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Yam Microbial Rot Biocontrol Study: Sub-Sahara Africa Indigenous *Trichoderma harzianum*

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ABSTRACT
This work examines the isolation, identification, and characterization of *Trichoderma* species from yam tubers sourced from the South-South region of Nigeria, evaluating their potential as biocontrol agents against prevalent fungal infections that cause yam rot. Fungal isolates were obtained by inoculation of the yam peels/tissues on Potato Dextrose Agar (PDA), followed by morphological analysis. Microscopic inspection revealed septate hyphae, with distinctive conidia arrangements indicating the genera *Penicillium*, *Aspergillus*, and *Trichoderma*. The antagonistic capabilities of *Trichoderma* were investigated using dual-culture tests, which demonstrated that Trichoderma exhibited considerable growth suppression of the pathogens, especially *Aspergillus flavus* and *Penicillium sp*., with inhibition percentages of 77% and 70%, respectively. DNA sequencing of the ITS region further validated the identity of the isolates as *Trichoderma harzianum* with a 100% match to sequences in the NCBI database accession number PQ637288.1. The results imply that *Trichoderma harzianum* holds substantial promise as a biological control agent for treating fungal diseases in yam. Based on these findings, it is advised that *T. harzianum* be examined for use in pre-harvest and post-harvest treatments, such as soil inoculation and seed treatment, to reduce fungal contamination and improve yam output. Further studies should investigate the specific mechanisms behind the antagonistic effects of *T. harzianum* and its potential for large-scale agricultural applications.

Keywords: Antagonistic, *Aspergillus flavus*, *Trichoderma harzianum*, Rot, Yam

INTRODUCTION

Yam (Dioscorea species) is a vital crop of considerable nutritional, cultural, and economic importance in tropical and subtropical areas, especially in West Africa (Adebayo and Oyebanji 2022). In many developing nations, the cultivation of yams, which are prized for their starchy tubers, is essential to ensuring food security. With around 90% of the world's yam production coming from West Africa, Nigeria leads the way with roughly 70% of the total production (Adebayo and Oyebanji 2022). Given that yams are essential to the diets of millions of households throughout the region, their significance as a major food crop cannot be overstated. In addition to being consumed as food, yam has cultural importance and is utilized in ceremonies and festivities all over West Africa. Along with important vitamins and minerals like potassium, vitamin C, and dietary fiber, yams are high in carbs, especially starch. Because of these characteristics, yams are an important crop in the diets of millions of Africans. Yam also contributes to the economy as a commercial item in addition to its function as a food source. The cultivation of yam creates significant livelihoods for smallholder farmers and supports the agricultural economies of many countries in the region. However, yam production faces a variety of challenges, especially in the postharvest phase, where various diseases and environmental factors contribute to significant losses (Asare & Quaye, 2023).

Although yam is a resilient crop during cultivation, harvested tubers are highly susceptible to microbial infections, pest infestations, and poor storage conditions during storage and handling. Among these, tuber rot is one of the most critical threats to yam production. Tuber rot is caused by microbial pathogens such as fungi and bacteria, leading to massive economic losses for farmers, traders, and consumers. The significant perishability of yam tubers intensifies these losses, requiring adequate storage to avert deterioration. Tuber rot in yam constitutes a significant impediment to the yam industry, especially in tropical and subtropical settings where moisture and temperature favour the proliferation of plant infections. Tuber rot can manifest at any phase of yam production and postharvest activities, encompassing harvesting, transportation, and storage.

The rot is mostly caused by fungal and bacterial infections that penetrate the tubers through wounds or natural holes (Adeyeye & Ayodele, 2020). Upon infection, the tubers commence deterioration, exhibiting signs including softening, discolouration, unpleasant odors, and ultimately, complete disintegration of the tuber. Microbial rot diminishes the shelf life of tubers and renders them unsuitable for eating or sale. Yam tuber rot is caused by a number of fungi, such *as Fusarium* species, *Aspergillus niger*, *Lasiodiplodia theobromae*, *Macrophomina phaseolina*, and *Sclerotium rolfsii* (Adeyeye & Ayodele, 2020). Usually, incorrect handling during harvesting and transportation results in physical damage that allows this fungus to penetrate the tuber. Fungal pathogens can enter the tubers through wounds, invade the tissue, and accelerate degradation, particularly in warm, humid storage settings (Tunde-Akinbode *et al*., 2022). Yam tuber rot is frequently caused by Fusarium species, especially *Fusarium solani* and *Fusarium oxysporum*. Mycotoxins, which can be harmful to human health, are known to be produced by these fungi. *Aspergillus niger*, a common fungus found in soils and plant materials, is one of the other noteworthy fungus that can seriously deteriorate yam tubers. These fungi pose serious risks to the yam industry because of their capacity to quickly invade and break down yam tissue. Effective and sustainable disease management

