



Research Article

Biocontrol Potentials and Pathogenicity of Fungi Associated with Leaf Spots of Groundnuts

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important legume, valued globally for its high protein and edible oil content. However, foliar fungal diseases continue to severely limit productivity, causing significant annual yield losses. This study evaluated the pathogenicity and biocontrol potential of fungi associated with groundnut leaf spots in Lafia, Nasarawa State, Nigeria. Fungi isolated from diseased groundnut leaf tissues were artificially inoculated on healthy groundnut leaves to evaluate pathogenicity for a period of 7 days. The biocontrol potential of the non-pathogens was assessed through dual culture tests. Pathogenicity tests revealed significant differences in the virulence of fungal isolates ($P < 0.05$). The highest leaf spots (45.80%) were produced by *Aureobasidium pullulans*, followed by *Lecanicillium lecanii* (38.30%), *Curvularia lunata* WF2F (30.83%), *C. lunata* NF2H (30.00%), and *Fusarium* sp. (28.30%). Dual culture assays demonstrated significant variations in antagonistic activity among the tested antagonistic fungi ($P < 0.05$). Non-pathogenic *A. pullulans* exhibited the highest inhibition of *L. lecanii* (42.33%) and *C. lunata* (26.58%), while *Trichoderma* sp. was most inhibited by *A. pullulans* (35.06%). Whereas *Mucor* sp. produced the highest inhibition of *Fusarium* sp. (38.8%). The study identified *A. pullulans*, *L. lecanii*, and *C. lunata* as highly virulent foliar pathogens of groundnut, requiring targeted management to minimize yield losses. Furthermore, non-pathogenic strains of *A. pullulans*, *Trichoderma* sp., *A. niger*, *Mucor* sp., and *C. rosea* showed promising antagonistic activity against leaf spot pathogens, highlighting their potential as effective biocontrol agents of pathogens causing fungal leaf spots of groundnuts.

Keywords: Biocontrol agents; Dual culture assays; Fungal pathogens; Groundnut; Lafia; Leaf spots

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an important legume and oilseed crop, valued around the world for its high protein content (roughly 25–30%), edible oil (45–50%), and numerous benefits, as food, animal feed, and industrial raw material. In Nigeria, it is one of the main food and cash crops, especially in the Guinea Savanna Zones such as Nasarawa State (Ajeigbe *et al.*, 2015; Mshelmbula *et al.*, 2025). Despite the crop's invaluable benefits, groundnut productivity in Nigeria is often threatened by biotic stressors, such as foliar fungal pathogens. Necrotic

lesions caused by these pathogens on leaves, petioles, and stems result in early defoliation, decreased photosynthetic capacity, and significant yield losses. Pod yield reductions of up to 70% have been reported in Nigeria and other parts of Africa, as a result of leaf spots caused by several fungi (Denwar *et al.*, 2021; Awoke *et al.*, 2025). Although the major causative agents are often species of *Passalora*, *Cercospora*, and *Phaeoisariopsis* (Giordano *et al.*, 2021; McDonald *et al.*, 1985), the pathogen complex, often involving other fungal species such as *Fusarium*, *Curvularia*, *Aureobasidium*, and *Aspergillus*, further

worsens the disease and complicates the implementation of effective management practices (Ndifon, 2022).

Although significant results have been achieved through the conventional use of synthetic fungicides in leaf spots management, they are often not easily affordable by the resource-poor smallholder farmers who are the major stakeholders in Nigeria's groundnut production. Furthermore, the concerns of residual toxicity, non-target environmental effect, and the possible emergence of fungicide-resistant pathogen populations invoke the urgent need for sustainable alternatives to synthetic fungicides in leaf spots management (Denwar *et al.*, 2021; Awoke *et al.*, 2025).

Biological control through the use of fungal biocontrol agents presents a promising, environmentally friendly alternative to synthetic fungicides (Thambugala *et al.*, 2020), and the successful use of various fungal antagonists in in vitro and in vivo biocontrol trials has been reported (Nartey *et al.*, 2022; El-Dawy *et al.*, 2023). The mechanisms of pathogen antagonism by these biological control agents include competition, mycoparasitism, antibiosis, and the induction of host resistance (Terna *et al.*, 2013; Terna, 2015; Yao *et al.*, 2023).

