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Full Length Research Article

From Fungus to Fertilizer: Unleashing the Biofertilizer Potential of *Aspergillus niger* for Improved Maize Productivity

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Abstract

Aspergillus niger, a fungus notorious for causing postharvest rot in *Annona squamosa* (sweetsop) fruits, has been discovered to play a dual role in agriculture. This research explores the dual role of *A. niger*: its involvement in sweetsop rot and its potential to boost *Zea mays* (maize) growth as a biofertilizer. The fungus was isolated using Standard Blotter Method and pure culture, was obtained on Potatoes Dextrose Agar (PDA). Morphological and molecular study (ITS-1 and 2 markers) identified *A. niger* (95.89% DNA similarity) and *Rhizoplaca sp.* (98.11% similarity) as the causative agents of sweetsop rot in Rivers State, Nigeria. *A. niger* was isolated and tested on maize (Oba Super 6) in a greenhouse using different doses: (20, 30, and 40 ml/bucket) at 0.08 spores/ml, controls that had no inoculation, and poultry manure (standard). The 30 ml *A. niger* treatment demonstrated notable efficacy in promoting maize growth, yielding a plant height of 91.6 cm, which represents a 52% increase over the control (60.3 cm). This performance was comparable to poultry manure (95.0 cm), a standard treatment. Notably, the 30 ml *A. niger* dose also resulted in the longest root length (24.1 cm), significantly outperforming other treatments ($p < 0.05$). These findings suggest that the 30 ml *A. niger* treatment is effective in enhancing both shoot and root growth, positioning it as a potential eco-friendly biofertilizer. *A. niger* shows promise as both a pathogen and an eco-friendly biofertilizer, boosting crop growth while reducing chemical use and environmental impact. Incorporating *A. niger* into farming practices is recommended to improve yields and promote eco-friendly food production methods, contributing to sustainable agriculture and reduced chemical usage.

Keywords: *Annona squamosa, Aspergillus niger*, Biofertilizer, sweetsop rot, maize, Sustainable agriculture

Introduction

The Green Revolution brought unprecedented food production, but its dark side - chemical fertilizers - now threatens our planet and health. Water pollution, soil degradation, and toxic crops are just a few of the devastating consequences (Kumar *et al.,* 2022; Sharma and Singh, 2017). This has led to increased incidence of diseases like cancer, respiratory disorders, and dermatological conditions, due to the accumulation of toxic compounds in crops (Gupta and Gupta, 2020).

In light of these challenges, biofertilizers have emerged as a promising alternative, offering a sustainable and environmentally friendly approach to agriculture. *Aspergillus niger*, a non-pathogenic filamentous fungus, has garnered attention for its exceptional phosphate-solubilizing abilities, organic acid secretion, and decomposition of organic matter, making it an ideal candidate for improving soil fertility and crop productivity (Vassilev *et al.,* 2006). In the pursuit of eco-friendly farming solutions, *Aspergillus niger* shines as a beacon of hope, poised to elevate crop yields and champion sustainable agriculture.

This study aims to explore the biofertilizer potential of *A. niger*, investigating its capacity to enhance maize productivity while reducing reliance on chemical fertilizers. By harnessing the power of this microorganism, we can contribute to the development of sustainable agricultural practices, promoting eco-friendly farming systems and improved crop yields.

Materials and Methods

Study Area

This research was conducted at the University of Port Harcourt, involving the Plant Science and Biotechnology Mycology/Pathology laboratory and the Regional Centre for Biotechnology and Bio-fuel Research Laboratory (Latitude: 4.9071° N, Longitude: 6.917° E), where DNA extractions were performed in collaboration with IITA, Ibadan, and the Centre for Ecological Studies for the field experiment.

Study design

The research comprised multiple phases: collection of rotten sweetsop fruits, preparation of Potato Dextrose Agar (PDA), isolation of fungal species via direct plating, subculturing for purification, slant transfer, microscopy, molecular characterization, biofertilizer production, and a controlled greenhouse experiment measuring growth parameters. The experiment used a one-way design with three replicates.