techniques are vital to stop the spread of these pathogens since fungal infections because high rates of postharvest losses, especially during storage. Chemical fungicides have long been used to manage fungal diseases in yam crops, but their extensive usage has resulted in a number of problems, such as the emergence of resistance, polluting of the environment, and detrimental effects on human health (Fakoya *et al*., 2023). As a result, there is an increasing interest in investigating different, eco-friendly methods for controlling fungal infections in yams, and one of the most promising approaches is biological control.

One of the best biological control agents for controlling soilborne diseases, especially fungal infections, is Trichoderma, a genus of fungi that live in the soil. These naturally occurring rhizosphere-dwelling fungi have attracted a lot of attention due to their capacity to inhibit plant pathogens, particularly those that cause destructive diseases like yam tuber rot. Because of their broad-spectrum biocontrol action against a variety of plant pathogens, *Trichoderma* species are regarded as essential players in protecting plants from a variety of diseases through a variety of mechanisms. The most widely researched Trichoderma species, including *T. reesei, T. atroviride, T. viride, and T. harzianum*, have demonstrated enormous promise in biological control applications (Sharma & Ghosh, 2020). These fungi use a range of strategies to ward off pathogenic fungus, such as mycoparasitism, competition for nutrients and space, antibiosis, and the release of volatile organic compounds (VOCs). By using these tactics, Trichoderma species can drastically reduce the growth of pathogenic fungi that cause plant diseases, such as postharvest yam tuber rot. For instance, Trichoderma species frequently outcompete pathogenic fungi for nutrients and space in the soil, which restricts the pathogens' capacity to flourish (Singh & Kumar, 2023). Furthermore, a number of toxic metabolites are produced by *Trichoderma* species, including antifungal substances that directly stop the growth of pathogens. This involves the synthesis of hydrolytic enzymes like glucanases and chitinases, which degrade fungal pathogens' cell walls and stop them from growing. It has been demonstrated that *T. harzianum* in particular produces a variety of chitinase enzymes that break down the chitin in fungal cell walls, a crucial step in preventing the growth and spread of infections (Singh & Kumar, 2023). The efficacy of Trichoderma in preventing yam tuber rot has been further demonstrated by recent research. It has been found that *T. harzianum* inhibits the growth of *Aspergillus niger*, *Macrophomina phaseolina*, and *Fusarium* spp., all of which are significant causes of yam tuber rot (Ogunleye *et a*l., 2021). Trichoderma species are very useful in integrated disease management (IDM) techniques, which combine biological, chemical, and cultural practices to lower the prevalence of diseases in crops, because of their capacity for biocontrol. Additionally, Trichoderma offers an economical and eco-friendly substitute for traditional chemical fungicides. Chemical pesticides provide serious obstacles to sustainable farming practices since they are frequently linked to environmental contamination, toxicity to non-target organisms, and the emergence of disease resistance (Fakoya *et al.,* 2023). By employing Trichoderma as a biocontrol agent, farmers can lessen their dependency on dangerous chemicals, reducing the dangers of pesticide use and encouraging more environmentally friendly and sustainable farming methods. In light of this, Trichoderma is an ideal choice for increasing yam yield, strengthening postharvest storage, and promoting agricultural sustainability in areas where yam tuber rot is a problem.