Despite the agricultural and food security significance of groundnut leaf spots, the pathogenicity and biological control potentials of fungi associated with the disease remain poorly understood, especially in Lafia (Joseph *et al.*, 2025). Therefore, this study was aimed at evaluating the pathogenicity and biological control potentials of fungi associated with leaf spot symptoms on groundnuts in Lafia, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Source of Fungal Isolates

Already identified fungal isolates from a previous study were collected from the plant pathology culture collection of the Department of Plant Science and Biotechnology, Federal University of Lafia and preserved on Potato Dextrose Agar (PDA) slants throughout the duration of the study.

Plant Samples

Healthy groundnut leaves for leaf bioassays were sourced from the Botanical Garden, Federal University of Lafia, and conveyed in sterile polyethylene bags to the Plant Science and Biotechnology Laboratory, Federal University of Lafia, for further experiments.

Pathogenicity Testing of Fungal Isolates from Groundnut Leaves

Pathogenicity tests were performed using the modified detached leaf bioassay method of Terna *et al.* (2020). The sampled leaves were surface sterilized by swabbing with 70% ethanol and kept hydrated throughout the experiment by wrapping the leaf petioles with sterile cotton balls moistened with sterile distilled water. The leaf tissues were wounded by pricking with a sterile needle, thereafter, 7 mm diameter agar discs obtained from actively growing mycelial regions of 7-day old cultures of potential leaf pathogens were aseptically placed at the wounded spots. The inoculated leaves were incubated for 7 days at 28°C, and observed for the development of disease symptoms. Disease was estimated using the standard area diagrams of Lage *et al.* (2015).

Biological Control Tests

Non-pathogenic fungi selected from the pathogenicity tests were considered as potential biocontrol agents and screened against the most virulent foliar fungal isolates. Dual culture assays were performed on PDA plates as reported by Terna *et al.* (2016). The test pathogen and antagonist were inoculated on opposite sides of the plate, and observed for a period of seven days for antagonistic activity. Pathogens and antagonists grown separately in single cultures served as the controls. The radial growths of the pathogens were measured using a meter rule, and percentage radial growth inhibition was computed using the formula:

$$PRG = \frac{PC - P}{PC} \times 100$$

Where, PRG = Percentage Radial Growth Inhibition; PC = Radial growth of pathogen in the control plate; P = Radial growth of pathogen in the presence of the antagonist.

Experimental Design and Data Analysis

Treatments were laid out using the Completely Randomized Design (CRD) with three replicates. Data obtained was subjected to Analysis of Variance (ANOVA) at 5% level of probability, using the Minitab Statistical Software, Version 19. The means were separated using Tukey's Honestly Significant Difference Test.

RESULTS

Results of pathogenicity test of fungi associated with diseased groundnut leaves in Lafia are presented in Table 1. Results showed that the representative isolates of different fungal species showed variations in pathogenic potential on artificially inoculated groundnut leaves. The strongly pathogenic isolates

were *A. pullulans* isolate SF4d, *L. lecanii* isolate WF4G, *C. lunata* isolate WF2F, *C. lunata* isolate NF2H, and *Fusarium* sp. isolate 2 SF4E, producing 45.80%, 38.30%, 30.83%, 30.00%, and 28.30% leaf spots, respectively, which differed significantly from the control ($P < 0.05$). Those classified as weak pathogens were *C. lunata* isolate SF3C, *A. niger* isolate WF3C, *A. niger* isolate WF4D, and *P. oxalicum* isolate EF2A, producing leaf spots ranging from 8.33% to 18.30%, which did not differ significantly from the uninfected control ($P \geq 0.05$). Isolates that did not produce disease symptoms on inoculated leaves were considered non-

pathogenic. They included *A. pullulans* isolates WF1B, EF1C, and NF2J, *C. lunata* isolates WF3A, and EF1B, *F. incarnatum* isolates WF2C, NF3B, SF3A, *Fusarium* sp. 1 isolate EF3A, *F. chlamydosporum* isolate NF1A, *A. niger* isolate WF4A, *A. terreus* isolates EF1F, SF4B, NF2A, and NF2E, *P. oxalicum* isolate EF2F, *Trichoderma* sp. 1 isolate WF3D, SF1E, EF3D, NF1C, and WF3B, *T. harzianum* isolate SF1B, WF1D, EF1A, *Trichoderma* sp. 2 isolate SF4H, *Trichoderma* sp. 3 isolate NF1E, *Colletotrichum* sp. isolate EF1E, *Mucor* sp. isolates EF2C, WF4C, SF1C, and SF3B, *C. rosea* isolates WF1C, SF2A, and NF3D.

