Original Article

PATHOGENICITY AND BIOLOGICAL CONTROL POTENTIALS OF A FOLIAR ENDOPHYTIC *Aspergillus* SPECIES FROM *Mangifera indica L.* (MANGO)

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ABSTRACT

Endophytic colonisation of plant tissues by microbes is an important determinant of plant health and productivity, yet, not much is known about the biological roles of endophytic fungi inhabiting mango leaf tissues. Therefore, a study was carried out to isolate, identify, and evaluate the pathogenicity and biological control potentials of foliar endophytic fungi of Mangifera indica L. (mango). An endophytic fungus isolated from disease-free leaves of mango collected from different parts of Lafia was morphologically identified and subjected to pathogenicity test. Thereafter, the endophyte was screened for biological control potentials using dual culture tests against two pathogenic Colletotrichum species. The endophyte was morphologically identified as A. flavus. Pathogenicity test results showed that the endophytic A. flavus was unable to produce disease symptoms on artificially inoculated mango leaves. Simultaneous pairing of the endophytic A. flavus with pathogenic Colletotrichum nymphaeae and Colletotrichum asianum strains in dual culture tests showed 16.95% and 27.94% radial growth inhibitions of the respective pathogenic fungi. The differences in radial growth inhibition of the pathogens by the endophytic A. flavus were significant ($P<0.05$). The findings of this study revealed that the endophytic A. flavus from mango leaves was non-pathogenic and also possessed appreciable biological control potentials, requiring further assessment to validate its suitability as a potent biological control agent against fungal pathogens of plants.

KEYWORDS: Aspergillus Flavus, Biocontrol Agent, Endophytic Fungi, Fungal Pathogens, Mango Leaves.

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INTRODUCTION

Mango (*Mangifera indica L.*) is one of the most widely cultivated and consumed fruits in the world (FAO, 2020). It is widely cultivated in countries like India, China, Brazil, and Nigeria, with global production increasing steadily (Saúco, 2019). According to the Food and Agriculture Organisation (FAO), the growing demand for mangoes worldwide is driven by their nutritional value, flavor, and versatility (FAO, 2020).

In Nigeria, mango cultivation is a vital part of the agricultural sector, providing income for many farmers, and the country's climate allows for mango production, particularly in regions like Nasarawa State (RMRDC, 2026). However, studies have shown that mango production in Nigeria faces challenges like diseases and pests, which can significantly impact yields and quality (Onyeani *et al.*, 2012).

Endophytic fungi are microorganisms that reside within plant tissues without causing visible symptoms of disease (Schulz and Boyle, 2006). These fungi have been found in various plant species, and have been known to promote plant growth, enhance stress tolerance, and produce bioactive compounds (Strobel and Daisy, 2003). Some endophytic fungi have also been shown to have pathogenic potentials, causing disease in plants under certain conditions (Saikkonen *et al*., 1998).

Although endophytic fungi are important determinants of plant health and productivity, not much is known about the biological roles of these fungi within mango leaf tissues. Therefore, the present study was carried out to isolate, identify, and evaluate the pathogenicity and biological control potentials of foliar endophytic fungi of *Mangifera indica* (mango) in Lafia, Nasarawa State Nigeria.

MATERIALS AND METHODS

Sample Collection

Mango leaves were collected from disease-free mango trees sampled from farm lands within the four cardinal locations of Lafia, namely Lafia East, Lafia North, Lafia South, and Lafia West. Four farms each were randomly selected from the four cardinal locations (Table 1), and from each farm, four healthy mango leaves were collected. The leaves were placed in sterile polythene bags and transported to the Plant Science and Biotechnology Laboratory, Federal University of Lafia, for processing.

Table 1: GPS Coordinates of the Sampled Locations

Location	Longitude	Latitude
Lafia North	8 °30'2.034" N	8 °31'20.226" E
Lafia South	8 °33'7.58" N	8 °32'12.887" E
Lafia East	8 °29'5.13" N	8 °32'42.073" E
Lafia West	8 °29'26.744" N	8 °30'46.408" E

Isolation of Endophytic Fungi

The protocol described by Terna (2022) was used for tissue processing and isolation of endophytic fungi from tissues of sampled mango leaves. Leaf 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 2 min, followed by 70% ethanol for 2 min. Thereafter, surface disinfected plant tissues were rinsed three times in sterile distilled water for 1 min each time, and blotted dry on sterile Whatman No.1 filter paper to remove excess water.

