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ISOLATION AND CHARACTERIZATION OF CELLULOSE-DEGRADING FUNGI FROM *Arachis hypogaea* Shells FOR POTENTIAL BIOMASS VALORIZATION

***Abdulmumini, S. A, Yusuf-Salihu, B. O. and Awe, S.**

Department of Microbiology, Kwara State University, Malete, Kwara State, Nigeria.

*Corresponding authors' email: shakirat.abdulmumini@kwasu.edu.ng Phone: +2347032916898
ORCID iD: https://orcid.org/0009-0003-7137-6902

ABSTRACT

The exploitation of agro-industrial waste provides a sustainable method for enzyme production and biomass use. This work examined the isolation and characterization of cellulolytic fungi from decomposed *Arachis hypogaea* (groundnut) shells, a lignocellulosic substrate abundant in cellulose. Four fungal isolates were procured, of which two (OPGS and OPBS) had notable cellulolytic activity, as indicated by distinct hydrolysis zones on carboxymethyl cellulose (CMC) agar stained with Congo red. The isolates were identified as *Aspergillus flavus* and *Aspergillus niger* through morphological and microscopic investigation. Quantitative screening in submerged fermentation demonstrated maximal cellulase activities of 86.7 ± 1.7 U/mL for *A. flavus* and 92.3 ± 1.1 U/mL for *A. niger*. Enzyme activity associated with a gradual decrease in pH, signifying active substrate metabolism. These findings emphasize the viability of groundnut shell as an economical substrate for cellulase production and demonstrate the appropriateness of *Aspergillus* strains in biomass bioconversion processes.

Keywords: Cellulolytic fungi, *Aspergillus flavus*, *Aspergillus niger*, Groundnut shell, Congo red staining

INTRODUCTION

The aggregation of agricultural leftovers poses environmental challenges and prospects for sustainable bioconversion. Groundnut (*Arachis hypogaea*) shells, a prevalent agro-industrial by-product, are abundant in lignocellulosic polymers, specifically cellulose, hemicellulose, and lignin, rendering them a viable substrate for microbial degradation (Arnthong et al., 2020; Dhiman et al., 2024). The inherent recalcitrance of these polymers requires pretreatment or the application of resilient microbial systems to improve the accessibility of cellulose and other fermentable constituents (Xu et al., 2015; Cheng et al., 2022).

Cellulose, the primary component of groundnut shells, is the most prevalent biopolymer on Earth and functions as a renewable feedstock for biofuels and high-value bioproducts (Dhiman et al., 2024). The microbial conversion is chiefly facilitated by cellulolytic fungi that release extracellular enzymes, which can infiltrate and hydrolyze the lignocellulose matrix, frequently hindered by interactions with hemicellulose and lignin (Arnthong et al., 2020; Fasiku et al., 2023). Recent studies highlight the importance of isolating fungi from agro-waste environments to identify novel strains exhibiting superior cellulase activity, particularly for resilient substrates such as groundnut shells (Fasiku et al., 2023; Dhiman et al., 2024).

Initiatives to extract cellulolytic fungi from lignocellulosic waste have intensified due to its dual function in biomass degradation and environmental waste management techniques, such as composting and fermentation (Cheng et al., 2022). These methodologies enhance comprehension of fungal enzymatic systems and facilitate the development of efficient microbial consortia and integrated bioprocesses (Xu et al., 2015).

This study aims to isolate and screen cellulolytic fungi from decayed *Arachis hypogaea* shells for potential application in the bioconversion of agricultural waste into renewable products.

MATERIALS AND METHODS

Isolation and culturing of fungi

Arachis hypogaea shells were collected from a peanut processing facility at Ago market in Ilorin, Kwara State, and stored in clean polyethylene bags. To induce spoilage, the samples were maintained at room temperature in transparent polythene bags for one week. Small portions of the deteriorated shells were then inoculated directly onto Potato Dextrose Agar (PDA) plates supplemented with streptomycin (50 µg/mL) to inhibit bacterial contamination. The plates were incubated at 28 ± 2°C for three days. Distinct fungal colonies were repeatedly sub-cultured to obtain pure isolates, which were subsequently maintained on PDA slants at 4°C for further characterization.