Table 1. Pathogenicity of Fungi Isolated from Leaf Tissues Showing Leaf Spot Symptoms

S/No.	Isolate Code	Identity	Incidence of Leaf Spots (%)	Inference
1.	SF4D	<i>A. pullulans</i>	45.80 ^a	Strong pathogen
2.	WF1B	<i>A. pullulans</i>	0.00 ^d	Non-pathogenic
3.	EF1C	<i>A. pullulans</i>	0.00 ^d	Non-pathogenic
4.	NF2J	<i>A. pullulans</i>	0.00 ^d	Non-pathogenic
5.	WF4G	<i>L. lecanii</i>	38.30 ^{ab}	Strong pathogen
6.	WF2F	<i>C. lunata</i>	30.83 ^{abc}	Strong pathogen
7.	NF2H	<i>C. lunata</i>	30.00 ^{abc}	Strong pathogen
8.	SF3C	<i>C. lunata</i>	8.33 ^{cd}	Weak pathogen
9.	WF3A	<i>C. lunata</i>	0.00 ^d	Non-pathogenic
10.	EF1B	<i>C. lunata</i>	0.00 ^d	Non-pathogenic
11.	SF4E	<i>Fusarium</i> sp. 2	28.30 ^{abc}	Strong pathogen
12.	WF2C	<i>F. incarnatum</i>	0.00 ^d	Non-pathogenic
13.	NF3B	<i>F. incarnatum</i>	0.00 ^d	Non-pathogenic
14.	SF3A	<i>F. incarnatum</i>	0.00 ^d	Non-pathogenic
15.	EF3A	<i>Fusarium</i> sp. 1	0.00 ^d	Non-pathogenic
16.	NF1A	<i>F. chlamydosporum</i>	0.00 ^d	Non-pathogenic
17.	WF3C	<i>A. niger</i>	18.30 ^{bcd}	Weak pathogen
18.	WF4D	<i>A. niger</i>	8.33 ^{cd}	Weak pathogen
19.	WF4A	<i>A. niger</i>	0.00 ^d	Non-pathogenic
20.	EF1F	<i>A. terreus</i>	0.00 ^d	Non-pathogenic
21.	SF4B	<i>A. terreus</i>	0.00 ^d	Non-pathogenic
22.	NF2A	<i>A. terreus</i>	0.00 ^d	Non-pathogenic
23.	NF2E	<i>A. terreus</i>	0.00 ^d	Non-pathogenic
24.	EF2A	<i>P. oxalicum</i>	16.67 ^{bcd}	Weak pathogen
25.	EF2F	<i>P. oxalicum</i>	0.00 ^d	Non-pathogenic
26.	WF3D	<i>Trichoderma</i> sp. 1	0.00 ^d	Non-pathogenic
27.	SF1E	<i>Trichoderma</i> sp. 1	0.00 ^d	Non-pathogenic
28.	EF3D	<i>Trichoderma</i> sp. 1	0.00 ^d	Non-pathogenic
29.	NF1C	<i>Trichoderma</i> sp. 1	0.00 ^d	Non-pathogenic
30.	WF3B	<i>Trichoderma</i> sp. 1	0.00 ^d	Non-pathogenic
31.	SF1B	<i>T. harzianum</i>	0.00 ^d	Non-pathogenic
32.	WF1D	<i>T. harzianum</i>	0.00 ^d	Non-pathogenic
33.	EF1A	<i>T. harzianum</i>	0.00 ^d	Non-pathogenic
34.	SF4H	<i>Trichoderma</i> sp. 2	0.00 ^d	Non-pathogenic
35.	NF1E	<i>Trichoderma</i> sp. 3	0.00 ^d	Non-pathogenic
36.	EF1E	<i>Colletotrichum</i> sp.	0.00 ^d	Non-pathogenic
37.	EF2C	<i>Mucor</i> sp.	0.00 ^d	Non-pathogenic
38.	WF4C	<i>Mucor</i> sp.	0.00 ^d	Non-pathogenic
39.	SF1C	<i>Mucor</i> sp.	0.00 ^d	Non-pathogenic
40.	SF3B	<i>Mucor</i> sp.	0.00 ^d	Non-pathogenic
41.	WF1C	<i>C. rosea</i>	0.00 ^d	Non-pathogenic
42.	SF2A	<i>C. rosea</i>	0.00 ^d	Non-pathogenic
43.	NF3D	<i>C. rosea</i>	0.00 ^d	Non-pathogenic
44.	Control	Control	0.00 ^d	Control