Sources of Fruits and poultry droppings

Oba Super 6 maize seeds (Plate 2) were sourced from Agricultural Development Programme (ADP) in May 2025. The maize seeds and fresh sweetsop fruit (Plate 1a) were authenticated at the Department of Plant Science and Biotechnology herbarium, Faculty of Science, University of Port Harcourt, with a reference number UPH/C/115. Decayed sweetsop (Plate 1b) was obtained from Umuanyagwu in Etche Local Government Area of Rivers State, located at Latitude 5° 0' 0.274" N and Longitude 7° 4' 0.269" E. Poultry droppings were collected from the Faculty of Agriculture Farm, University of Port Harcourt, on July 4th, 2025



Plate 1a and b: a). Fresh sweetsop **b).** Decayed sweetsop

Isolation of Fungi from decayed sweetsop

Fungal organisms were isolated from decayed sweetsop using a modified streaking plate method (Sanders, 2012). The samples were surface-sterilized with 70% ethanol for 3 minutes and rinsed with sterile water. Prepared PDA medium was poured into Petri dishes and inoculated with samples of the fungal organisms using a flamed loop. Plates were incubated upside down at 25±2°C for 5 days. Growing fungi were sub-cultured at 25±2°C for 7 days to obtain pure cultures.

Molecular characterization of the fungal isolate using the Internal Transcribed Spacer (ITS) marker and identification

The genomic DNA of isolates VF1 and VF4, was extracted at the Regional Centre for Biotechnology and Bioresources, University of Port Harcourt, using the Quick DNA™ Fungal/Bacterial MiniPrep Kit. DNA quantity and purity were measured with a Nanodrop 2000c spectrophotometer and assessed via agarose gel The Fungal/Bacterial MiniPrep Kit was used to extract fungal DNA from pure culture mycelium, with some methodological changes. The quantity and purity of the isolated DNA were measured with a NanoDrop 2000c Spectrophotometer. electrophoresis. The DNA was amplified and sequenced at the IITA Bioscience Centre, utilizing ITS1 and ITS4 primers. Sequencing was performed on an ABI 3500 sequencer, with subsequent analysis conducted using MEGA 7.0.26 and alignment via BLASTN from the NCBI database. *Aspergillus niger* and *Rhizoplaca sp*. were the predominant fungal pathogens associated with the decay of sweetsop fruits, with *A. niger* being selected for use as a biofertilizer.

Biofertilizer formulation

Biofertilizer was manufactured using a modified approach from Anubrata and Rajendra (2014). Broth was made by filling a sterilized 1000ml conical flask with 500ml of distilled water, then dissolving 5g of peptone and 20g of dextrose in 500ml of the water. The broth flask was marked, autoclaved, and cooled. The soup was inoculated with 7-day-old fungal organism and shaken periodically. The broth was allowed to ferment for 18 days and after the 18 days, fungal organisms in the broth had proliferated. A biomass count was performed to determine the number of fungal organisms in the broth.

Biomass Count

Biomass was determined by drying 25ml *A. niger* broth in an oven, then weighing the dried mass. Calculations showed 2g of *A. niger* in 25ml broth, equivalent to 0.08g *A. niger* per 1ml broth.

Cultivation of *Zea mays* (oba super 6 variety)

Zea mays was cultivated at the Centre for Ecological Studies, University of Port Harcourt (Greenhouse). Seeds obtained from ADP Port Harcourt were directly sown into loamy soil in 15 replicates within the greenhouse environment. Three replicates were established for each of the 5 treatments: control, *A. niger* broth (20ml, 30ml, and 40ml applications) and poultry droppings. Seeds were singularly planted to prevent overcrowding and competition. Germination occurred 5 days post-planting.



Plate 2: Maize seeds (oba super 6 variety)

Application of *A. niger* for Plant Growth: For the application of *A. niger*, a modified technique outlined by Khan *et al.* (2008) was used. In week two, the foliage of *Zea mays* was treated with eighteen-day-old broth containing *A. niger* as shown in plate 3, at a concentration of 0.08 spores per millilitre (mL) spores/ml at a rate 20ml, 30ml, and 40ml /bucket, *Zea mays* stands that did not have *A. niger* was used as the control, and poultry dung the standard.



Plate 3: Application of *Aspergillus niger* broth

Agronomical Assessment of the maize plant

Following the emergence of genuine leaves at week 2, measurements of plant height, leaf number, and leaf area were obtained weekly for five (5) weeks, beginning with week two (2) following the application of *A. niger* and poultry droppings to the soil. Root length was measured after harvesting. The plants were plucked, and the roots were thoroughly cleansed to eliminate soil particles before being measured with a 30cm metre rule. A 30cm metre rule was used to measure the plant height, as well as the leaf length and breadth. The number of leaves was counted manually, and leaf discolouration was evaluated by eye inspection of the leaves. To calculate the average of each parameter, the various parameters of each

plant were combined together and then divided by the total number of plants in the stand.

