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Identification and Pathogenicity of Fungi Responsible for Foliar Diseases of Groundnut (*Arachis hypogaea* L.) in Lafia

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Abstract: Foliar diseases of groundnuts are important determinants of yield and productivity of the crop. The aim of this study was to identify andevaluate the pathogenicity of fungi responsible for foliar diseases of groundnut in Lafia. Tissues of groundnut leave showing signs of discoloration, and spots, were cultured on potato dextrose agar (PDA) for isolation of in-dwelling fungi. A total of 48 isolates belonging to five genera, namely *Fusarium, Rhizomucor, Curvularia, Epicoccum,* and *Aureobasidium* were recovered. The identified species were *Fusarium incarnatum, Rhizomucor* spp., *Curvularia lunata, Epicoccum nigrum,* and *Aureobasidium pullulans.* Results of pathogenicity test showed that *Rhizomucor* spp. produced the highest leaf spots (60.00%), followed by *Aureobasidium pullulans* (55.00%), *Epicoccum nigrum* (30.00%), *Curvularia lunata* (16.67%), and *Fusarium incarnatum* (12.33%). However, differences in the severity of leaf spots caused by the different fungal pathogens were not significant (P>0.05). The study revealed that fungi isolated from symptomatic leaves of groundnuts were pathogenic, producing varying percentages of leaf spots on inoculated leaves. Therefore, there is a need to control fungal contamination of groundnut leaves in order to improve crop health and enhance yield of groundnuts.

Keywords: Identification, pathogens, fungi, foliar, groundnut, leaf spots

Introduction

Groundnut (*Arachis hypogaea* L.) originated in Latin America and was introduced to the African continent from Brazil by the Portuguese in the 1600's [1]. It is an important food and fodder crop in Nigeria, also known as peanut or earthnut. The genus and species names *Arachis hypogaea* are derived from Greek words *Arachos*, meaning weed, and *hypogaea*, meaning underground chamber [2]. It is an annual herbaceous plant growing up to 30 to 50 cm tall, and cultivated inmore than 100 countries in all the six continents around the world in semi-arid tropical areas in subsistence andcommercial farming systems [3]. In Nigeria, the crop is popularly grown in the northern parts of the country, in states such as Benue, Kano, Borno, and Nasarawa States.

Fungal diseases are among themajor constraints to productivity and availability of healthy groundnut produce, resulting in huge annual yield loses worldwide [4]. The present study investigates the etiology of fungi associated with ground nut leaves showing various disease symptoms such as leaf spots, leaf necroses, and leaf chloroses. Findings of this study shall contribute valuable information that will facilitate efforts aimed at the improvement of crop health and yield of groundnuts in the study area.

Materials and Methods

Sample collection

Groundnut leaves showing symptoms of infections were collected from 12 groundnut fields comprising three farms each from the North, South, East, and West cardinal locations of Lafia. The collected samples were conveyed in sterile polyethylene bags to the Plant Science and Biotechnology Laboratory, Federal University of Lafia, for further processing.

Isolation and identification of fungi associated with diseased groundnut leaves

The methods of Abdulla [5] and White *et al.* [6] were adopted. The infected tissues of groundnut leave were cut to sizes of about 2 cm^2, and surface-sterilized to remove debris by dipping completely in 10% Sodium Hypochlorite (NaOCl) solution for 1 min. Thereafter, the tissues were rinsed three times in sterile distilled water (SDW), and plated on sterile PDA at the rate of five tissues per plate, and three replicates per sampled location. Inoculated plates were incubated at room temperature and monitored daily for the emergence of fungal growth for a duration of seven days. Fungal growths were sub-cultured separately to freshly prepared PDA plates and incubated for a period of three days, to obtain pure cultures.

Identification of isolates

The fungal isolates were morphologically identified based on colony morphology, shapes and configuration of conidia, hyphal septation and branching pattern, conidiophore and conidiogenous cells [7].

