Original Article

CHARACTERISATION OF ENDOPHYTIC FUNGI FROM BELL PEPPER *(Capsicum annuum L.)* IN LAFIA, NIGERIA

Ugal, E., and *Terna, T.P.
Department of Plant Scnence and Biotechnology, Federal University of Lafia, PMB 146 Lafia, Nasarawa State, Nigeria.
Corresponding Author's Email: terna.paul@science.fulafia.edu.ng
Tel: 07030350853

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Correspondence;

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ABSTRACT

Tissue colonisation by endophytic microbes is an important determinant of plant health and produce quality. This study was carried out to identify and evaluate the pathogenicity and biological control potentials of endophytic fungi from bell pepper in Lafia, Nigeria. Endophytic fungi were isolated from disease-free pepper fruits collected from various pepper farms, and morphologically identified through cultural and microscopic examination. The identified endophytes were further subjected to pathogenicity test on disease-free pepper fruits for 7 days. Results showed that F. mangiferae was the most occurring (50%) species, followed by Candida sp. (30%), and both Clonostachys rosea (10%) and Bipolaris sp. (10%). Pathogenicity test showed that the endophytic F. mangiferae was the most pathogenic, producing 45.00% of tissue damage, followed by Candida sp. (25.00%), and Clonostachys rosea (5.00%), while pepper fruits inoculated with endophytic Bipolaris sp. showed no disease symptoms after five days of inoculation. Simultaneous pairing of the endophytic Bipolaris sp. with the rot-causing endophytes showed significant radial growth inhibition of F. mangiferae ($P<0.05$). These findings reveal that bell peppers play host to both pathogenic and non-pathogenic fungi which play critical roles in determining fruit health and yield quality. The study highlights C. rosea as a promising candidate for further evaluation as a biocontrol agent against Fusarium-associated fruit rots in pepper under greenhouse and field conditions.

KEYWORDS: Bell Pepper, Biocontrol Potentials, Endophytic Fungi, Fruit Rot, Pathogens.

INTRODUCTION

Pepper (*Capsicum annuum* L.) is a fruiting plant that belongs to the genus *Capsicum* and family *Solanaceae* (nightshades) (Liu *et al.,* 2023). The plant yields berries that are red, green, and yellow in color, and they frequently have a strong flavor. With over 200 variations within the species, it also has various common names and varieties, such as bell pepper, paprika, chili pepper, jalapeño, and cayenne (Barboza *et al.,* 2022). Additionally, domestication of this crop dates back around 6,000 years, making it one of the earliest crops to be grown (Kraft *et al*., 2014).

In Nigeria, over 200 different varieties of pepper are grown, and while the crop grows well in the South Western States of Nigeria, pepper is predominantly produced in the Northern States (Idowu-Agida *et al.,* 2012). Nutritionally, pepper has high levels of vitamins A and E, more vitamin C than many other vegetable crops, helpful in preventing flu and colds, and in a lot of homes, pepper is needed to enhance the flavour of several meals (Kantar *et al*., 2016). Despite the importance and frequent usage of pepper in Nigeria, the yield of pepper in Nigeria is low compared to other parts of the world. This low yield in pepper production has been attributed to some production challenges such as diseases, pests and poor management practices (NAERLS, 2016).

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 (Rodriguez *et al*., 2009; Hardoim *et al*., 2015). However, certain endophytic fungi can turn pathogenic under certain conditions, leading to detrimental effects on plant health and productivity.

Endophytic fungi play important roles in determining the health and productivity of several crops including pepper (Terna *et al*., 2024). In recent years, the study of endophytic fungal species has gained prominence as researchers have recognised their potential role in the complex interactions occurring within plant-microbe communities. Understanding the diversity of these endophytic fungi is crucial for managing crop diseases effectively and developing sustainable strategies for crop protection. Therefore, the present study was carried out to characterise endophytic fungi from bell pepper in Lafia, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Sample Collection

The study was carried out in Lafia, Nasarawa State Nigeria. Disease-free pepper fruits were sampled from farm lands within the four cardinal locations of Lafia, namely Lafia East, Lafia North, Lafia South, and Lafia West. Two farms each were randomly selected from the four cardinal locations, and from each farm, four pepper fruits were collected and transported in paper bags to the Department of Plant Science and Biotechnology Laboratory, Federal University of Lafia, Nasarawa State, for further analysis. A total of 32 pepper fruits were collected.