After surface sterilization, the imprint technique was used to confirm the efficacy of surface sterilisation before leaf tissues were plated on Potato Dextrose Agar (PDA) supplemented with 0.5% chloramphenicol, to inhibit bacterial growth. Two tissues each were plated from each sampled leaf, 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 emerged. Pure cultures of fungal isolates were obtained by transferring single hyphal tips to fresh PDA plates.

Fungal Identification

The isolated fungus was identified based on their macro and micro morphological characteristics. The macro morphological characteristics were colony color, texture, margin, pigmentation and size, while micro morphological characteristics included shape of conidia, hyphal structure, and orientation of conidiophore (Samson *et al.,* 2010).

Pathogenicity Test

The leaf bioassay method of Terna *et al.* (2020) was used for pathogenicity tests. Healthy Mango leaves were surface sterilized by swabbing with 70 % ethanol, and wounded slightly with a sterilised needle. Wounded portions were inoculated by placing mycelial plugs (7 mm diameter) of the fungal isolate taken from the actively growing hyphae. Inoculated Mango leaves were placed in sterile plastic containers and monitored for 72 h for the development of disease symptoms. Two sterile cotton balls moistened with sterile water were kept in the plastic container to keep the container humid throughout the duration of the pathogenicity test. Disease incidence was assessed visually by estimating the percentage of diseased tissue in relation to the entire leaf area as reported by James (1971).

Source of Fungal Pathogens

Pathogenic *Colletotrichum nymphaeae* and *Colletotrichum asianum* used for biocontrol assays were obtained from the plant pathology culture collection of the Department of Plant Science and Biotechnology, Federal University of Lafia. The pathogens produced necrotic lesions on citrus leaves in previous studies.

Biological Control Assay

The biological control potential of the isolated endophytic fungus was evaluated through in vitro antagonism test, where the endophyte was tested for its ability to inhibit the growth of plant pathogenic fungi. The isolated fungus was grown in dual culture with plant pathogenic fungi on sterile PDA plates to evaluate the biological control potentials of the isolate. The diameters of the two different cultures

were measured with a meter rule and used to compute percentage radial growth inhibition using the formula:

$$PRGI = \frac{\text{Growth of pathogen in control} - \text{Growth of pathogen paired with antagonist}}{\text{Growth of pathogen in control}} \times 100$$

Where PGRI = Percentage of Radial Growth Inhibition

Experimental Design and Data Analysis

Experiments were set up using the Completely Randomized Design (CRD) with two replicates. The data collected were subjected to Analysis of Variance (ANOVA) at 5% level of probability using the Minitab statistical software, Version 20. Means were separated using the Fisher's Least Significant Difference (LSD).

RESULTS

Results of morphological identification of the endophytic fungus isolated from mango leaves in Lafia are presented in Table 2 and Plates 1 to 3. 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 isolate was identified as *Aspergillus flavus.*

Results of pathogenicity test of endophytic *A. flavus* recovered from mango leaves are presented in Table 3. Mango leaves showed no disease symptoms after three days of inoculation with endophytic *A. flavus*, signifying that the isolate was non-pathogenic on the tested plant.

Figure 1 and Plate 2 presents the results of antagonistic activity of endophytic *A. flavus* against a pathogenic strain of *Colletotrichum nymphaeae* in dual culture test. The results revealed that the radial growth of the pathogen were inhibited by 16.95% which was significantly different from the control ($P<0.05$). The formation of a clear zone of growth inhibition (Plate 4) may be indicative of the production of growth inhibitors by the antagonist (*A. flavus*) against the pathogen (*C. nymphaeae*).

Figure 2 and Plate 3 presents the results of antagonistic activity of endophytic *A. flavus* against a pathogenic strain of *Colletotrichum asianum* in dual culture test. The results revealed that the radial growth of the pathogen were inhibited by 27.94% which was significantly different from the control ($P<0.05$). The presence of degraded hyphae on the pathogen's mycelia (Plate 3) may be indicative of mycoparasitic activity of antagonist (*A. flavus*) against the pathogen (*C. asianum*).

