodium hypochlorite for 2 min, and plated on potato dextrose agar (PDA) for three days for the emergence of endophytic fungi. Endophytic fungi were morphologically identified through observation of their macro and micromorphological chracteristics. A total of 15 isolates were identified, two isolates each (20%) belonging to *C. rosea*, *A. niger*, *P. corticola*, *A. flavus*, and *M. ruber*, while one isolate each (10%) of *A. viridistratum*, *A. calidoustus*, *Fusarium* sp., *Penicillium* sp., and *Aspergillus* sp. were recovered. Ombi-Anzaku had the highest number of isolates 6(40.00%), comprising one isolate of *A. viridistratum* (6.67%), two isolates of *A. niger* (13.33%), and one isolate each (6.67%) of *A. calidoustus*, *Fusarium* sp., and *Penicillium* sp., followed by Akoko with 5 isolates (33.33%), comprising two *A. flavus* (13.33%), one *Aspergillus* sp. (6.67%), and two *M. ruber*(13.33%). Bakin-Rijiya and Bukan-Kwato had two isolates each (13.33%), comprising *P. corticola* in Bakin-Rijiya, and *C. rosea* in Bukan-Kwato. The identification of genera with known biocontrol, bioactive, and growth-promoting properties underscores the potential application of these fungi in sustainable agricultural practices.

Keywords: Endophytes, tomato, morphological identification, distribution, fungi

Introduction

Endophytic fungi are a group of microorganisms that colonize the internal tissues of plants without causing any apparent disease symptoms. They establish a symbiotic relationship with their host plants, often conferring beneficial effects such as enhanced growth, nutrient acquisition, and protection against various stresses [1-2]. However, certain endophytic fungi can turn pathogenic under certain conditions, leading to detrimental effects on plant health and productivity [3].

In recent years, the study of endophytic fungi has gained prominence as researchers have recognized their potential role in the complex interactions occurring within plant-microbe communities [4]. The identification and characterization of endophytic fungi is essential for understanding their biology, ecology, and pathogenicity. Accurate species identification provides crucial information for effective disease management strategies, including the development of resistant cultivars and targeted fungicide applications [5-6]. Furthermore, the study of endophytic fungi can provide valuable insights into their role as potential biocontrol agents or beneficial endophytes [7].

Materials and Methods

Sample collection

A purposive sampling technique was employed to select tomato fields representing different geographical locations within Lafia. Leaf, stem, and root tissues samples were collected from healthy-looking tomato plants showing no apparent disease symptoms. A total of 100 samples were collected from different fields and stored in sterile plastic bags and transported to the Plant Science and Biotechnology Laboratory, Federal University of Lafia, for further analysis. The GPS coordinates of the sampled locations are provided in Table 1.

Table 1: GPS coordinates of the sampled locations

S/N	Name of Location	GPS Coordinate
1.	UAC Road	8°32'36.258'' N 8°30'0.666'' E
2.	Bakin-Rijiya	8°31'20.256'' N 8°37'9.438'' E
3.	Shendam Road	8°31'33.786'' N 8°36'33.132'' E
4.	Bukan Kwatu	8°28'24.327'' N 8°33'35.685'' E

Isolation of endophytic fungi

The collected tomato samples were processed for endophytic fungal isolation using the protocol described by Zakaria and Ning [8]. Briefly, tissue segments of the plant samples about 2 cm^2 in size were cut using a sterile scalpel, and surface sterilized for two minutes each in 70% ethanol and 1% sodium hypochlorite, thereafter, the sterilized tissues were rinsed in two changes of sterile distilled water for one minute in each change of water. The sterilized samples of stem, leaf, and root tissues were plated on potato dextrose agar (PDA) supplemented with about 0.01 mL of stremptomycin antibiotics to inhibit bacterial growth. The plates were incubated at room temperature for 4-10 days until fungal colonies emerged. Pure cultures of fungal isolates were obtained by transferring single hyphal tips to fresh PDA plates. The isolated fungal colonies were identified to the species level based on morphological characteristics [9].

Morphological identification

Pure cultures of isolated fungi were subjected to detailed morphological characterization, including colony color, texture, elevation, pigmentation, growth rate, conidial size, and shape, hyphal structure, and orientation of conidiophore [10].

Results and Discussion

Results of morphological identification of endophytic fungi isolated from tomato leaf, stem, and root in Lafia are presented in Table 2. The fungi were identified in seven genera, namely *Annulohypoxylon, Clonostachys, Aspergillus, Fusarium, Perenniporia, Monascus,* and *Penicillium.* Based on morphological characteristics such as colony appearance, texture, hyphal structure, conidia shape and pigmentation, and orientation of conidiophores, the fungi in the seven genera were identified as *Annulohypoxylonviridistratum, Clonostachys rosea, Aspergillus niger, Aspergillus calidoustus, Aspergillus flavus, Aspergillus* sp., *Fusarium* sp., *Perenniporiacorticola, Monascusruber,* and *Penicillium* sp.