Screening for cellulolytic activity

Cellulolytic activity of fungal isolates was tested using carboxymethyl cellulose (CMC) agar medium consisted of (g/L): $NaNO_3$ (2.0), KH_2PO_4 (1.0), $MgSO_4 \cdot 7H_2O$ (0.5), KCl (0.5), yeast extract (0.5), CMC (10.0), and agar (20.0) (Helal et al., 2022). A fungal plug from each isolate was inoculated at the center of a pre-poured CMC agar plate and incubated at 28 ± 2°C for 3 days. Following incubation, the plates were flooded with 1% Congo red solution and allowed to stand for 10 minutes. Following incubation, the plates were filled with 1% congo red solution and let to stand for 10 mins. Hydrolyzed zones emerged as distinct clear halos against a reddish background, indicating cellulose degradation. The diameter of each clear zone and accompanying fungal colony was measured. Hydrolyzed zones emerged as distinct halos over a dark background, indicating cellulose degradation. The diameter of each clean zone and accompanying fungal colony was measured.

Morphological identification of fungal isolate
Isolates demonstrating substantial cellulolytic activity were evaluated macroscopically (colony color, pigmentation, texture) and microscopically using lactophenol cotton blue staining. Microscopic inspection was employed to identify hyphal structures, conidiophores, vesicle kinds, and conidial configurations.

Secondary screening of fungal isolates
The fungal isolates were subjected to quantitative screening in submerged fermentation using the previously described basal medium supplemented with 10 g of powdered groundnut shells as the primary carbon source (Biaragi, 2016). The pH of the medium was adjusted to 6.5 prior to sterilization. Precisely, 100 ml of the medium was poured into 250 mL Erlenmeyer flasks, sterilized at 121°C for 15 minutes, and inoculated with a 6.0 mm mycelial disc from a 72-hour-old culture of each isolate. The flasks were incubated at 30°C on a rotary shaker at 120 rpm for 5 days. Samples were aseptically extracted at 24-hour intervals for pH monitoring and enzyme extraction. The culture broth was filtered using Whatman No. 1 filter paper, and the resultant filtrate was centrifuged at 3000 rpm for 10 minutes. The clear supernatant was collected and used as the crude extracellular enzyme source.

Enzyme Assay
Cellulase activity was assessed using the 3,5-dinitrosalicylic acid (DNS) technique. In summary, 0.5 mL of properly diluted enzyme extract was combined with 0.5 mL of 1% (w/v) carboxymethyl cellulose (CMC) produced in 0.05 M citrate buffer (pH 5.0). The reaction mixture was incubated at 50°C for 30 minutes. The reaction was halted by adding 3 mL of DNS reagent, followed by boiling at 100°C for 10 minutes. The mixture was cooled, and absorbance was read at 540 nm using a spectrophotometer. One unit (U) of cellulase activity was defined as the quantity of enzyme that releases 1 µg of reducing sugar (as glucose equivalent) per minute under the experiment conditions (Biaragi, 2016). The formation of a reddish-brown color indicated the presence of reducing sugars released from CMC hydrolysis (Figure 1).

Figure 1: DNS assay tubes showing color development due to the release of reducing sugars from CMC hydrolysis by fungal cellulase

RESULTS AND DISCUSSION

Isolation and Screening of Cellulolytic Fungi
This investigation identified four unique fungal isolates from decayed *Arachis hypogaea* shells, of which only two designated OPGS and OPBS demonstrated discernible hydrolysis zones on CMC agar after Congo red staining (Table 1). The presence of distinct halos surrounding the fungal colonies signifies active enzymatic degradation of cellulose, as congo red exclusively stains intact cellulose, leaving degraded areas unstained (Ahmed et al., 2022; Ezeagu et al., 2023). The distinct hydrolytic zones found align with existing cellulolytic screening methods, so affirming the efficacy of the congo red assay in detecting active cellulose-degrading fungi (Ahmed et al., 2022; Ezeagu et al., 2024).

Table 1: Clear Zone Diameters of Fungal Isolates on CMC Agar

Isolate code	Clear zone diameter (mm)	Colony diameter (mm)
OPGS	44.00	38.00
OPBS	43.00	39.00

Morphological and Microscopic Identification
The macroscopic features of isolate OPGS comprised rapidly proliferating colonies on PDA with greenish-yellow coloring, creamy white edges, a granular surface texture, and considerable sporulation. These characteristics align with the established morphology of *A. flavus* (Okayo et al., 2020; Seerat et al., 2022). Comparable characteristics have also been documented in research concerning aflatoxigenic strains of *A. flavus* (Shang et al., 2025).
Microscopic analysis utilizing lactophenol cotton blue staining demonstrated septate hyphae and rough, branching conidiophores ending in globose vesicles. Biseriate phialides were noted extending from the vesicle surface, generating chains of rough-walled, spherical conidia. The intricate morphological characteristics correspond closely with both ancient and contemporary descriptions of *A. flavus* (Seerat et al., 2022; Shang et al., 2025). The integration of macroscopic and microscopic characterizations is essential for precise fungal identification and supports the classification of isolate OPGS as *Aspergillus flavus (*Figure 2).