Statistical Analysis

The data collected during the experiment were analyzed using one-way analysis of variance (ANOVA), with statistical significance determined at a p-value threshold of 0.05. Least significant difference (LSD) was computed to separate mean differences.

Result

DNA Successfully Isolated from Fungal Cultures:

Genomic DNA was successfully isolated from all samples, with spectrophotometry revealing concentrations of 28.1, 36.8, and 33.3 ng/µL for isolates VF1 and VF4, respectively (Table 1). Agarose gel electrophoresis showed distinct, sharp bands, indicating intact DNA (Figure 1).

Table 1. Table of DNA Concentration

S/N	Code	DNA	PCR	Sequencing	DNA Concentration (ng/ul)	Sequenced
1	VF1	Yes	ITS	Yes	28.1 ng/µL	Yes
2	VF4	Yes	ITS	Yes	33.3 ng/µL	Yes

Fungal DNA Successfully Detected via PCR:

PCR amplification of the fungal ITS region was successful across all samples. Agarose gel electrophoresis revealed distinct bands at ~200-420 bp (Figure 1), confirming amplification of the target ITS genes. The consistent band intensity and uniformity indicated robust amplification using FirePol Master Mix, validating the PCR product quality and specificity.

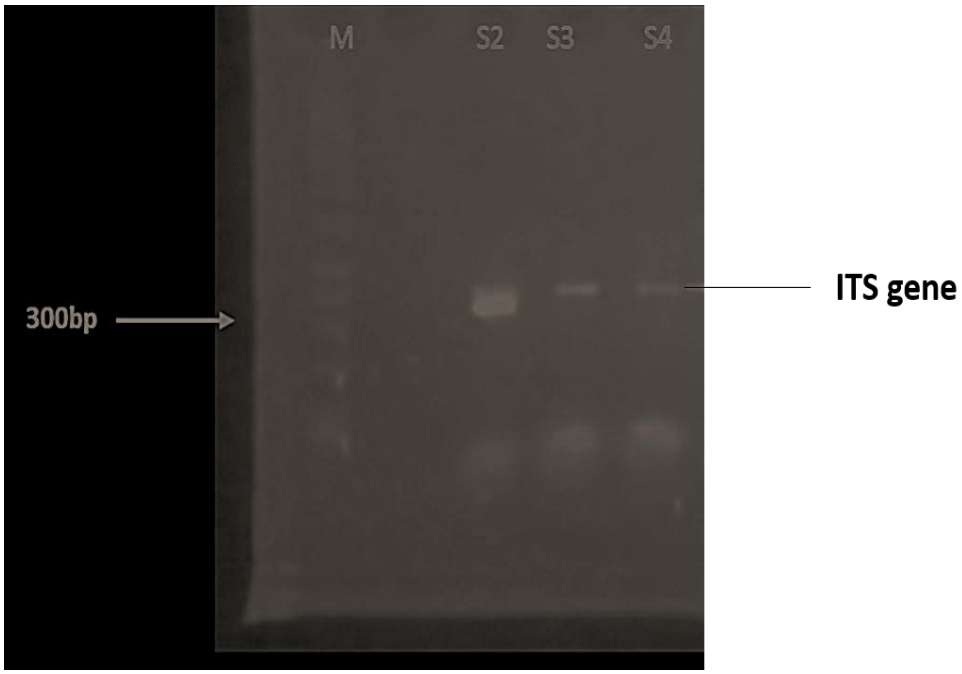


Figure 1: Representative agarose gel showing PCR-amplified fungal DNA. Lane M: 100 bp DNA ladder; S1 (VF1) and S3 (VF4): samples positive for ITS gene (~400 bp).

Fungal Species Identified via Sanger Sequencing

Sanger sequencing produced high-quality chromatograms, and bioinformatics analysis including BLAST searches facilitated species identification for each sample.

BLAST result and phylogenetic tree construction

BLAST examination of sequences VF1 and VF4 yielded different levels of identification confidence (Table 2). VF1 aligned strongly with *Aspergillus* species (*A. niger* ~96% identity, ~92% coverage),

suggesting accurate species-level classification. VF4 matched with fungi (*Rhizoplaca* sp.) with ~98% identity and ~16-17% coverage, indicating unclear classification due to database limits or sequence divergence. Thus, VF1 used as biofertilizer, was confidently identified as *Aspergillus* spp.

