

First Report of *Aspergillus Niger* Causing Collar Rot of Groundnut (*Arachis Hypogaea* L.) in Ghana

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Abstract

Groundnut (*Arachis hypogaea* L.) is a vital legume and oilseed crop of major economic importance. In Ghana, an emerging rot disease poses an increasing threat to groundnut production. This study investigated the causal pathogen of the rot symptoms observed at CSIR–Crops Research Institute, Fumesua, during the 2023 minor cropping season. Seventy-five symptomatic plants were collected from three research farms, and tissues from seed, root, and collar regions were cultured on Chloramphenicol-amended Potato Dextrose Agar (PDA). Fungal colonies initially appeared white to yellowish later turned black with yellowish margins. Microscopic examination revealed morphological features consistent with *Aspergillus niger*, including septate hyaline mycelia, elongated conidiophores terminating in vesicles, and brown-to-black globose conidia. Pathogenicity tests conducted using eleven isolates successfully reproduced collar rot symptoms, confirming *A. niger* as the causal agent, while control plants remained symptomless. The fungus re-isolated from inoculated plants exhibited morphological characteristics identical to the original isolates. The findings confirm the widespread occurrence and variable pathogenicity of *A. niger* in groundnut fields and highlight the need for integrated disease management strategies and the development of resistant varieties.

Keywords: Groundnut; Collar rot disease; *Aspergillus niger*.

1.0 Introduction

Groundnut, regarded as the “king of oilseed crops,” is an economically important food legume and edible oilseed crop cultivated in over one hundred countries worldwide (Desmae *et al.*, 2019). It contributes approximately 12% of the recommended daily nutrient intake and is enriched with seven of the thirteen essential vitamins and seven of the twenty minerals required for growth and development (Kumaret *al.*, 2020). Although Ghana is a relatively minor player in global groundnut production, it accounts for less than 2% of total worldwide output (Owusu-Adjei *et al.*, 2017).

Groundnut production is constrained by numerous diseases (Kumar and Thirumalaisamy, 2016). In Ghana, an emerging rot disease has increasingly contributed to yield losses, affecting the crop at all growth stages. Since 2020, Research and Extension Linkage Committees (RELCs) in the Ashanti, Bono, Bono-East, Ahafo, and Northern Regions have reported the occurrence of this emerging groundnut rot disease. Therefore, this study was conducted to determine the causal pathogen of the emerging rot disease by collecting samples from suspected research fields at CSIR–Crops Research Institute (CRI), Fumesua, isolating the pathogen, and identifying it based on its morphological characteristics.

2.0 Materials and Methods

2.1 Sampling method

Seventy-five groundnut plants exhibiting rot disease symptoms were collected from three research farms located 2–3 km apart at the Council for Scientific and Industrial Research (CSIR), Crops Research Institute (CRI), Fumesua. The study was conducted during the 2023 minor cropping season (November), as disease symptoms were

observed across the research fields during this period.

A purposive sampling method was employed to select symptomatic plants due to the patchy distribution of the emerging rot symptoms in the fields. From each of the three research farms, 25 symptomatic plants were sampled, resulting in a total of 75 plants. Seed, root, and collar tissues were collected separately for laboratory analysis.

For each farm, relevant information including farm location and groundnut variety grown was recorded. All sampled fields were cultivated under sole groundnut cropping systems.

2.2 Isolation and identification of *Aspergillus niger* from samples collected from Fumesua research fields

Chloramphenicol-sulphate-amended Potato Dextrose Agar (CPDA) was used for fungal isolation. Potato Dextrose Agar (PDA) was prepared from commercial dehydrated powder (Oxoid, United Kingdom) according to the manufacturer’s instructions. For every 1 L of medium prepared, 500 mg of chloramphenicol sulphate was added to suppress bacterial growth. The antibiotic was incorporated prior to autoclaving at 121°C and 1.2 kg/cm² pressure for 15 minutes. After sterilization, the medium was cooled to approximately 45°C before pouring.

20 ml of CPDA were dispensed into sterile 85 mm diameter Petri plates under laminar airflow conditions and allowed to solidify for 24 hours.

Seed, root, and collar tissues were surface sterilized in 10% sodium hypochlorite for 30 seconds, rinsed in sterile distilled water, and blotted dry on sterile paper towels. Four tissue pieces were plated per Petri dish. Tissues were plated separately according to genotype and farm location. Plates were sealed, labelled appropriately,

and incubated at room temperature (22–30°C) for seven days.