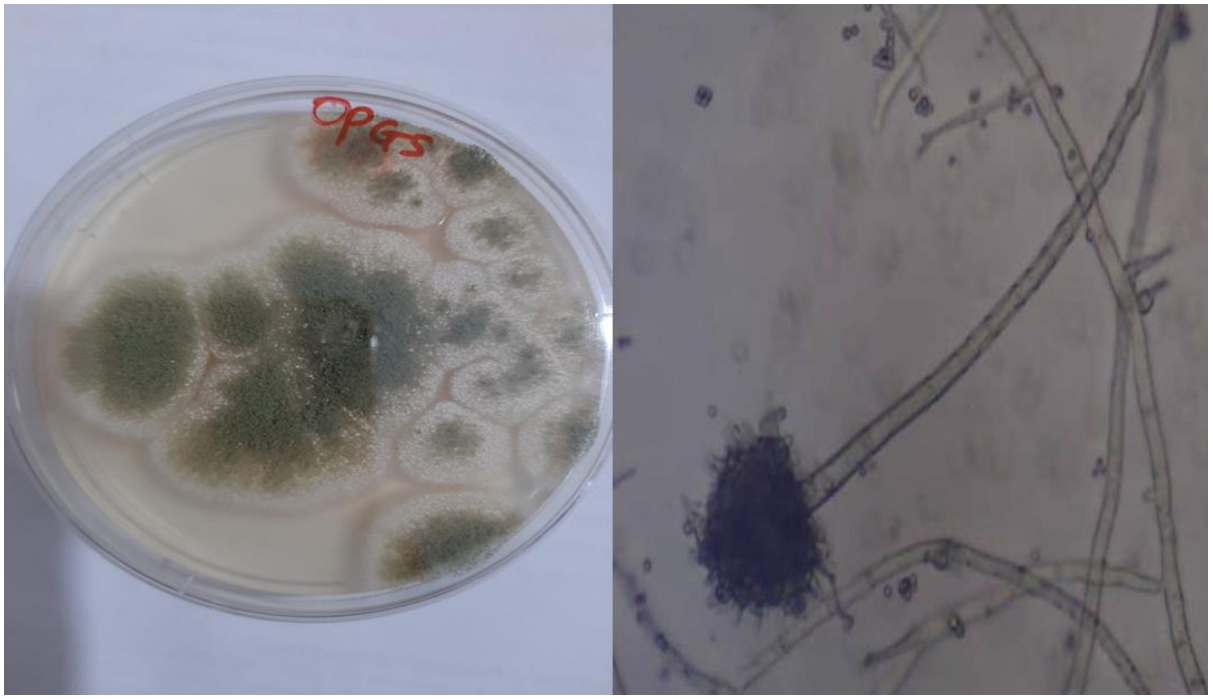

Figure 2: Macroscopic and microscopic feature of *A. flavus*

The macroscopic morphology of isolate OPBS on Potato Dextrose Agar (PDA) exhibited big, dense, velvety colonies characterized by black pigmentation and distinct radial grooves. These traits align with the standard morphological characteristics of *A. niger* (Ejimofor, 2022).

Microscopic analysis disclosed septate hyphae and smooth-walled conidiophores culminating in spherical vesicles. Radiating chains of black conidia were noted, closely conforming to established taxonomic descriptions for *A. niger* (Tariq, 2017).

The results indicate that standard phenotypic techniques, when used with appropriate medium like PDA, are dependable for the precise identification of *A. niger* in clinical and environmental samples (Figure 3) (Ejimofor, 2022; Tariq, 2017).