Table 2: BLAST results summary for isolates VF1, VF3 and VF4

Parameter	**VF1**	**VF4**
Name of organisms	*Aspergillus. niger*	*Rhizoplaca* sp.
GenBank closest match	*A. niger* LayoO8	*Rhizoplaca* sp. AA-2021a
Percent Identity with closest GenBank match	95.89	98.11

Phylogenetic Placement of Isolate VF1

In Figure 2, VF1 is positioned within the *Aspergillus niger/welwitschiae* complex, clustering with *A. niger* and *A. welwitschiae* strains (bootstrap support: 69-78). Due to close genetic relatedness, VF1 is best classified as *A. niger/welwitschiae* complex, with stronger affinity to *A. niger*.



Figure 2: Neighbor-joining evolutionary relationship of the fungal isolate VF1

Phylogenetic Placement of Isolate VF4

VF4's phylogenetic analysis (Figure 3) shows it is distinct from reference taxa, branching externally to clades like *Abrothallus, Hausknechtia, and Rhizoplaca* with 100% bootstrap support in neighboring groups. It's likely an uncharacterized fungus or divergent lineage; ITS data is not enough. Closest match is *Rhizopus* sp., but more markers (LSU, TEF1-α, RPB2) are needed for clarity.

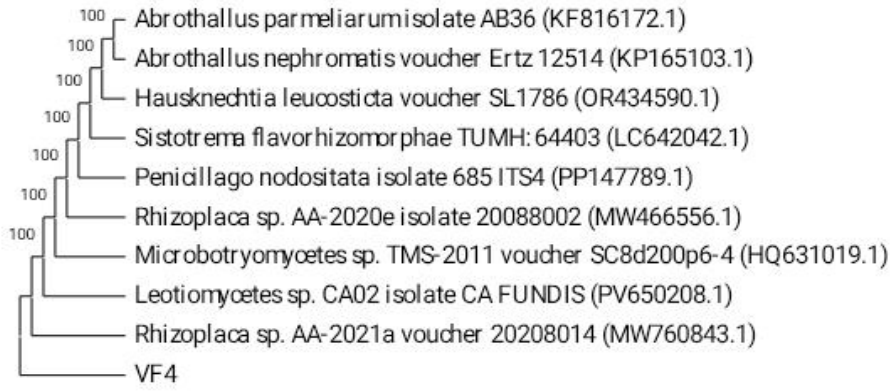


Figure 3: Neighbor-joining evolutionary relationship of the fungal isolate VF4

The fungal organism isolated from sweetsop, as depicted in Plate 4, exhibited a black, non-cracked reverse and brown powdery spores. For comparison, *Aspergillus niger* typically produces a black, velvet-like, dense colony with a yellowish to colourless reverse side, and is characterized by abundant black, powdery spores. Growth was typically rapid, covering the plate within 3-5 days on optimal media such as Potato Dextrose Agar (PDA).

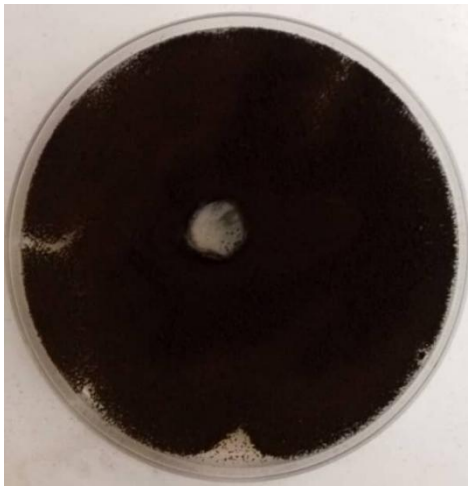


Plate 4: a pure culture of fungal isolate

The micrographs of the fungal isolate are presented in Plate 5 below. Macroscopic and microscopic descriptions, along with inferences of the pure culture used in biofertilizer production, are summarized in Table 3.