Isolation and Identification of Endophytic Fungi from Pepper Tissues

The protocol of Terna *et al*. (2024) was used for tissue processing and isolation of endophytic fungi from tissues of sampled pepper fruits. Fruit tissue segments measuring about 1 cm^2 were cut using a sterile scalpel and rinsed with running water to remove dirt. Washed tissues were surface-sterilised by submerging in 1% sodium

hypochlorite solution for two min, followed by 70% ethanol for two min. Thereafter, surface disinfected plant tissues were rinsed three times in sterile distilled water for 1 min each time, and blotted dry on sterilised Whatman No.1 filter paper to remove excess water. After surface sterilisation, the imprint technique was used to confirm the efficacy of surface sterilisation before pepper tissues were plated on Potato Dextrose Agar (PDA) supplemented with 0.5% streptomycin to inhibit bacterial growth. Two tissues each were plated from each sampled fruit, at the rate of two tissues per plate, spaced about 5 cm apart. The plates were incubated at room temperature for 3-4 days until fungal colonies emerge. Pure cultures of fungal isolates were obtained by transferring single hyphal tips to fresh PDA plates. The pure cultures of isolated endophytic fungi were subjected to morphological characterisation to determine their macroscopic and microscopic features. Colony characteristics, such as growth rate, color, and texture, were recorded. Microscopic features, including shape and pigmentation of conidia, structure of hyphae, and conidiophore, were examined using light microscopy, following the methods outlined by Damm *et al*. (2012). The isolated fungi were identified with the aid of appropriate identification guides (Leslie and Summerell, 2006; Zalar *et al*., 2008; Samson *et al*., 2011; Visagie *et al*., 2014).

Pathogenicity Testing

Pathogenicity tests were conducted using the methods of Terna *et al*. (2024). The endophytic fungi were used to artificially inoculate pepper fruits by placing 7 mm diameter mycelial plugs on wounds created on pepper fruits by pricking twice with a sterile needle, while control experiments were inoculated with sterile PDA plugs. Disease symptoms were observed and recorded over a period of seven days, by measuring disease diameters with a transparent meter rule. Three treatment replicates were used for each fungal species.

Biological Control Tests

Potential biocontrol agents were screened against the most virulent endophytic species identified in the pathogenicity assays. Dual culture assays were performed on PDA plates, where the test organisms were inoculated on opposite sides of the plate, and observed for a period of seven days for antagonistic activity. The radial growth inhibition of the pathogenic strain was measured and used as an indicator of the antagonistic activity of the biocontrol agent.

Experimental Design and Data Analysis

The experiment was set up using the Completely Randomised Design (CRD) with three replications. Data obtained was subjected to Analysis of Variance (ANOVA) at 5% level of probability, using the Minitab Software, Version 17. Means were separated using the Tukey's Honestly Significant Different Test.

RESULTS

Results of morphological identification of endophytic fungi recovered from pepper fruits in Lafia are presented in Table 1 and Plate 1. On the basis of morphological characteristics such as colony colour, texture, elevation, margins, pigmentation, diameter, hyphal structure, type and structure of conidiophore, in addition to shape and texture of conidia, the endophytic isolates were identified as *Fusarium mangiferae* (5 isolates), *Clonostachys rosea* (1 isolate), *Candida* sp. (3 isolates), and *Bipolaris* sp. (1 isolate). A total of 10 isolates of endophytic fungi were recovered from the pepper fruits.

Table 2 presents the occurrence of the different endophytic fungi of pepper fruits in the four studied locations in Lafia. Of the isolated fungi, *F. mangiferae* was the most occurring (50%), followed by *Candida* sp. (30%), and both *C. rosea* (10%) and *Bipolaris* sp. (10%). Of the studied locations, Lafia East had the highest number of fungal species (n=3) and fungal isolates (n=9), followed by Lafia West, with one fungal species, and one fungal isolate. The fungal species identified in pepper fruits from Lafia East were *F. mangiferae*, *C. rosea*, and *Candida* sp., while only *Bipolaris* sp. was identified in Lafia West. No endophytic fungi were recovered from Lafia North and Lafia South.

