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Identification and Pathogenicity of Fungi Associated with Anthracnose Disease of Pepper (*Capsicum annuum* L.) in Lafia, Nasarawa State

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Abstract: Anthracnose disease is a critical challenge in pepper cultivation, particularly in tropical and subtropical regions like Nigeria, leading to significant yield losses and reduced fruit quality annually. A study was conducted to identify and control pathogens associated with anthracnose disease of pepper fruits in Lafia. Fungi associated with infected fruits were isolated and identified using morphological characteristics. Identified fungi were subjected to pathogenicity tests to evaluate their disease producing potentials in healthy pepper fruits. Results of morphological identification of the fungi isolated from anthracnosed pepper fruits in Lafia showed that the fungi were identified in two genera, namely *Aspergillus* and *Fusarium.* Results of pathogenicity test showed that fruit rot diameters were higher in fruits inoculated with *A. niger* (5.12 cm), compared to fruits inoculated with *Fusarium* sp. (1.90 cm diameter) after six days. The differences in rot diameters among the pepper fruits inoculated with the two fungi were significant ($P \leq 0.05$). Control measures targeted at the prevention of fruit contamination by *A. niger* will go a long way to minimize yield losses of tomato in the study area.

Keywords: Anthracnose, fungi, pepper, morphological identification, Lafia

Introduction

Anthracnose disease is a critical challenge in pepper (*Capsicum annuum* L.) cultivation, particularly in tropical and subtropical regions like Nigeria [1]. The disease, primarily caused by various species of the fungus *Colletotrichum*, is notorious for leading to significant yield losses and reduced fruit quality [2]. In several cases, anthracnose symptoms are made worse by opportunistic infections by other associated fungi. The warm and humid conditions in parts of Nigeria such as Lafia create an ideal environment for the proliferation of *Colletotrichum* species, making anthracnose a persistent challenge for pepper farmers in the region [3-5].

Accurate identification of these fungal species is essential for effective disease management [2, 6]. The present study was aimed at identifying and evaluating the pathogenicity of fungi associated with pepper fruits showing anthracnose-like symptoms in Lafia. The information obtained from the findings of this will be useful in enhancing the development of control measures aimed at reducing the predisposition of the crops to the disease, as well as reducing the inoculum potential in the area, as a means of improving the health and yield of the pepper crop.

Materials and Methods

Survey of incidence and severity of pepper anthracnose

Survey of incidence and severity of anthracnose disease was carried out in 4 fields each within the four cardinal locations of Lafia, namely Lafia North, South, East, and West, using the method of Terna *et al.* [7]. Following the disease survey, two fruits each of anthracnose-infected pepper plants were excised and conveyed in sterile polyethylene bags to the Plant Science and Biotechnology Laboratory, Federal University of Lafia, for further processing.

Isolation of fungi from infected pepper fruits

The method of Yadav *et al.* was used as follows [8]: Fruit tissues measuring about 2 cm^2 were cut at the intersection between infected and healthy tissues, washed in sterile distilled water to remove dirt, and surface sterilized by soaking in 70% ethanol 2 min, and rinsed with three changes of sterile distilled water. Surface sterilized tissues were blotted dry on sterile filter paper, plated on Potato Dextrose Agar (PDA) for 3 to 7 days and monitored for emergence of fungal mycelia. Isolated fungi were sub-cultured separately unto sterilized PDA for the development of pure cultures. Pure cultures were preserved on sterilized PDA slants until required.

Identification of fungal isolates

Isolated fungi were morphologically identified through examination of their macro morphological characteristics such as colony texture, appearance and pigmentation, and micro morphological features such as shape of conidia, hyphal structure, and conidiogenous cells. The isolates were identified by comparing the observed morphological features with relevant identification guides [9].

Pathogenicity test of fungal isolates

The fruits bioassay method of Terna *et al.* was used for pathogenicity tests [7]. Healthy pepper fruits were surface sterilized by swabbing with 70 % ethanol, and wounded slightly with a sterilized needle. Wounded portions were inoculated by placing mycelia plugs (7 mm diameter) of the tested fungi taken from the actively growing hyphae. Inoculated pepper fruits were placed in sterile plastic containers and monitored for 72 hours for the development of anthracnose symptoms. Two sterile cotton balls moistened with sterile water were kept in the plastic container to keep the container humid during the pathogenicity test duration. Disease incidence was assessed visually by estimating the percentage of diseased tissue in relation to the entire fruit area as reported by Sharma [10].