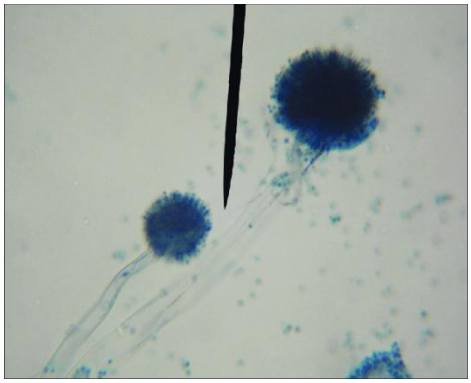


Plate 5: Microscopic appearance of fungal isolate using a light microscope

Table 3: Macroscopic Description and Microscopic Description

Isolate Code	Macroscopy	Microscopy	Possible organisms
VF1	Colonies are black, velvet-like, and dense, with abundant black, powdery conidia; reverse is yellowish to colourless.	The isolate exhibits conidiophores, hyaline hyphae, and globose vesicles.	*Aspergillus niger*

The following results presented below were observed in the field experiment which was carried out at the Greenhouse:

Agronomic Characteristics after Application of Biofertilizer and Poultry Manure from Week 2 to Week 6: The application of *A. niger* and poultry manure significantly affected plant height ($p < 0.05$) from Week 3 onwards. Poultry manure resulted in the highest plant height (95 cm at Week 6), followed by the 30 ml *A. niger* treatment (91.6 cm), both significantly higher than the control (60.3 cm). The 30 ml *A. niger* treatment showed a 52% increase in plant height compared to the control, indicating its effectiveness in promoting plant growth (Table 4).

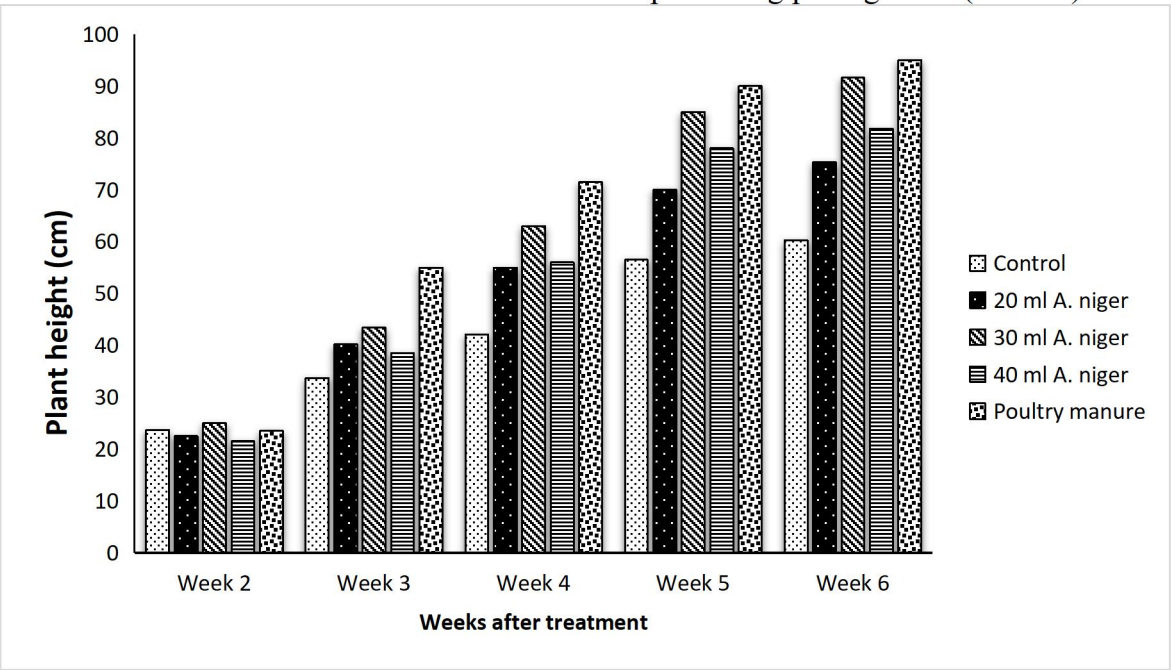


Figure 4: Average plant height

Number of Leaves:
The effects of biofertilizer and poultry manure on maize leaf number, is shown in Figure 5. The 30 ml *A. niger* treatment resulted in a notable increase in number of leaves, peaking at 7 cm in week 4, indicating a positive effect on growth. However, this effect declined to 6.3 cm by week 6, which was not statistically significant compared to the control (5.8 cm) ($p > 0.05$). Notably, this treatment outperformed the control and other *A. niger* doses, although poultry manure remained the most effective, resulting in a leaf number of 9.3 at week 6, significantly higher than the control (5.8) ($p < 0.05$).