Results of pathogenicity tests of the endophytic fungi recovered from pepper fruits are presented in the Table 3 and plate 2. The highest tissue damage of pepper fruits (45.00%) was caused by *F. mangiferae*, followed by *Candida* sp. (25.00%), and *C. rosea* (5.00%). Pepper fruits inoculated with endophytic *Bipolaris* sp. showed no symptoms of disease. Differences in tissue damage caused by the different endophytic fungi were not significant ($P \geq 0.05$).

Results of the radial growth inhibitions of rot-causing *Candida* sp. and *F. mangiferae* by endophytic *Bipolaris* sp. are presented in Table 4. The results revealed that *Candida* sp. was the most inhibited (5.18%), followed by *F. mangiferae* (2.47%). Differences in radial growth of *Candida* sp. by the endophytic *Bipolaris* sp. were significantly different from the control ($P<0.05$).

Table 5 presents the results of the radial growth inhibitions of rot-causing *Candida* sp. and *F. mangiferae* by endophytic *C. rosea*. The results showed that although *Fusarium mangiferae* was the most inhibited (21.70%), followed by *Candida* sp. (0.00%), differences in growth inhibition of the fruit rot fungi by the endophytic *C. rosea* were not significant ($P<0.05$).

Table 1: Morphological Characteristics of Endophytic Fungi Recovered from Pepper Fruits in Lafia

	Macromorphology				**Micromorphology**			**Morphological Identity**
Group	Colony Colour	Texture, Elevation and Margins	Mean Colony Diameter after 5 days on PDA	Pigmentation	Hyphae	Conidiophore	Conidia/spores	
1	Pinkish purple	Flat, circular colonies, with a slightly wooly texture, and filiform margins	6.6 cm	Pink to orange	Septate and sparsely branched	Slender monophialides and false conidial heads were found	Abundant macroconidia, microconidia and mesoconidia were found	*Fusarium mangiferae* (5 isolates)
2	Cream to yellow	Flat, slightly wooly to powdery, irregular, with slightly entire margins	7.45 cm	Pale cream to yellow	Septate and highly branched	Abundant short and slender monophialides	Conidia were transparent, and ranged in shape from bean-shaped, cylindrical, to oval	*Clonostachys rosea* (1 isolate)
3	Cream	Slimy, irregular cultures, with a flat elevation, and filiform margins	7.48 cm	Cream to yellow	Hyphae not found	Budding yeast cells	Abundant yeast cells with shapes ranging from cylindrical, oval, to sub-oval	*Candida* sp. (3 isolates)
4	Grayish green	Slightly raised, velvety, almost circular to irregular, with filiform margins	4.4 cm	Blackish green mixed with gray	Septate, with slander and long branching pattern	Conidiophore not properly differentiated.	Conidia are elongated, subcylindrical, highly segmented and pigmented	*Bipolaris* sp. (1 isolate)