Means followed by different superscripts within the same column are significantly different ($P < 0.05$)

Results of the growth suppression of foliar pathogens of groundnuts by fungal antagonists are presented in Table 2. The highest radial growth inhibition of *L. lecanii* (WF4G) (42.33%) was produced by *A. pullulans* (WF1B) (42.33%), followed by *A. niger* (WF4A) (34.33%), *Trichoderma* sp. 2 (SF4H) (33.33%), *Mucor* sp. (WF4C) (28.33%), *Trichoderma* sp. 1 (WF3D) (26.67%), and *C. rosea* (WF1C) (26.00%). Similarly, the highest radial growth inhibition of *C. lunata* (NF2H) was produced by *A. pullulans* (WF1B) (26.58%), followed by *Trichoderma* sp. 2 (SFH) (21.92%), *A. niger* (WF4A) (20.13%), *Mucor* sp. (WF4C) (15.83%), *C. rosea* (WF1C) (15.47%), and *Trichoderma* sp. 1 (14.04%).

Radial growth inhibition of *A. pullulans* (SF4D) was highest (35.06%) when challenged with *Trichoderma* sp. 2 (SF4H), followed by *A. niger* (WF4A) (27.44%), then the non-pathogenic *A. pullulans* (WF1B) (26.83%), both *Mucor* sp. (WF4C) and *C. rosea* (WF1C) with 21.95% each, and *Trichoderma* sp. 1 (WF3D) (17.68%). Whereas, *Fusarium* sp. 1 (SF4E) was most inhibited by *Mucor* sp. (WF4C) (38.80%), followed by *Trichoderma* sp. 1 (WF3D) (36.20%), *A. pullulans* (WF1B) (35.87%), *A. niger* (WF4A) (34.29%), *Trichoderma* sp. 2 (SF4H) (32.29%), and *C. rosea* (WF1C) (27.73%). Differences in radial growth inhibition of the pathogens by the various antagonists were significant ($P < 0.05$).

Table 2. Radial Growth Suppression of Foliar Pathogens of Groundnuts by Fungal Antagonists

Antagonists	% Radial Growth Inhibition of Pathogens			
	<i>L. lecanii</i> (WF4G)	<i>C. lunata</i> (NF2H)	<i>A. pullulans</i> (SF4D)	<i>Fusarium</i> sp.1 (SF4E)
<i>A. pullulans</i> (WF1B)	42.33 ^a	26.58 ^a	26.83 ^{ab}	35.87 ^{ab}
<i>A. niger</i> (WF4A)	34.33 ^{ab}	20.13 ^{bc}	27.44 ^{ab}	34.24 ^b
<i>Trichoderma</i> sp. 2 (SF4H)	33.33 ^{ab}	21.92 ^{ab}	35.06 ^a	32.29 ^b
<i>Mucor</i> sp. (WF4C)	28.33 ^b	15.83 ^{bc}	21.95 ^{ab}	38.80 ^a
<i>Trichoderma</i> sp. 1 (WF3D)	26.67 ^b	14.04 ^c	17.68 ^{bc}	36.20 ^{ab}
<i>C. rosea</i> (WF1C)	26.00 ^b	15.47 ^c	21.95 ^{ab}	27.73 ^c
Control	0.00 ^c	0.00 ^d	0.00 ^c	0.00 ^d

Means followed by different superscripts within the same column are significantly different ($P < 0.05$)