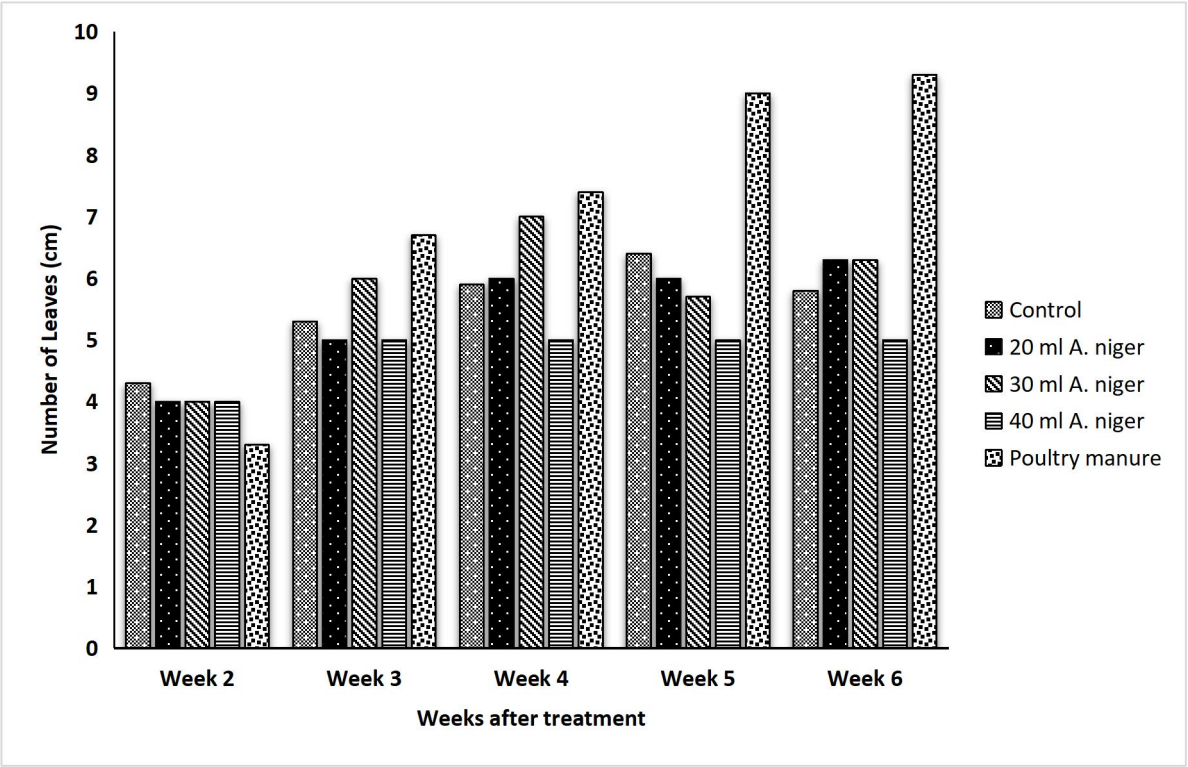


Figure 5: Average Leaf Number

Leaf length: Poultry manure resulted in the highest leaf length (75 cm at Week 6), significantly outperforming the control (45 cm) ($p < 0.05$). The 30 ml *A. niger* treatment also significantly increased leaf length (62.3 cm) compared to the control ($p < 0.05$), though less than poultry manure as shown in Figure 6.