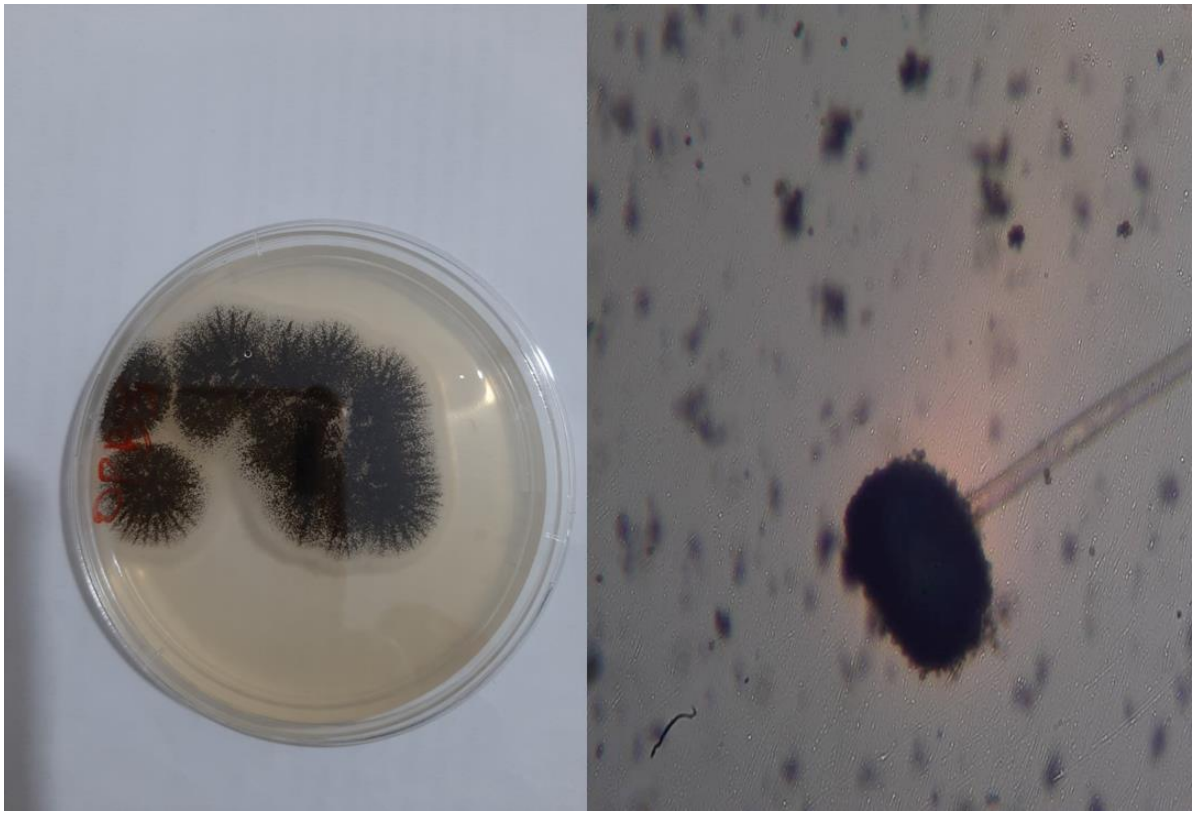

Figure 3: Macroscopic and microscopic feature of *A. niger*

The isolation of cellulolytic *Aspergillus* species from groundnut shells highlights the potential of degraded agro-wastes as sources for industrially significant fungus. These strains may be advantageous for biomass valorization, bioethanol production, composting, and enzyme industries, in accordance with global initiatives for sustainable bioconversion technologies (Kumar et al., 2008; Dhiman et al., 2024).

Quantitative cellulase screening

Figure 4 displays the cellulase activity of *A. flavus* and *A. niger* over a 5-day incubation. *A. niger* demonstrated peak activity at 48 h (92.3 ± 1.1 U/mL), while *A. flavus* peaked later at 72 h (86.7 ± 1.7 U/mL). Both strains demonstrated a reduction in activity beyond 72 h, likely due to food depletion or enzyme degradation, consistent with earlier results (Naher et al., 2021).

The earlier peak by *A. niger* suggests quick metabolic adaptation, but *A. flavus* maintained enzyme production longer, suggesting more stable secretion kinetics (Moubasher et al., 2016). These findings indicate their potential in lignocellulose bioconversion processes.