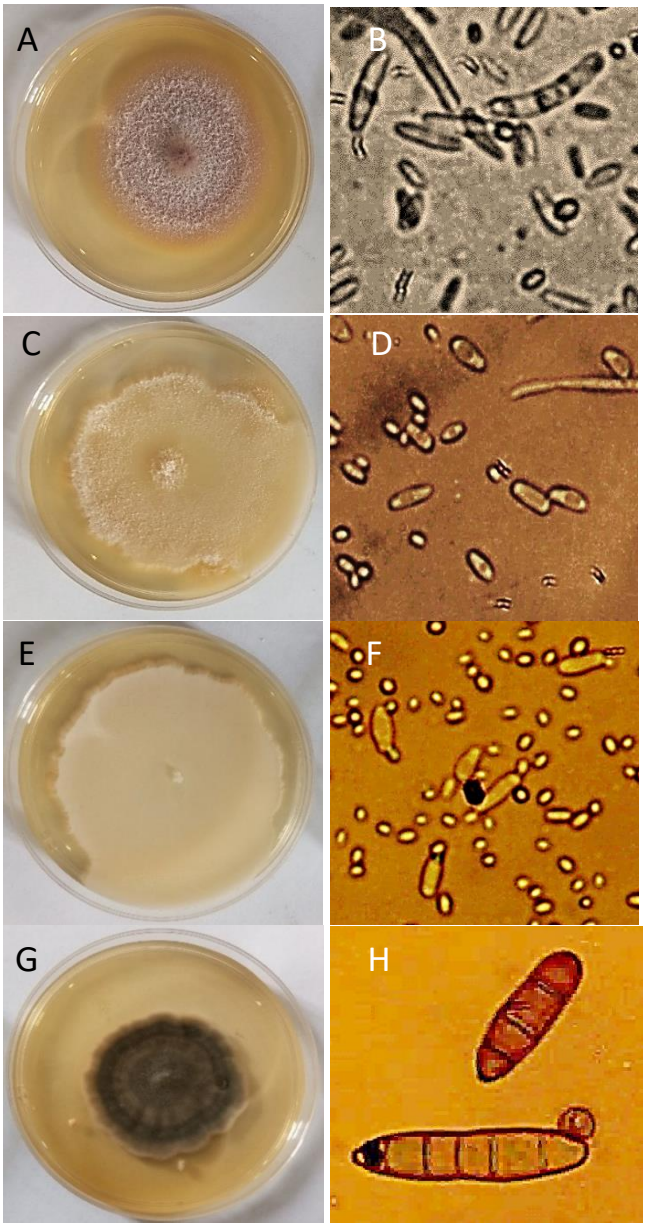


Plate 1. *Colony Appearance and Spore/Conidial Morphology of F. mangiferae (A-B), C. rosea (C-D), Candida sp. (E-F), and Bipolaris sp. (G-H) (×40).*

Table 2: Occurrence of Endophytic Fungi of Pepper Fruits in the Studied Locations

Location	*F. mangiferae*	*C. rosea*	*Candida* sp.	*Bipolaris* sp.	Total
Lafia North	-	-	-	-	0(0.00%)
Lafia South	-	-	-	-	0(0.00%)
Lafia East	5(50.00%)	1(10.00%)	3(30.00%)	-	9(90%)
Lafia West	-	-	-	1(10.00%)	1(10.00%)
Total	5(50.00%)	1(10.00%)	3(30.00%)	1(10.00%)	10.00

Table 3: Pathogenicity of Endophytic Fungi Recovered from Pepper Fruits

Isolates	% Tissue Damage
F. mangiferae	45.00[a]
Candida sp.	25.00[a]
C. rosea	5.00[a]
Bipolaris. sp.	0.00[a]
Control	0.00[a]

Means followed by the same superscripts within the same column are not significantly different (P≥0.05).

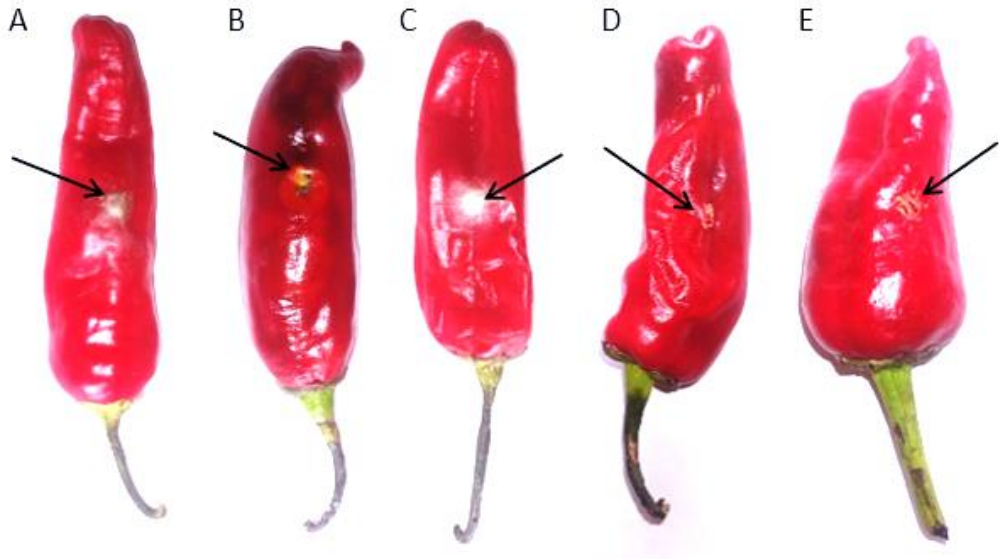


Plate 2: Pathogenicity of Endophytic Fungi Isolated from Pepper Fruits. (A) Bipolaris sp.; (B) C. rosea; (C) F. mangiferae; (D) Candida sp.; (E) Control. Black arrows indicate points of inoculation.