After incubation, fungal colonies were examined for morphological characteristics. Microscopic identification was performed using a compound microscope based on conidial shape, size, and other structural features, and comparisons were made with descriptions in Muñoz et al. (2000). The number of *Aspergillus niger* colonies on each plate was recorded separately for each tissue type and expressed as a percentage of the four tissue pieces plated per plate.

2.3 Pathogenicity test to confirm *Aspergillus niger* as the causal organism

Pathogenicity testing was conducted in the plant house of the Plant Pathology Laboratory, Kwame Nkrumah University of Science and Technology (KNUST).

A spore suspension of *Aspergillus niger* adjusted to 5.0×10^6 spores/ml was used for inoculation. Sterilized plastic pots (20 cm diameter $\times$ 40 cm depth) containing sterilized soil were used for the experiment. Seeds of the susceptible groundnut variety ‘Obolo’ were sown in the pots. Control pots received no fungal inoculum.

The experiment was arranged in a completely randomized design (CRD) with four replications. Soil moisture was maintained at field capacity through regular watering.

Plants were observed daily for 28 days after sowing for symptom development, including yellowing and wilting of lower branches, brown lesions on the hypocotyl and stem, soft and rotted collar regions,

and complete plant death. The fungus was re-isolated from symptomatic plants and compared morphologically with the original isolates to confirm Koch’s postulates.

Results were presented using photographs.

3.0 Results

3.1 Morphological characterization of fungal pathogen causing collar rot disease of groundnut

The fungal pathogen isolated from groundnut infected samples and pathogenicity test was morphologically identified as *Aspergillus niger* based on the colony color, spore shape among others. Seven days after inoculation, colony of the fungal pathogen appeared black with yellowish margins on the amended PDA medium (Plate 3.1).

Microscopic examination of pure cultures of *A. niger* isolates showed septate, hyaline mycelia, with conidiophores that were long and had spherical vesicles at the tips. The conidia were globose and ranged in color from brown to black (Plate 3.1).

Twenty-eight days after sowing, the experiment revealed significant differences in disease severity (Plate 3.2). Most plants showed symptoms of collar rot disease, such as wilting of leaves and branches, soft and rotten collar region (Plate 3.3), while the control plants remained healthy (Plate 3.3). The plants were uprooted, and the pathogen was re-isolated. After re-isolation, it was compared with the original isolates, and using cultural appearance it was confirmed that *A. niger* is responsible for causing groundnut collar rot disease in Ghana.

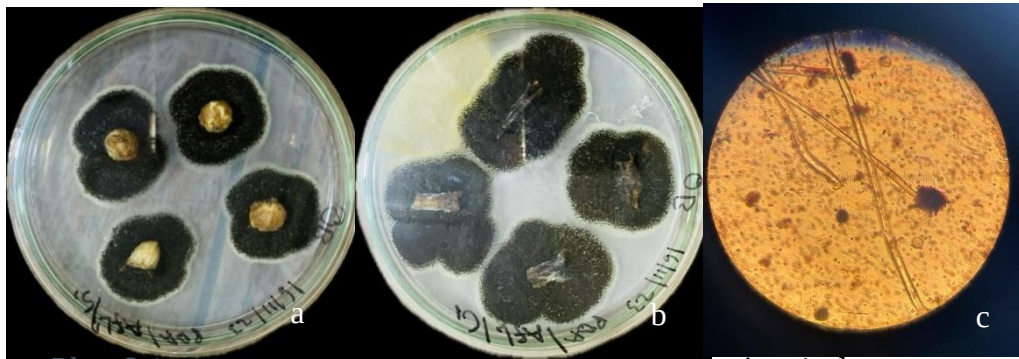


Plate 3.1 Cultural and microscopic appearance of *A. niger* isolates
a = isolates from diseased groundnut seeds. b = isolates from diseased groundnut collar region. c = Microscopic view of *A. niger*