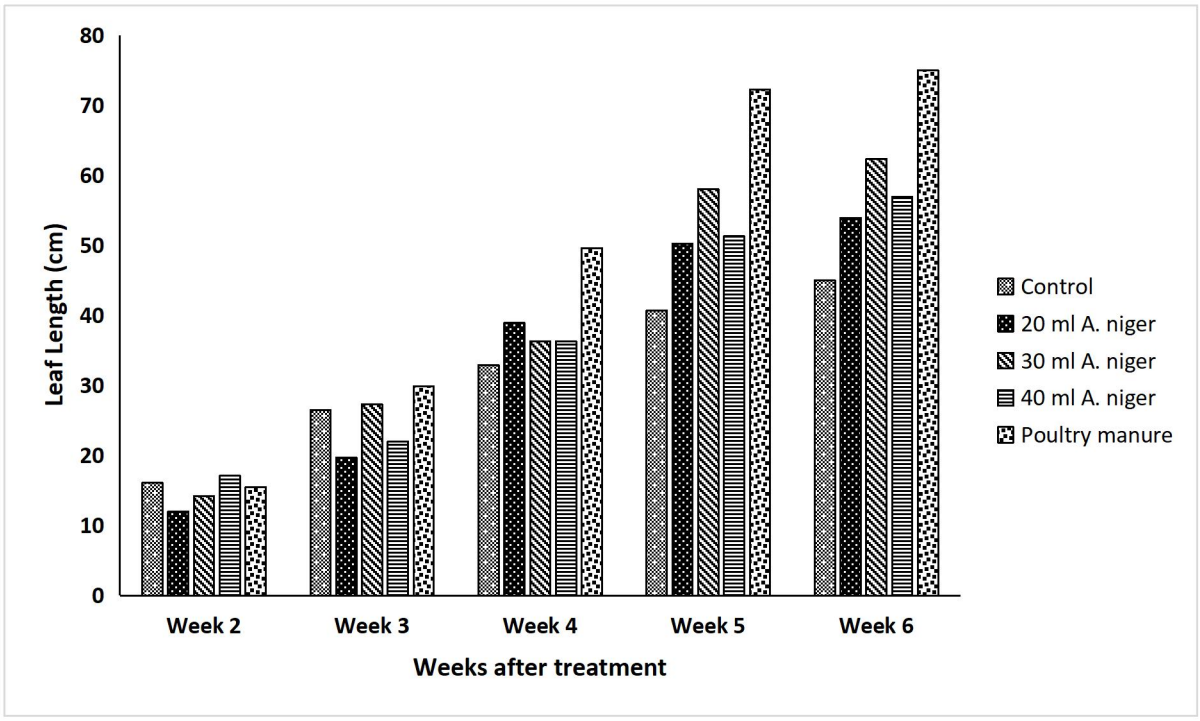


Figure 6: Average leaf length at week 6

As shown in Figure 7, 30 ml *A. niger* treatment (24.1) was significantly higher than the control (22.7) ($p < 0.05$). Other treatments were not significantly different from the control.