Table 2: Morphological Characteristics of Endophytic Fungi Isolated from Tomato Leaves, Stems, and Roots

Isolate Code	Morphological characteristics				Morphologically assigned identity
	Cultural characteristics	Conidia	Conidiogenous cells	Hyphae	
A1-L1P1R1	Pinkish-brown mixed with white colonies, raised, wooly, and irregular mycelia, with filiform margins	Cylindrical, smooth-walled, and hyaline	Endoconidia were found within hyphae	Septate, branched, and pigmented	*Annulohypoxylon viridistratum*
A1-L4P1R1	Cream to pink, mixed with white colonies, raised, circular, with powdery texture, surrounded with entire margins	Conidia were oval, bean-shaped and hyaline	Slender monophialides were observed	Highly septate and pigmented	*Clonostachys rosea*
A2-LIPIL1	Coffee brown colonies, with powdery texture, slightly raised, irregular, with whitish filiform margins	Conidia were globose, pigmented, and rough-walled	Conidiophores were radial, with globose vesicles	Hyphae were scantily branched and hyaline	*Aspergillus niger*
A2-L1P2L2	Yellowish-white colonies, raised, powdery, circular, with filiform margins	Conidia were smooth-walled, Sub-oval and pyriform	Conidial head was radiate, with uniseriate flask-shaped phialides borne on subclavate vesicles	Hyphae were scantily branched and septate	*Aspergillus calidoustus*
A2-L1P5L5	Pinkish white colonies, raised, woolly, almost circular, with filiform margins	Septate macroconidia and oval-shaped microconidia present	Abundant monophialides were present	Hyphae were scantily branched and septate	*Fusarium* sp.
A-L3P3L3	Colonies were lemon green, flat, and circular, with a powdery texture and filiform margins	Conidia were thick-walled, oval and sub-oval	Conidial heads were columnar, comprising subclavate vesicles that bore flask-shaped phialides	Hyphae were branched and contained thick-walled septations	*Aspergillus flavus*
A-L3P4R4	Grayish-brown colonies, with a scant wooly texture, raised mycelia, with circular, filiform margins	Absent	Terminal and intercalary chlamydospores present	Hyphae were irregular, branched, pigmented, and highly septate with constrictions and points of septation.	*Monascus ruber*
A-L3P5S5	Bluish green colonies, raised, woolly, irregular, with whitish filiform margins	Oval and pigmented	Biverticillate conidiophores bore slender and flask-shaped phialides.	Hyphae unbranched, non-septate, and hyaline	*Penicillium* sp.
B1-L3P4RA	Cultures were flat, coffee brown, and circular, with filiform margins	Pigmented, smooth-walled, oval and sub-oval	Uniseriate flask-shaped phialides on sub-globose vesicles	Hyphae septate and extensively branched.	*Aspergillus* sp.

Table 3: Distribution of endophytic fungi associated with tomato leaves, stems, and roots in Lafia

Endophyte	No. of Isolates			
	Ombi-Anzaku	Bakin-Rijiya	Akoko	Bukan-Kwato
A. viridistratum	1(6.67%)	-	-	-
C. rosea	-	-	-	2(13.33%)
A. niger	2(13.33%)	-	-	-
A. calidoustus	1(6.67%)	-	-	-
A. flavus	-	-	2(13.33%)	-
Aspergillus sp.	-	-	1(6.67%)	-
Fusarium sp.	1(6.67%)	-	-	-
P. corticola	-	2(13.33%)	-	-
M. ruber	-	-	2(13.33%)	-
Penicillium sp.	1(6.67%)	-	-	-
Total	**6(40.00%)**	**2(13.33%)**	**5(33.33%)**	**2(13.33%)**

– = Fungal species not found

The distribution of endophytic fungi associated with tomato leaf, stem, and root in Lafia is presented in Table 3. A total of 15 fungal isolates were obtained from tomato leaf, stem, and root during the study. Of the recovered fungi, two isolates each (20%) belonged to *C. rosea, A. niger, P. corticola, A. flavus,* and *M. ruber,* while one isolate each (10%) of *A. viridistratum, A. calidoustus, Fusarium* sp., *Penicillium* sp., and *Aspergillus* sp. were recovered. Ombi-Anzaku had the highest number of isolates 6(40.00%), comprising one isolate of *A. viridistratum* (6.67%), two isolates of *A. niger* (13.33%), and one isolate each (6.67%) of *A. calidoustus*, *Fusarium* sp., and *Penicillium* sp., followed by Akoko with 5 isolates (33.33%), comprising two *A. flavus* (13.33%), one *Aspergillus* sp. (6.67%), and two *M. ruber* (13.33%). Bakin-Rijiya and Bukan-Kwato had two isolates each (13.33%), comprising *P. corticola* in Bakin-Rijiya, and *C. rosea* in Bukan-Kwato.