Pathogenicity of isolated fungi

Pathogenicity of fungi isolated from diseased leaf tissues was determined using the modified detached leaf assay method reported by Terna *et al.* [8]. Healthy leaves of groundnuts collected at the Botanical Garden of the Federal University of Lafia were surface sterilized as earlier described, and plated separately in five sterile petri dishes kept humid by placing on Whatman No. 1 filter paper moistened with 2mL drops of sterile distilled water. The leaf tissues were wounded slightly with a sterile 3 mm diameter cork borer, thereafter, 7 mm diameter agar discs obtained from actively growing mycelial regions of 7 d old cultures of

potential leaf pathogens were aseptically placed at the wounded spots. The inoculated leaves were incubated for 5 d at 28^0C, and observed for the development of disease symptoms. Disease was estimated using the standard area diagrams of Lage and Capucho [9].

Experimental design and data analysis

Treatments were laid out using the Completely Randomized Design (CRD) with three replicates. Data obtained were 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 and Discussion

Table 1 presents the morphological identification of the fungi isolated from diseased groundnut leaves collected from farms in Lafia. Based on cultural and microscopic morphology, fungi isolated from diseased leaves of groundnuts were identified under five genera, namely *Fusarium*, *Rhizomucor*, *Curvularia*, *Epicoccum*, and *Aureobasidium.* Species identified were *F. incarnatum*, *Rhizomucor* spp., *Curvularialunata*, *Epicoccum nigrum*, *Aureobasidium pullulans.* A total of 48 isolates were recovered, of which *Rhizomucor* spp. were the highest number of isolates, 16(33.33%), followed by *C. lunata* 14(29.17%), *A. pullulans* 12(25.00%), *F. incarnatum* 4(8.33%), and lastly *E. nigrum* 2(4.17%).

Table 1: Morphological characteristics and identities of fungi isolated from diseased groundnut leaves

Isolate Group	Cultural characteristics	Conidia	Conidiogenous cells	Hyphae	Morphologically assigned identity
G1	Colonies were grayish-brown, with a raised elevation, filiform margins, and orange-brown pigmentation on reverse	Abundant 3-septate canoe-shaped macroconidia, with a few microconidia in false conidial heads	Abundant mono-and polyphialides found, often slender and elongated	Hyphae were sepatate. Coiled hyphae also found	*Fusarium incarnatum* (4 isolates)
G2	Colonies were blackish-brown, raised, with fuzzy growth, filiform margins, and brown pigmentation on reverse	Conidia were near transparent, oval shaped and smooth-walled	Conidiophore were slender, almost colourless with numerous sporangia	Hyphae were highly septate and sparingly branched	*Rhizomucor*spp. (16 isolates)
G3	Colonies were grayish-brown, slightly raised, irregular, with a pale to brown pigmentation on reverse	Conidia were smooth-walled, pigmented and septate	Conidophores were highly septate and lacked proper differentiation. Conidia were borne either terminally or intercalarily along the length of the conidiophore	Hyphae were highly septate and sparingly branched	*Curvularialunata* (14 isolates)
G4	Colonies were yellowish-brown, flat, with filiform margins, and a bright yellow tobrown pigmentation on reverse	Small irregularly shaped endoconidia were found within the hyphae	Chlamydospores were found along the length of the hyphae	Hyphae were highly pigmented, septate, and branched	*Epicoccum nigrum* (2 isolates)
G5	Cultures appeared gray mixed with brown, raised, filiform, with a pale pigmentation on the reverse	Conidia were transparent and oval. Abundant endoconidia were found within haphae	Conidiophores lacked proper differentiated	Hyphae were transversely septate, showing very little branching	*Aureobasidium pullulans* (12 isolates)