Data analysis

Data obtained from the study was subjected to Analysis of Variance (ANOVA) at 5% level of probability using Minitab Statistical Software, Version 19. Means were separated using the Tukey's Honestly Significant Test.

Table 1: Incidence of pepper anthracnose on tomato farms in Lafia

Location	Incidence (%)
North	27.83^{b}
South	41.33^{ab}
East	42.67^{a}
West	44.67^{a}

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

Results and Discussion

Results of the incidence of pepper anthracnose in Lafia are presented in Table 1. The highest incidence of pepper anthracnose was observed in Lafia West (44.67%), followed by Lafia East (42.67%), Lafia South (41.33%), and Lafia North (27.83%). The differences in incidence of pepper anthracnose were significant among the surveyed locations (P≤0.05).

Results of morphological identification of the fungi isolated from anthracnosed pepper fruits in Lafia are presented in Table 2 and Plates 1 – 2. The fungi were identified in two genera, namely *Aspergillus* and *Fusarium.* Based on morphological characteristics such as colony appearance, texture, pigmentation, hyphal structure, conidia shape and pigmentation, and orientation of conidiophores, the fungi in the two genera were identified as *A. niger* and *Fusarium* sp.

Table 2: Morphological characteristics of fungi recovered from pepper fruits showing symptoms of anthracnose in Lafia

Group	Macromorphology			Micromorphology			Morphological Identity
	Colony Colour	Texture, Elevation & Margins	Pigmentation	Hyphae	Conidiophore	Conidia	
1	Brownish-black	Powdery, slightly raised, with irregular and slightly undulating filiform margins	Yellowish-brown	Highly septate, but sparsely branched	Cylindrical Phialides in uniseriate and biseriate conidial heads	Pigmented, smooth-walled, sub-globose to globose	*A. niger*
2	Brown mixed with white	Wooly, raised, with circular filiform margins	Blackish-brown	Septate and scantily branched	Few monophialides found	Boat-shaped mesoconidia found	*Fusarium* sp.