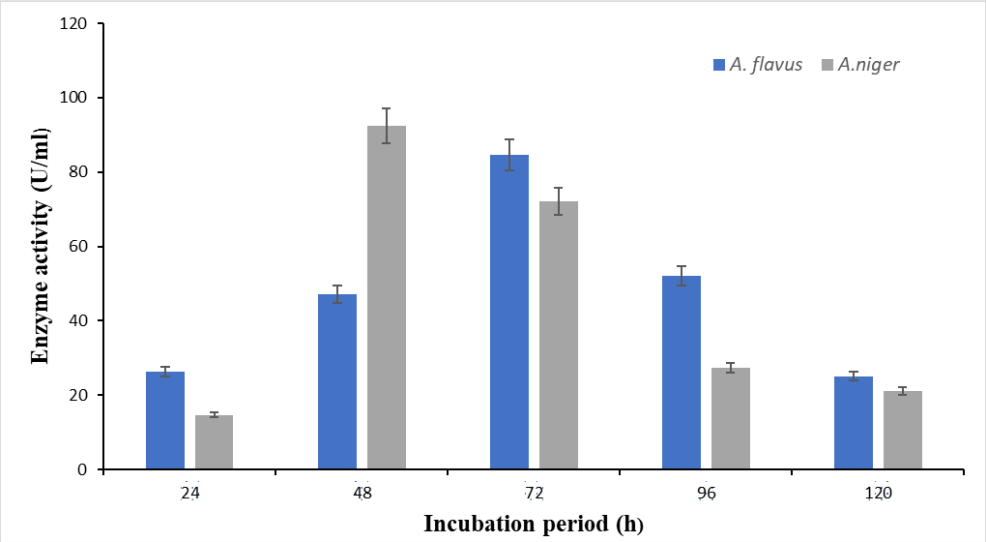

Figure 4: Time-course profile of cellulase activity by fungal isolates

pH Variation During Fermentation

As shown in Figure 5, the pH of the culture medium for both *A. flavus* and *A. niger* declined steadily from 6.1 at 24 hours to 4.4 at 120 hours. This acidification reflects fungal metabolic activity and organic acid release during cellulose degradation (Weimer et al., 2022). The trend aligns with optimal pH ranges (4.0–6.0) for fungal cellulase activity (Bakare et al., 2022) and may explain the drop in enzyme production after 72 hours.

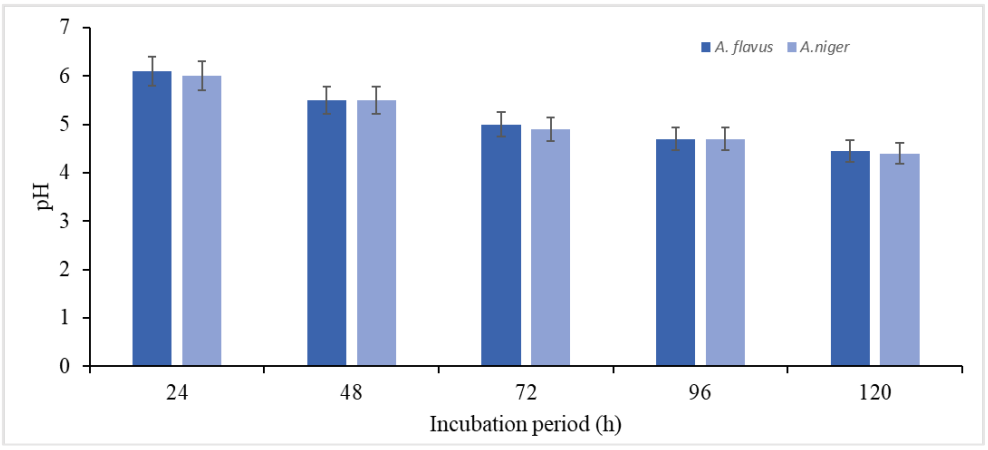

Figure 5: Course of pH during incubation

CONCLUSION

This work effectively illustrated the viability of decomposed groundnut shells as a medium for the isolation and growth of cellulolytic fungus. Of the four isolates obtained, *Aspergillus flavus* and *Aspergillus niger* demonstrated notable cellulose-degrading ability, as indicated by both qualitative and quantitative assessments. Both strains generated significant quantities of extracellular cellulase, with maximum activity corresponding to ideal fungal growth conditions and culture acidity. These findings validate the biotechnological significance of fungi originating from agro-waste in enzyme synthesis and endorse their utilization in sustainable biomass valorization methods, including composting, bioethanol generation, and the bioprocessing of lignocellulosic substances.

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