Table 2: Morphological Characteristics of Endophytic Fungi Recovered from Mango Leaves in Lafia

Macromorphology				**Micromorphology**			**Identity**
Colony Colour	Texture, Elevation and Margins	Pigmentation	MCD	Hyphae	Conidiophore	Conidia	
Deep to yellowish green	Colonies were flat, powdery, circular, with entire margins	Pale to orange	6.08 cm	Branched and extensively septate	Conidial heads were mostly oval shaped, while conidiophores were monoverticillate, bearing slender flask-shaped phialides and globose vesicles.	Oval, and mostly smooth-walled	*Aspergillus flavus* (1 isolate)

MCD = Mean Colony Diameter after 5 days on PDA

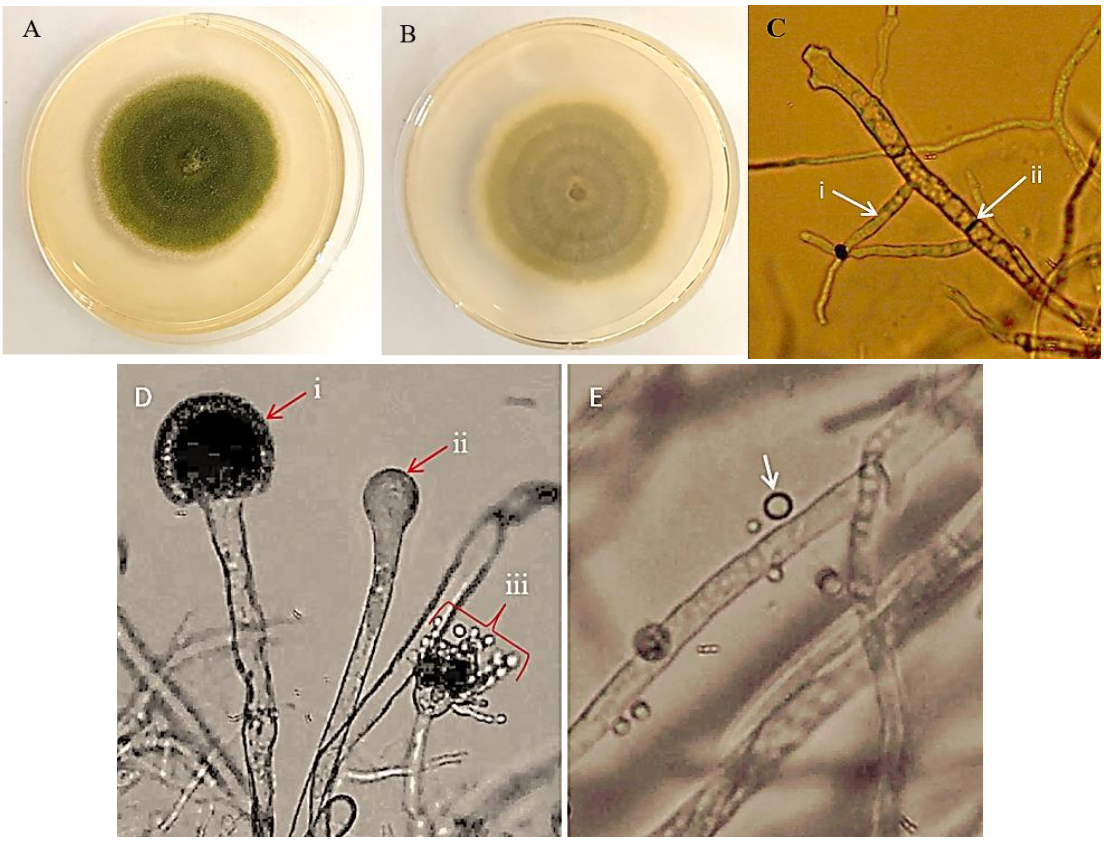


Plate 1. Micromorphological Characteristics of Endophytic *A. flavus* Recovered from Mango Leaves. A). Colony appearance; B). Pigmentation on reverse; Ci). Hyphal branching; Cii). Hyphal septation; Di). Monoverticillate conidial head; D(ii): Globose vesicle; D(iii) Monoverticillate conidial head bearing phialides and conidia; E: Oval conidium (×40).