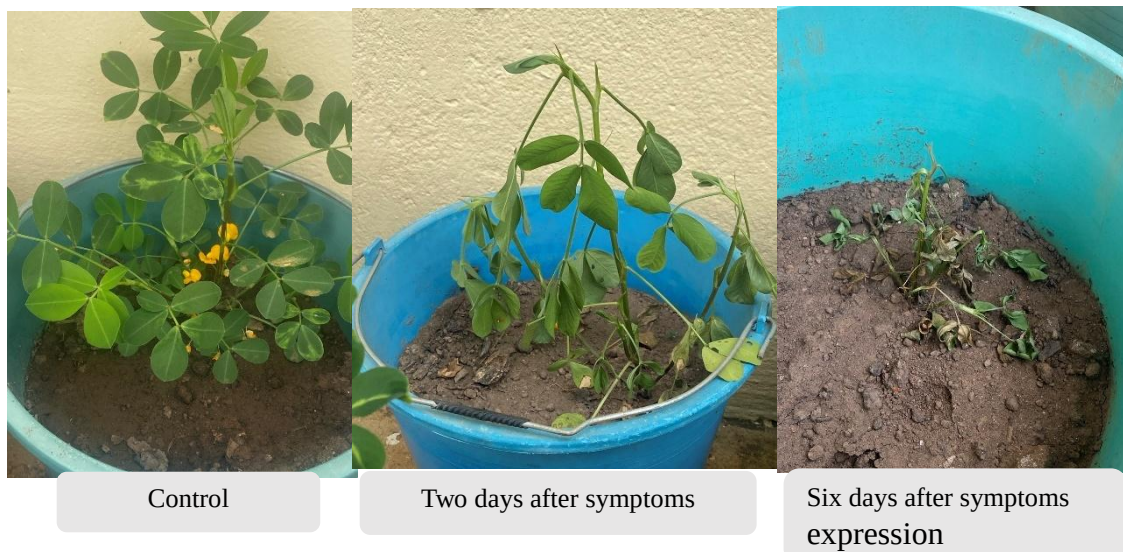


Plate 3.2: Response of groundnut plants to *Aspergillus niger* under plant house condition



Plate 3.3:



Plate 3.4:

A = Diseased groundnut plant from pathogenicity test

B = Healthy groundnut plant from pathogenicity test

4.0 Discussion

The identification was based on the morphological characteristics observed during a seven-day incubation period. Initially, 24 hours after incubation, the mycelium of the isolates appeared white to yellowish on the medium. This early stage was followed by a change in the mycelium colour, which turned black with yellowish edges as the incubation progressed. The exhibition of initial white to yellowish mycelial growth that turned black with age is a characteristic consistent with findings by Klich (2009) who emphasized the importance of colony morphology, including the colour of the reverse side, in the identification process. Additionally, Samson *et al.* (2006) noted that the reverse colony colour of *A. niger* can range from pale yellow to almost uncoloured, depending on the medium and incubation conditions. This was similarly documented by Klich (2009),

The colonies of *A. niger* on the PDA Petri plates were circular with irregular margins, a distinguishing characteristic that aligns with descriptions by Zohriet *al.* (2020), who noted similar features when *A. niger* was isolated from various substrates and added that this characteristic helps differentiate *A. niger* from other *Aspergillus* species, which may exhibit more regular colony margins. The transition from a white or yellowish mycelium to a black colour is consistent with the sporulation of *A. niger*, where the conidial heads, initially white, become black as the conidia mature. This observation is crucial for the identification of *A. niger*, as the black conidia are one of the hallmark features of this species. The growth patterns observed over the seven days post-incubation, with colonies expanding and turning black, are in agreement with the general growth behaviour of *A. niger* reported by Pitt (2009).

The microscopic examination of *A. niger* isolates from the current study revealed distinct features such as septate, hyaline mycelia, long conidiophores with spherical vesicles at the tips, and globose conidia ranging in colour from brown to black.

According to Samson *et al.* (2006), the hyaline nature of the mycelia and the septate structure are fundamental traits used to distinguish *Aspergillus* species, including *A. niger*. The brown-to-black colouration of the conidia observed in this study aligns well with other findings. For example, a study by Kochkina *et al.* (2012) indicated that the pigmentation of conidia, ranging from light brown to dark black, is linked to the maturation of the fungal spores and indicates spore viability. The globose shape of the conidia, as noted in this research, was similarly documented by Visagie *et al.* (2014), where the spherical nature of the conidia was a distinguishing morphological characteristic of *A. niger*.

Conclusion

The identification of *Aspergillus niger* isolates in this study was effectively achieved through a combination of morphological and microscopic characteristics. Over a seven-day incubation period, the isolates exhibited distinct colony morphology, beginning with white to yellowish mycelial growth that transitioned to black with yellowish edges, an indication of conidial maturation. Colony features such as irregular margins and colour transformation were consistent with literature reinforcing the reliability of these morphological traits in distinguishing *A. niger* from other *Aspergillus* species. Microscopic examinations further validated the identification, revealing septate, hyaline mycelia; long conidiophores with terminal spherical vesicles; and brown-to-black globose conidia.

The use of a recognized laboratory manual (Muñoz *et al.*, 2000) for confirmation added credibility to the identification process. Overall, the findings of this study reaffirm the diagnostic importance of both colony and microscopic features in the accurate identification of *A. niger*, which is critical for further studies on its control strategies in groundnut production.

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