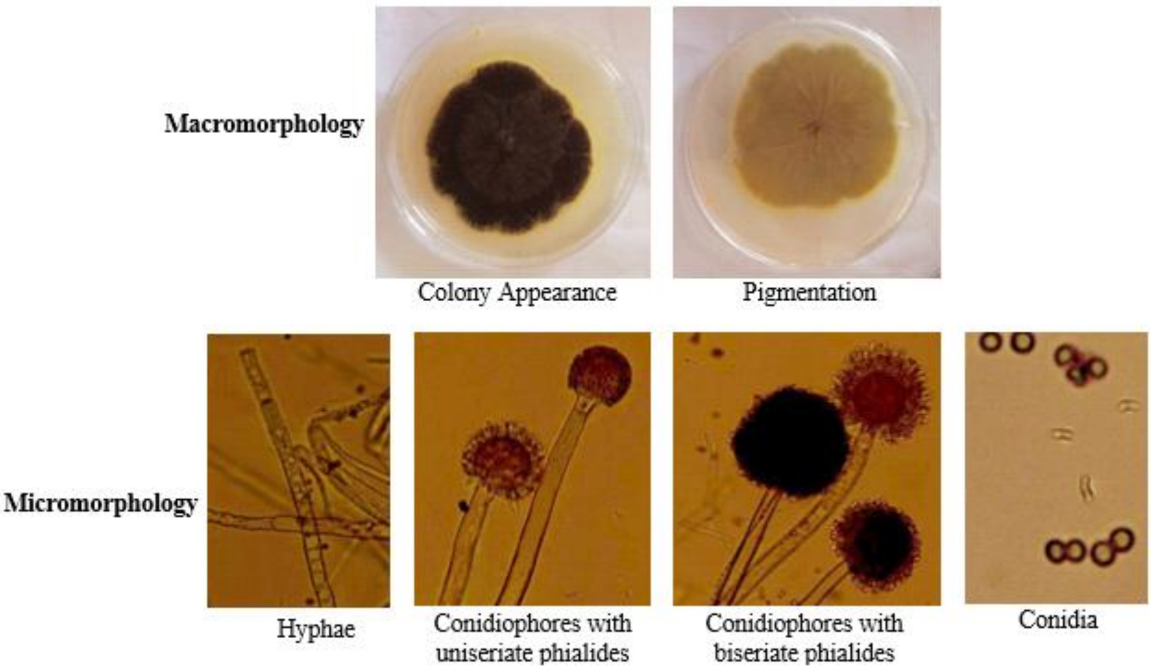

Plate 1: Macroscopic and microscopic features of *A. niger* isolated from pepper fruits showing symptoms of anthracnose disease

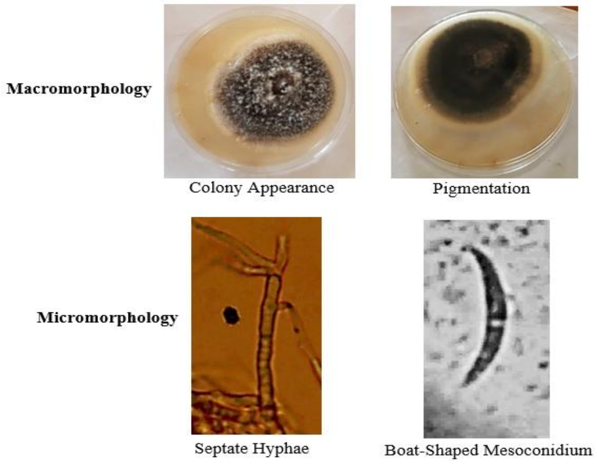

Plate 2: Macroscopic and microscopic features of *Fusarium* sp. isolated from pepper fruits showing symptoms of anthracnose disease

The distribution of fungi associated with anthracnose disease of pepper in Lafia is presented in Table 3. A total of 14 fungal isolates were obtained from the infected pepper fruits during the study. Of the recovered fungi, only one isolate of *A. niger*(7.14%) was found in infected pepper fruits collected from Lafia East, while 13 (92.86%) isolates of *Fusarium* sp. were obtained from infected pepper fruits, comprising four each from Lafia North and South (28.57% each), three from Lafia West (21.43%), and two from Lafia East (14.28%).

Table 3: Distribution of fungi associated with anthracnose disease of pepper in Lafia

Location	No. of Isolates	
	A. niger	***Fusarium* sp.**
Lafia North	-	4 (28.57%)
Lafia South	-	4 (28.57%)
Lafia East	1 (7.14%)	2 (14.28%)
Lafia West	-	3 (21.43%)
Total	**01 (7.14%)**	**13 (92.86%)**

= Fungi absent

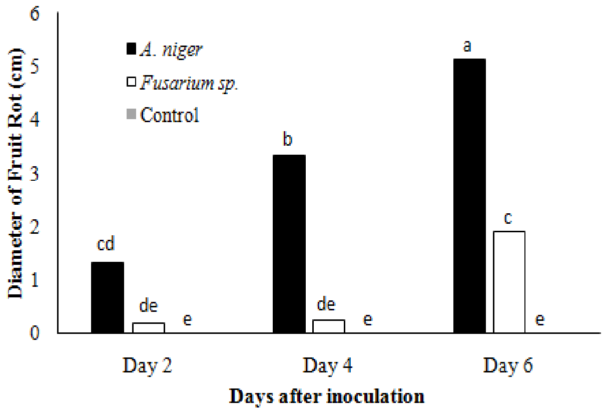

Figure 1: Pathogenicity of fungi isolated from anthracnosed pepper fruits

Plate 3: Disease responses of pepper fruits inoculated with *A. niger*(A) and the uninoculated control (B) after six days of post-inoculation

Figure 1 shows the results of the pathogenicity of *A. niger* and *Fusarium* sp. isolated from anthracnosed pepper fruits. Fruit rot commenced after two days in fruits inoculated with the two fungal species, however, fruit rot diameters were higher in fruits inoculated with *A. niger* after two days (1.33 cm), four days (3.33 cm), and six days (5.12 cm), compared to fruits inoculated with *Fusarium* sp. which showed 0.20 cm rot diameter after two days, 0.25 cm diameter after four days, and 1.90 cm diameter after six days. The differences in rot diameters among the pepper fruits inoculated with the two fungi were significant ($P \leq 0.05$). Rot was not observed in the uninoculated pepper fruits (Plate 3).