Table 3: Pathogenicity of Endophytic A. flavus Recovered from Mango Leaves

Isolates	% Leaf necrosis	% Leaf chlorosis
A. flavus	0.00[a]	0.00[a]
Control	0.00[a]	0.00[a]

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

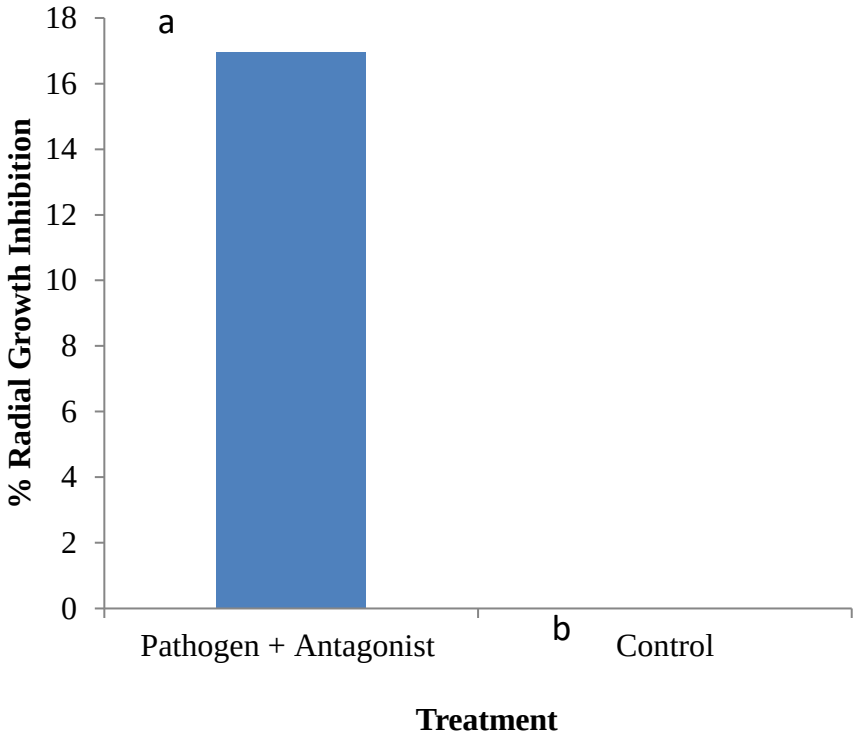


Figure 1. Biological Control Activity of Endophytic *A. flavus* Against Pathogenic *C. nymphaeae* (Bars carrying different alphabets are significantly different (P<0.05)).

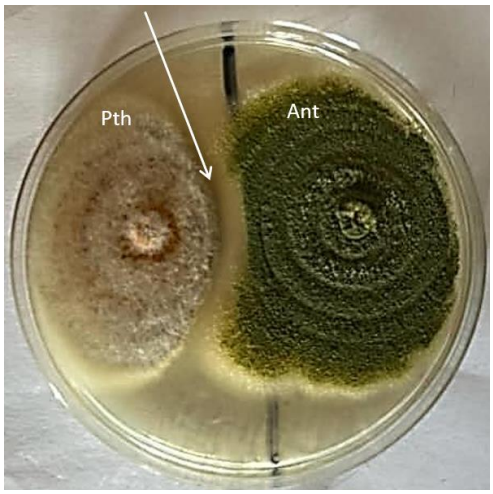


Plate 2. Antagonistic activity of endophytic *A. flavus* against a pathogenic strain of *C. nymphaeae* in dual culture test. (Pth) Pathogen; (Ant) Antagonist. White arrow indicates zone of growth inhibition.