DISCUSSION

Results of the pathogenicity tests showed considerable variations in the virulence of fungal isolates associated with groundnut leaf spot disease in Lafia. Among the tested isolates, *A. pullulans*, *L. lecanii*, *C. lunata*, and *Fusarium* sp. produced the highest tissue damage, whereas several isolates of *Trichoderma* spp., *Mucor* sp., *C. rosea*, *P. oxalicum*, *A. terreus*, and some isolates of *A. pullulans*, *A. niger*, *C. lunata*, and *Fusarium* sp. did not induce disease symptoms. This variation in pathogenicity suggests that fungal isolates belonging to the same species may differ in their virulence due to genetic diversity, physiological adaptation, and environmental influences (Agrios, 2005; Leslie and Summerell, 2006). The study presents the first report of leaf spot initiation by *L. lecanii* on groundnuts in Nigeria. This finding is new, and requires further investigation to unravel the factors responsible for the seemingly emerging pathogenic status of *L. lecanii* which has been previously reported as a non-pathogen of groundnuts by several researchers (Subrahmanyam *et al.*, 1990; Mamadou *et al.*, 2022; Nana *et al.*, 2022). The high pathogenicity exhibited by *C. lunata* and *Fusarium* species agrees with previous reports from Nigeria, where these fungi have been identified as important pathogens responsible for foliar diseases of groundnut and other cultivated crops (Kabir *et al.*,

2024). Similarly, in a recent study conducted in Lafia, Joseph *et al.* (2025) reported that isolates of *Curvularia*, *Fusarium*, and *Aureobasidium*, differed significantly in their pathogenic potential, with only a few isolates producing severe disease symptoms. The similarity between the present findings and those reported in Lafia confirms that pathogenic variability exists even among isolates of the same fungal species. The observation that several isolates were non-pathogenic despite being isolated from diseased tissues is also consistent with earlier studies in Nigeria, which have shown that diseased plant tissues often harbour secondary colonizers, endophytes, and saprophytes in addition to the primary causal pathogens (Ezekiel *et al.*, 2020; Joseph *et al.*, 2025). This finding highlights the importance of pathogenicity testing in fulfilling Koch's postulates and distinguishing true pathogens from opportunistic fungi.

The dual culture assay further demonstrated that non-pathogenic fungal isolates differed significantly in their ability to suppress the growth of pathogenic fungi. *A. pullulans*, *Trichoderma* spp., *C. rosea*, and *Mucor* sp. consistently inhibited the radial growth of *L. lecanii*, *C. lunata*, *A. pullulans*, and *Fusarium* sp. These findings are consistent with numerous reports from Nigeria showing that *Trichoderma* species and *C. rosea* possess strong antagonistic activities through

mechanisms such as competition for nutrients, production of antifungal metabolites, mycoparasitism, and secretion of cell wall-degrading enzymes (Terna, 2015). Likewise, recent work in Lafia identified non-pathogenic isolates of *A. pullulans* as effective antagonists against foliar fungal pathogens of groundnut (Joseph *et al.*, 2025), supporting the present results.

CONCLUSION

The study revealed that groundnut tissues showing leaf spot symptoms harbour a diverse community of fungi with varying pathogenic potentials. Among the pathogens, *A. pullulans* produced the highest percentage of leaf spots, followed by *L. lecanii*, *C. lunata* WF2F, *C. lunata* NF2H, and *Fusarium* sp. Dual culture assays demonstrated significant variations in antagonistic activity among the tested antagonistic fungi, with Non-pathogenic *A. pullulans* exhibiting the highest inhibition of *L. lecanii* and *C. lunata*, while *Trichoderma* sp. was most effectively suppressed by *A. pullulans*, whereas, *Mucor* sp. produced the highest inhibition of *Fusarium* sp. The study identified *A. pullulans*, *L. lecanii*, and *C. lunata* as highly virulent foliar pathogens of groundnut, requiring targeted management to minimize yield losses. Furthermore, non-pathogenic strains of *A. pullulans*, *A. Trichoderma* sp., *A. niger*, *Mucor* sp., and *C. rosea* showed promising antagonistic activity against leaf spot pathogens, highlighting their potential as effective biocontrol agents of pathogens causing fungal leaf spots of groundnuts. The study presents the first report of leaf spot initiation by *Lecanicillium lecanii* on groundnuts in Nigeria. Further studies are required to unravel the factors responsible for the seemingly emerging pathogenic status of *L. lecanii* which has been previously reported as a non-pathogen of groundnuts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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