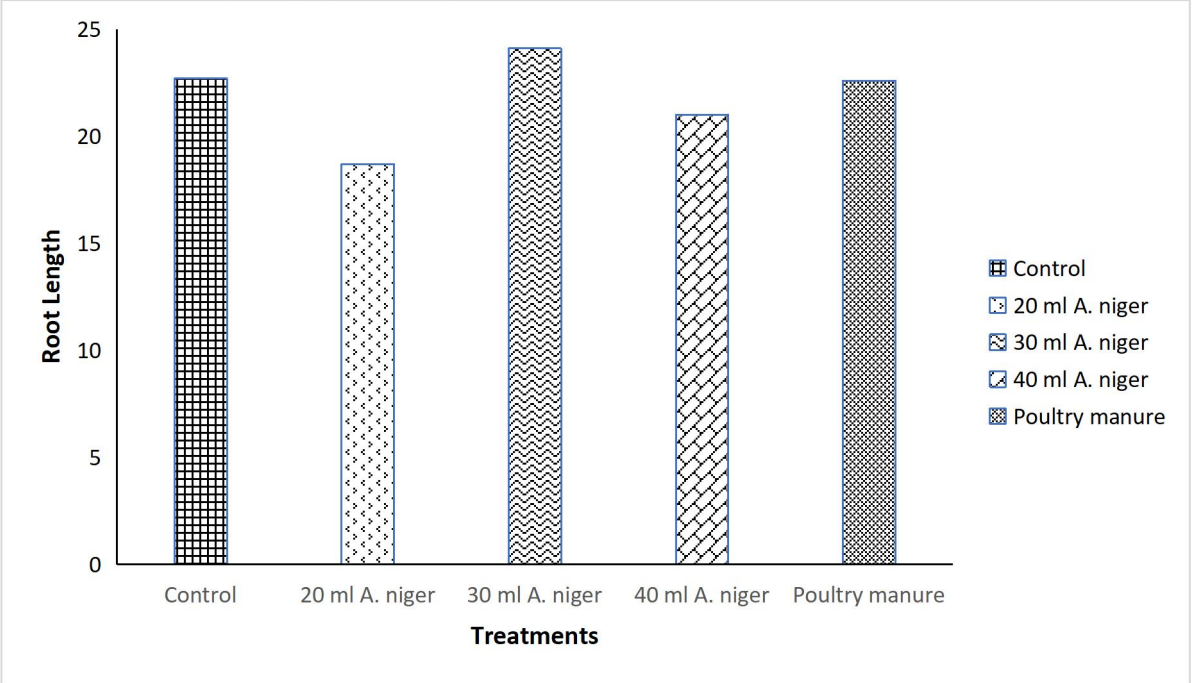


Figure 7: Average root length at week 6

Discussion

The present work highlights the dual role of *Aspergillus niger*, a fungus traditionally linked with postharvest rot in *Annona squamosa* (sweetsop) fruits, as a possible biofertilizer for *Zea mays* (maize). *A. niger* (95.89% DNA similarity) and *Rhizoplaca sp.* (98.11% similarity) were identified morphologically and molecularly as the culprits responsible for sweetsop rot in Rivers State, Nigeria.

Soil microorganisms play a pivotal role in shaping plant growth and health, influencing nutrient cycling and ecosystem balance (Bhattacharyya & Jha 2012). Fungal species, in particular, are gaining recognition for their soil quality enhancement capabilities (Frac *et al*., 2015; Bagyaraj & Ashwin, 2017). In line with this, our study demonstrates that applying *A. niger* to maize plants yields a positive concentration-growth relationship. This aligns with existing research on fungal biofertilizers, underscoring the potentials of *A. niger* as a valuable bioresource for boosting agricultural productivity (Vessey, 2003; Harman *et al*., 2004; and Singh *et al*., 2011). The growth-promoting effects of *Aspergillus niger* can be attributed to its ability to produce plant growth-promoting substances, such as auxins and gibberellins, and solubilize phosphorus, making it accessible to plants (Vassilev *et al.,* 2006; Gupta *et al.,* 2012; Jain & Pandey, 2016). This dual mechanism enhances nutrient availability and uptake, driving improved plant growth.

This study emphasizes the dual role of *A. niger* and suggests it as an ecologically friendly biofertilizer that adheres to sustainable agricultural principles (Altieri, 1999; Pretty & Hine, 2001). Its implementation could minimize dependency on chemical fertilizers, slow environmental degradation, and help to promote more ecologically friendly food production techniques. Further research and incorporation of *A. niger* into agricultural systems may increase crop yields while also promoting environmentally friendly farming practices.

The findings support the growing body of research on fungal biofertilizers and highlight the potential of *A. niger* for sustainable agriculture.

Our results align with that of Mundim *et al.* (2022) study, which demonstrated that inoculating the soil with *A. niger* significantly affected seedling growth of lettuce, pepper, scarlet eggplant, watermelon, and tomato, notably in terms of height and shoot mass, compared to uninoculated controls. Our findings are consistent with those of Oluwole *et al*. (2023), who found that *A. niger* and *P. chrysogenum* show potential as mycofertilizer for improving grain crop growth, particularly in crops that do not fix nitrogen.

Aspergillus niger has become a promising mycofertilizer with several advantages for crop development (Ibrahim *et al.,* 2024; Vassileva *et al.,* 2024). One of its main benefits is that it increases the availability of phosphorus by solubilizing it through the creation of organic acid. Additionally, it stimulates the growth of roots and shoots by synthesizing auxins, gibberellins, and cytokinins. Furthermore, *A. niger* demonstrates biocontrol qualities, suppressing plant pathogens and lowering disease incidence in specific strains, and enhances soil health by increasing soil structure, organic matter, and beneficial microbial communities. Many advantages make *A. niger* a useful tool for sustainable agriculture, increasing crop yields and fostering ecosystem health.

Recent research by Wang *et al*. (2025) has elucidated a molecular mechanism underlying the plant growth-promoting effects of *A. niger* strain 129B, revealing its activation of the SAUR32-PP2C72 pathway in host plants. This activation facilitates proton efflux at the root surface, stimulating root elongation and underscoring potential of *A. niger* as a valuable tool for advancing sustainable agriculture and enhancing crop productivity.

Conclusion

In conclusion, this study reveals the dual role of *Aspergillus niger* in agriculture, highlighting its potential as a biofertilizer for maize while acknowledging its pathogenic effects on *Annona squamosa*. The significant growth promotion observed in maize, particularly at the 30ml treatment, underscores the ability fungus to enhance agricultural productivity sustainably. Further research is warranted to optimize application methods and mitigate potential risks, paving the way for eco-friendly farming practices and reduced chemical usage.

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Conflict of Interest: The authors declared no conflict of interests

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