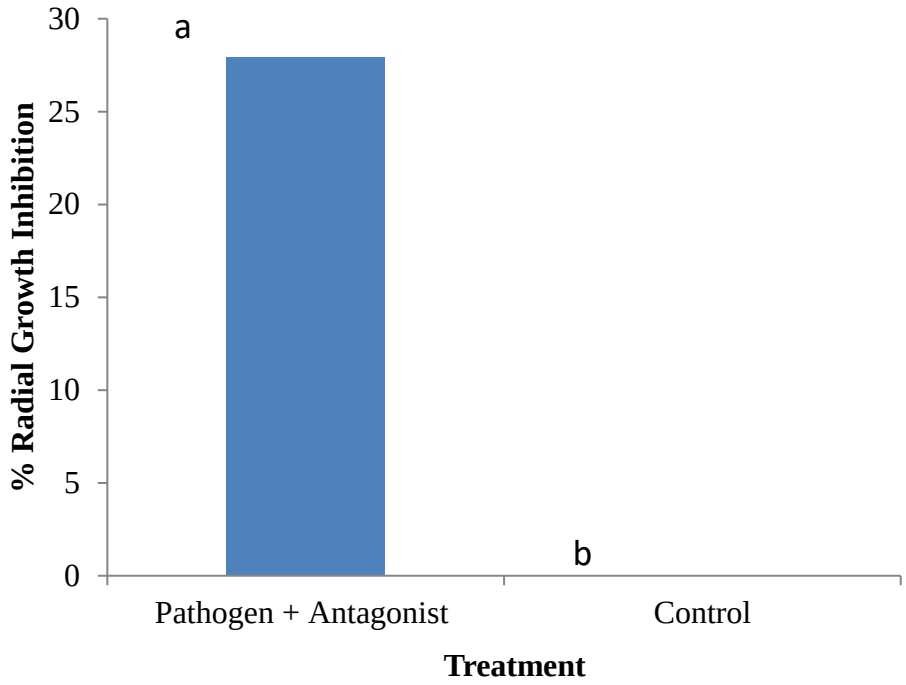


Figure 2. Biological Control Activity of Endophytic *A. flavus* Against Pathogenic *C. asianum* (Bars carrying different alphabets are significantly different (P<0.05)).

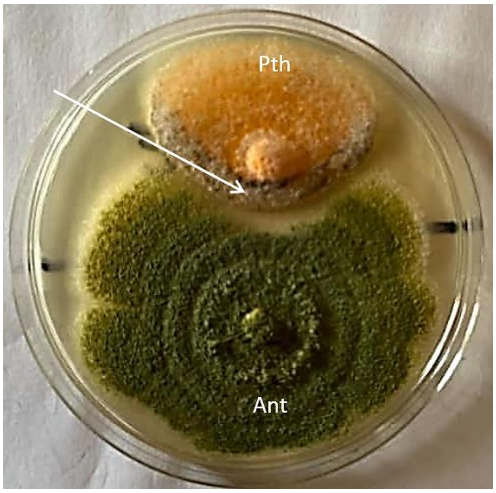


Plate 3. Antagonistic activity of endophytic *A. flavus* against a pathogenic strain of *C. asianum* in dual culture test. White arrow shows degraded pathogen mycelia.

DISCUSSION

Then endophytic fungus isolated in the present study was identified as *A. flavus,* based on the morphological descriptions of Samson *et al.* (2010). Although reports on the isolation of endophytic *A. flavus* from leaves of mango are generally lacking, Garuba *et al.* (2018) identified endophytic *A. flavus* from the pulp of mango, while other studies have reported *A. flavus* occurring endophytically in leaves of plants such as *Aegle marmelos* (Indian bael) (Patil *et al.*, 2015), *Dillenia indica* (Elephant apple) (*Gupta et al*., 2025), and *Zea mays* (corn) (Terna *et al.*, 2024). The success of *A.*

flavus as an endophyte of several plants can be attributed to its wide host range, especially in warm, humid regions where mango and several other tropical plants are grown. The warm temperatures and humid conditions prevalent in these regions are also favourable for the survival and dispersal of *A. flavus* (Zingales *et al.,* 2022; *Gupta et al*., 2025).

Results of pathogenicity tests indicated that *A. flavus* was not pathogenic in the inoculated plant tissues, which contradicts traditional perceptions of this species as pathogenic on several plants (Jackson-Ziems *et al*., 2012; Terna *et al.,* 2024). This finding aligns with the broader ecological understanding of *A. flavus* as a versatile organism capable of asymptomatic endophytic colonisation in tropical plants, while retaining the potential to shift to a pathogenic lifestyle under stress conditions such as elevated temperature and carbondioxide concentration (Maia *et al.,* 2024).

Further screening of the isolate for biocontrol activity against the foliar pathogens of citrus, *Colletotrichum nymphaeae* and *Colletotrichum asianum* demonstrated that the endophyte exhibited significant antagonistic activity. This finding corroborates literature asserting that certain strains of *A. flavus* can produce bioactive compounds that impede the growth of plant pathogens (Loi *et al*., 2020). This attribute is of paramount importance in sustainable agriculture. The utilization of such endophytic fungi is an increasing focus amidst rising concerns over chemical fungicides impacting human health and the environment (Bacon *et al*., 2019). The study revealed that *A. flavus* was the prevalent endophytic fungal species in *Mangifera indica* leaves sampled in Lafia. Test of pathogenicity also showed that the endophytic *A. flavus* was not pathogenic when inoculated on healthy mango leaves, while biocontrol assay revealed that the endophytic *A. flavus* isolate showed inhibitory activity against plant pathogenic strains of *C. nymphaeae* and *C. asianum.* The findings reveal that the endophytic *A. flavus* from mango leaves was non-pathogenic and also possessed appreciable biological control potentials, requiring further assessment to validate its suitability as a potent biological control agent against fungal pathogens of plants.

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