The results of this study showed that the highest incidence of pepper anthracnose was observed in Lafia west, followed by Lafia east, Lafia south and Lafia north respectively. Anthracnose is widespread and considered an important disease in most countries. The disease initiation, growth and development of the pathogen are influenced greatly by the nutritional and environmental factors of the host plant. Among the nutritional factors are sources of nitrogen and carbon whereas the environmental factors for disease development include wet, humid, hot weather as well as pH and Temperature, and the pathogen is also considered generally inactive in dry weather. This explains the reduced incidence of anthracnose in the dryer northern Lafia than the wetter regions of the West, East and South. Similarly, Bozic and Kanduc reported that wet conditions or high relative humidity are necessary for the disease to flourish [11].

Based morphological characteristics, the fungi isolated from anthracnosed pepper fruits in Lafia were identified in two genera, namely *Aspergillus* and *Fusarium*. The prominent morphological characteristic for the identification of *Aspergillus* was the presence of radial conidial heads bearing cylindrical phialides in both uniseriate and biseriate orientations. A study by Hong *et al.* [12] highlighted the presence of conidiophores that bear conidia in distinctive flask-shaped structures called phialides, as the key features for identification of *Aspergillus* species. On the other hand, the formation of a boat-shaped or sickle-shaped conidia considered as the major delineating characteristic for the *Fusarium* genus in the present study was also reported by Leslie and Summerell [13]. The authors emphasized that *Fusarium* fungi produce macroconidia that are typically sickle-shaped, multicellular, and contain distinct septa. Welideniva *et al.* have also reported the occurrence of members belonging to the *Aspergillus* and *Fusarium* genera on pepper fruits showing anthracnose-like symptoms [14].

Results of the study also showed that while only one isolate of *A. niger* was found in infected pepper fruits, 13 isolates of *Fusarium* sp. were obtained from the pepper fruits showing symptoms of anthracnose. Reports indicate that *Fusarium* spp. are widespread in tropical and subtropical regions where pepper cultivation is prominent. A study by Dewing *et al.* [15] also reported that certain *Fusarium* species exhibit host specificity, which influences their distribution. For instance, *F. oxysporum* F. sp. capsici is particularly associated with pepper plants and has been isolated frequently from affected crops in various regions, including Latin America and Asia. *Fusarium* species, particularly *F. solani* and *F. oxysporum*, are also significant in anthracnose development in peppers.

Fruit rot caused by *A. niger* in artificially inoculated pepper fruits was higher and significantly different from pepper fruit rot caused by *Fusarium* sp. ($P \leq 0.05$). *Aspergillus* species, particularly *Aspergillus niger* and *Aspergillus flavus*, have been linked to post-harvest spoilage and may contribute to anthracnose symptoms in peppers. Their pathogenicity can be attributed to several factors. For instance, *Aspergillus niger* secretes various enzymes (e.g., cellulases, pectinases) that degrade plant cell walls, facilitating invasion [16]. This enzymatic degradation is crucial for the colonization of host tissues and production of disease symptoms in infected plants. *Fusarium* species, such as *Fusarium oxysporum* and *Fusarium solani*, are also prominent in pepper diseases. Their pathogenic mechanisms include colonization of the xylem, leading to water stress and nutrient deficiencies [17]. The symptoms of anthracnose in peppers, including leaf spots, stem lesions, and fruit rot, can be exacerbated by the synergistic effects of multiple fungal pathogens [18]. The *A. niger* and *F. oxysporum* isolates recovered from anthracnosed tissues of pepper in this study could also play important roles in advancing the anthracnose disease complex.

Conclusion

The results of this study revealed that although *A. niger* and *Fusarium* sp. were the fungal species associated with anthracnose disease-like symptoms on pepper fruits in Lafia, *A. niger* was more pathogenic, producing the highest and most significant severity of fruit rot in infected fruits. Efforts targeted at the prevention of fruit contamination by *A. niger* will go a long way to minimize yield losses of tomato in the study area.

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