The study on morphological identification of endophytic fungi isolated from tomato leaves, stems, and roots in Lafia revealed the presence of seven genera;*Annulohypoxylon*, *Clonostachys*, *Aspergillus*, *Fusarium*, *Perenniporia*, *Monascus*, and *Penicillium*. These findings align with previous studies that highlight the diverse and significant roles of endophytic fungi in plant health, protection, and growth enhancement. The genus *Annulohypoxylon*, belonging to the family Xylariaceae, has primarily been associated with decaying wood in natural ecosystems. However, its identification as an endophyte in tomato plants in Lafia suggests a possible mutualistic relationship. Zhao *et al*. [11] reported that *Annulohypoxylon* species produce bioactive secondary metabolites, such as antifungal compounds and antioxidants, which may contribute to plant defense mechanisms.

The ability of this genus to adapt as an endophyte might be due to its metabolic versatility and capacity to form symbiotic associations. *Clonostachys* species are recognized for their significant biocontrol potential against plant pathogens. Sun *et al*. [12] demonstrated that *Clonostachys rosea* can colonize plant tissues and protect them by parasitizing pathogenic fungi, including *Botrytis cinerea* and *Fusarium* spp. The presence of *Clonostachys* in tomato plants in Lafia could be attributed to its competitive ability to occupy ecological niches within plant tissues and its potential to offer protection against fungal pathogens. The identification of *Aspergillus* as an endophyte in tomato plants in Lafia aligns with numerous studies highlighting its widespread occurrence in various environments, including plant tissues.

According to Khan *et al*. [13], *Aspergillus* species are known for producing secondary metabolites, including plant growth-promoting compounds and stress-resistance enzymes. The adaptability of *Aspergillus* as an endophyte could be due to its ability to utilize diverse carbon sources within plant tissues, providing both ecological and functional advantages. *Fusarium* species are notorious as plant pathogens but have also been reported as endophytes in numerous plants [14]. Non-pathogenic strains of *Fusarium* have been found to promote plant growth and induce resistance to environmental stresses. The occurrence of *Fusarium* in tomato tissues in Lafia may suggest the presence of non-pathogenic endophytic strains capable of coexisting with the plant without causing disease. Yuan *et al*. [14] emphasized the dual nature of *Fusarium*, noting that environmental factors and host plant conditions often determine whether it behaves as a pathogen or beneficial endophyte.

The genus *Perenniporia*, typically known for its wood-decomposing properties, has been increasingly identified as an endophyte in recent studies. Zhao *et al*. [11] highlighted thatmembers of the orderPolyporales, including *Perenniporia*, play roles in nutrient cycling and enhancing plant defense mechanisms. The isolation of *Perenniporia* from tomato plants in Lafia may point to its involvement in symbiotic nutrient exchanges and structural strengthening of plant tissues. The identification of *Monascus* as an endophyte in tomato plants is significant, given its traditional role in the fermentation industry for the production of pigments and bioactive compounds. Zhang *et al*. [15] noted that *Monascus* species can produce antifungal compounds that protect plants from pathogenic fungi. Its presence in tomato plants in Lafia may be linked to its ability to produce bioactive secondary metabolites, which could play a role in plant health and disease resistance.

The genus *Penicillium* is widely recognized for its production of bioactive compounds, including the antibiotic penicillin. In plant tissues, *Penicillium* species have been reported to function as endophytes that promote plant growth and enhance stress tolerance [13]. The detection of *Penicillium* in tomato plants in Lafia is consistent with findings from other studies where it was associated with improved plant health and resistance to pathogens. The diversity of fungal genera identified in tomato plants in Lafia may be influenced by several factors, including the environmental conditions of the region, the physiological characteristics of the tomato plant, and the microbial diversity in the surrounding soil.

The adaptability of these fungi as endophytes is likely due to their ability to colonize internal plant tissues without causing harm while contributing to plant health through various mechanisms such as pathogen suppression, stress tolerance, and nutrient exchange [11, 14]. The detection of genera like *Aspergillus*, *Penicillium*, and *Fusarium* may be attributed to their ubiquitous nature and capacity to thrive in diverse environments. Additionally, the isolation of *Clonostachys* and *Monascus* underscores the potential biocontrol and bioactive properties these fungi may confer to tomato plants [12. 15].

Conclusion

The study revealed that the endophytic fungal species prevalent in tissues of tomato plants in Lafia were *Annulohypoxylon viridistratum, Clonostachys rosea, Aspergillus niger, Aspergillus calidoustus, Fusarium* sp., *Perreniporia corticola, Aspergillus flavus, Monascus ruber, Penicillium* sp., and *Aspergillus* sp. The identification of genera with known biocontrol, bioactive, and growth-promoting properties underscores the potential application of these fungi in sustainable agricultural practices.

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