Table 4: Radial Growth Inhibitions of Rot-Causing Candida sp. and F. mangiferae by Endophytic Bipolaris sp. in Dual Culture Tests

Isolates	% Growth Inhibition
Candida sp.	5.18[a]
F. mangiferae	2.47[ab]
Control	0.00[b]

Means followed by the same superscripts within the same column are not significantly different (P≥0.05)

Table 5: Radial Growth Inhibitions of Rot-Causing *Candida* sp. and *F. mangiferae* by Endophytic *C. rosea* in Dual Culture Tests

Isolates	% Growth Inhibition
Candida sp.	0.00[a]
F. mangiferae	21.70[a]
Control	0.00[a]

Means followed by the same superscripts within the same column are not significantly different (P≥0.05)

DISCUSSION

The present study identified *Fusarium mangiferae*, *Clonostachys rosea*, *Candida* sp., and *Bipolaris* spp. as endophytes of pepper, with *Fusarium mangiferae* occurring most frequently. This finding is consistent with earlier studies on *Capsicum* worldwide, which generally report *Fusarium* spp. as dominant endophytes, alongside other fast-growing Ascomycetes (Paul *et al*., 2012). Yeasts such as *Candida* have also been recovered as seed or tissue endophytes of pepper, while *Clonostachys rosea* is widely recognized as an endophyte with strong biocontrol potential (Sun *et al*., 2020). Conversely, *Bipolaris* is more common in cereals and only occasionally reported as an endophyte in non-grass hosts, which supports its low frequency in this study. Within Africa, particularly Ethiopia and other pepper producing regions, *Fusarium* wilt remains a major problem, explaining its prevalence in endophyte isolations. In West Africa and Nigeria, *Fusarium* has been reported from pepper fruits (Terna *et al*., 2025), further supporting the observations in the present study.

Results of the pathogenicity assay showed that *Fusarium mangiferae* induced the highest level of tissue damage on artificially inoculated pepper fruits, followed by *Candida* sp. In contrast, *Clonostachys rosea* caused only minimal damage, while *Bipolaris* sp. and the uninoculated control produced no measurable tissue damage. *Fusarium* species are well-documented pathogens of *Capsicum*, frequently associated with fruit rots and internal infections. Furthermore, the moderate pathogenicity observed for *Candida* sp. is consistent with reports that certain yeast-like fungi can act as opportunistic postharvest pathogens under favourable conditions. These findings highlight the ecological continuum that exists among endophytic fungi, ranging from latent or opportunistic pathogens to non-pathogenic or mutualistic species (Rodriguez *et al*., 2009).

Dual culture test results showed that *Clonostachys rosea* displayed a more pronounced and selective antagonistic effect against *F. mangiferae*, while showing no measurable inhibition against *Candida* sp. This selective activity is consistent with documented biocontrol profile of *C. rosea*, which frequently exhibits stronger antagonism toward *Fusarium* species than toward other fungal taxa (Sun *et al*., 2020; Kulik *et al*., 2024). Recent dual-culture studies have reported inhibition rates of *Fusarium* by *C. rosea*, ranging from moderate to high, often accompanied by mycoparasitic interactions such as hyphal coiling and degradation. The selective inhibition of *F. mangiferae* by endophytic *C. rosea* in the study suggests an isolate- and pathogen-specific variation in antagonistic capacity.

CONCLUSION

The study identified *Fusarium mangiferae*, *Clonostachys rosea*, *Candida* sp., *and Bipolaris* sp. as the endophytic fungi associated with pepper fruits in Lafia, with *F. mangiferae* emerging as the most dominant species. Pathogenicity tests confirmed endophytic *F. mangiferae*, *C. rosea*, and *Candida* sp. as latent pathogens, causing appreciable tissue damage of pepper, while *Bipolaris* sp. was non-pathogenic. In dual-culture assays, *C. rosea* showed moderate but selective antagonism against *F. mangiferae*, while *Bipolaris* sp. displayed only limited inhibitory activity. The study highlights *C. rosea* as a promising candidate for further evaluation as a biocontrol agent against Fusarium-associated fruit rots in pepper under greenhouse and field conditions.

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