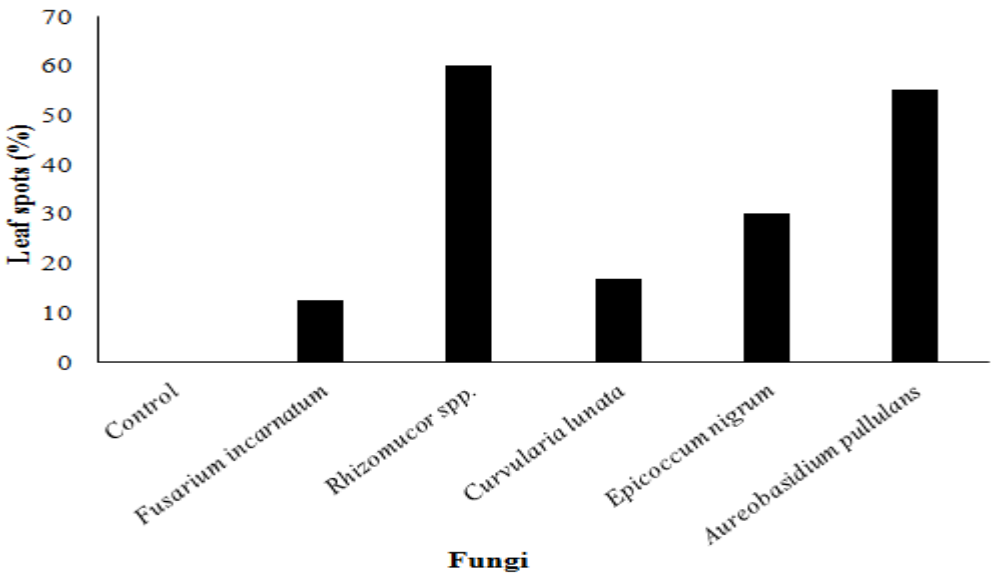

Figure 1: Pathogenicity of fungi isolated from diseased groundnut leaves

Figure 1 presents the results of the pathogenicity of different fungi isolated from diseased groundnut leaves. *Rhizomucor* spp. produced the highest leaf spots (60.00%), followed by *Aureobasidium pullulans* (55.00%), *Epicoccum nigrum* (30.00%), *Curvularialunata* (16.67%), and *Fusarium incarnatum* (12.33%). Leaf spots were not observed (0.00%) in uninoculated leaves (control). Differences in the amount of leaf spots caused by the different fungal pathogens on leaves of groundnuts were not significant ($P \geq 0.05$).

This study identified *Fusariumincarnatum*, *Rhizomucor* spp, *Curvularialunata*, *Epicoccumnigrum* and *Aureobasidiumpullulans* to be the predominant fungi associated with groundnut leaf diseasesin Lafia. Similarly, Muthukumar [10] identified some fungal pathogens of groundnut including *Fusarium*, *Choanephora*, *Colletotrichum*, *Curvularia*, and *Rhizomucor*. In the same vein, Pal *et al.* [11] stated that groundnuts are susceptible to fungal colonization because of their intimate contact with soil. Furthermore, Subrahmanyam *et al.* [12] reported that fungal pathogens attack any above-ground portions of the plant, but leaf spots are the most conspicuous symptoms, and depending upon weather conditions and cropping history, leaf symptoms usually appear between 30 to 50 days after planting.

The results of pathogenicity test indicated that *Rhizomucor* spp. produced the highest leaf spots, followed by *Aureobasidium pullulans*, *Epicoccum nigrum*, *Curvularialunata*, and *Fusarium incarnatum*. Similarly, Lindsey *et al.* [13] revealed that *Rhizomucor* spp. is ubiquitous with a wide host range, and found in all major peanut- growing areas of the world. In the same vein, Lima *et al.* [14] reported that *E. nigrum*often appears as second colonisers of leaves in places with temperature range of 23-28^{0}C. Buddenhagen*et al.* [2] also reported that *Epicoccum* spp. are opportunistic pathogens causing black spotson plant leaves.

Conclusion

The study revealed that fungi isolated from symptomatic leaves of groundnuts were pathogenic, producing varying percentages of leaf spots on inoculated leaves. Results of pathogenicity test showed that *Rhizomucor* spp. produced the highest leaf spots, followed by *Aureobasidium pullulans*, *Epicoccum nigrum*, *Curvularialunata*, and *Fusarium incarnatum*, however, differences in the severity of leaf spots caused by the different fungal pathogens were not significant ($P>0.05$). There is a need to control fungal contamination of groundnut leaves in order to improve crop health and enhance yield of groundnuts.

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