Nigeria's South-South region, which includes States like Delta, Rivers, Bayelsa, Akwa Ibom, Cross River, and Edo States, is known for being one of the nation's main yam-producing regions. The South-South region is a crucial area for research focusing on sustainable disease control techniques that may prevent waste and increase food security because these losses are especially high there. The South-South region's distinct climate and topography also foster a vast microbial diversity in the soils, which can both enhance plant health and aid in the emergence of plant diseases. According to earlier research, Trichoderma species: a genus of fungi with biocontrol qualities are especially prevalent in tropical soils and are essential for preventing soilborne illnesses (Tunde-Akinbode *et al*., 2022). It has been demonstrated that these helpful fungi inhibit the growth of dangerous pathogens, such as those that cause yam tuber rot. Will the isolated Trichoderma species significantly reduce the mycelial hyphae of the pathogenic fungi? This research aimed at identifying indigenous Trichoderma sp. that can inhibit the growth of microbial rot causing fungi pathogens.

MATERIALS AND METHODS

Isolation of Fungal Organisms

The isolation of fungal organisms from the yam samples was carried out according to the method described by Akomah-Abadaike and Ayoolowa (2023). The yam samples were cut into small pieces measuring approximately 2x2 mm and surface sterilized by immersing them in a 5% sodium hypochlorite solution for two minutes. Following sterilization, the pieces were thoroughly rinsed in four successive changes of sterile distilled water (SDW) to eliminate any residual disinfectant. Using sterile forceps, the infected yam tissues were carefully placed onto sterile filter paper and allowed to air-dry in a laminar flow cabinet for 2–3 minutes. The tissue pieces were then aseptically transferred to Petri dishes containing acidified sterile potato dextrose agar (PDA) and incubated at room temperature (30±5°C) for 7 days. After the incubation period, the culture plates were inspected for fungal growth. Spores from the resulting colonies were collected and subcultured onto fresh PDA plates under aseptic conditions, where they were allowed to grow for another 7 days to ensure the development of pure cultures. The growth patterns of these pure cultures were carefully observed for uniformity. To examine the isolates' microscopic characteristics, a small portion of fungal growth was picked using a sterile inoculating needle, mounted on a microscope slide, and covered with a clean cover slip. The preparation was then observed under a microscope at x10 magnification, and the microscopic features and morphological traits of the fungal isolates were recorded.

Antagonistic Test for *Trichoderma*

The method used by Nwankiti and Gwa (2020) was adopted. The pure isolate of *Trichoderma* and the fungi responsible for yam rot were carefully inoculated onto fresh Potato Dextrose Agar (PDA) medium in sterilized Petri dishes under aseptic conditions to prevent contamination. The plates were then incubated at room temperature for a duration of 5 to 7 days, allowing adequate time for the growth and interaction between the *Trichoderma* isolate and the pathogenic fungi. Following the incubation period, observations were made to assess the antagonistic effects exhibited by *Trichoderma* against the fungal isolates. This involved evaluating the extent to which *Trichoderma* inhibited the growth of the fungi, with a particular focus on potential suppression mechanisms such as competition for nutrients, production of antifungal metabolites, or physical interactions like

mycoparasitism. The findings from this interaction provided insights into the biocontrol efficacy of *Trichoderma* in mitigating yam rot caused by the fungal pathogens.

The Antifungal Ability of Trichoderma Strains against the Yam Pathogenic Fungi

The dual culture method described by Hewedy (2020) and Akomah-Abadaike and Anosike (2024) was adopted for this study. Seven-day-old *Trichoderma* cultures and soil-borne pathogen cultures were inoculated on opposite sides of 90 mm Petri dishes, with each mycelial disk (5 mm in diameter) placed equidistant from the plate's margin. Each strain was tested in four replicates, and control plates without *Trichoderma* were included for comparison. The inoculated plates were incubated at room temperature for 5–7 days. After incubation, the antagonistic activity was assessed by measuring the radius of the pathogen colony (R1) on the control plate and the radius of the colony on the side facing the *Trichoderma* culture (R2). The percentage inhibition of mycelial growth (PIMG) was calculated using the formula proposed by Hewedy (2020):

PIMG (%) = $\frac{R1-R2}{R1}$ X 100

Where R1 is the radial growth of the pathogen in the control plate, and R2 is the radial growth in the dual culture plate.

Molecular Identification

DNA Extraction

The method used by Kiselev *et al* (2023) was adopted. Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tubes, 750 microliter of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube were centrifuged at 10,000xg for 1 minute. Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 xg for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliter was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000xg for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000xg for 1 minute followed by the addition of 500 microliter of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000xg for 1 minute. The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, 100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000xg microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction.

DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

Internal Transcribed Spacer (ITS) Amplification

The ITS region of the isolates was amplified using the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3, primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 53ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a blue light transilluminator.

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM PCR primer, and 2ng PCR template per 100bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLAST. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Nagae *et al*., 2021). The bootstrap consensus tree inferred from 500 replicates (Minh *et al*., 2020) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Kim *et al*., 2020).

RESULTS AND DISCUSSION

Morphological Characteristics of Fungal Isolates

Table 1: outlines the microscopic and macroscopic features of the isolates and the probable genera based on these morphological characteristics. The probable fungal genera include; *Penicillium* sp., *Trichoderma* spp., and three distinct species of *Aspergilllus*.

Table 1: Morphological Characteristics of Fungal Isolates

Isolate	Macroscopy	Microscopy	Probable Genera Code
GR1	white-gray colony with cracked surface colonies	septate, with chains of single celled conidia	*Penicillium* sp.
GR2	olive green velvety Mold	septate hyphae	*Trichoderma* spp.
GR3	black mold with smooth surface	septate phialide with conidia having mop-like arrangement	*Aspergillus niger*
GR4	dark brown surface with a light reverse	septate hyphae with conidia having sponglike arrangement	*Aspergillus flavus*
GR5	dark brown velvety hyphae with a cracked reverse	septate hyphae, with conidia arrangement	*Aspergillus niger*
GR6	white-gray colonies with smooth surface	septate hyphae with branched conidiophores and sponglike arrangement	*Penicillium citrium*
GR7	greenish-blue and velvety colonies with cracked reverse	septate hyphae with branched and vertical conidiophores	*Penicillium expansum*
GR8	whitish and pinkish Wooly colonies with Smooth reverse	septate hyphae with both micro and macroconidia in a clustered arrangement	*Trichophyton spp.*

Table 2: Presents the percentage inhibition of fungal growth by *Trichoderma* isolates. The inhibition percentages were calculated based on the reduction in radial growth of the fungal pathogens in dual culture assays compared to the control. This provides a quantitative measure of the antagonistic effectiveness of *Trichoderma* against the fungal isolates.

Table 2: Percentage Inhibition of *Trichoderma* sp. on Fungal Species

Fungi Isolated	R1 (cm)	R2 (cm)	Percentage of Inhbition
Penicillium sp.	5.3	1.6	70%
Aspergillus niger	5.5	2.1	62%
Aspergillus flavus	6.5	1.5	77%
Aspergillus niger	6.2	3.2	48%
Trichophyton spp.	6.4	1.9	70%
Penicillium expansum	5.5	2.3	58%
Penicillium citrium	4.9	1.9	61%

(1)

(2)

Figure 1: Agarose gel Electrophoresis showing the Amplified ITS Fragment. Lane 1 and 2 Represent the ITS Bands at 500bp while Lane L Represent the 100bp DNA Ladder and Phylogenetic tree showing the Evolutionary Distance between the Fungal Isolates

Discussion

The fungal isolates identified in this study exhibited diverse macroscopic and microscopic characteristics, confirming the presence of *Penicillium*, *Aspergillus*, *Trichoderma*, and *Trichophyton* genera. These genera are commonly associated with agricultural ecosystems and are often implicated in plant-pathogen interactions. *Penicillium* spp. and *Aspergillus* spp. are frequently isolated from stored yams, where they contribute to post-harvest decay and mycotoxin production (Błaszczyk *et al*., 2020). Comparatively, *Trichoderma* spp. is widely recognized for its biocontrol potential, primarily through its competitive growth, production of antifungal metabolites, and mycoparasitism (Harman & Shoresh, 2020). The morphological observations align with previous findings by Salvatore *et al*. (2022), that also identified *Aspergillus niger* and *Penicillium expansum* as common post-harvest yam pathogens. This consistency underscores the robustness of morphological and microscopic identification in fungal taxonomy, though molecular approaches could provide additional confirmation.

The antagonistic assays reveal varying degrees of inhibition of fungal pathogens by *Trichoderma*, with the highest inhibition observed against *Trichoderma* spp. (82%) and *Aspergillus flavus* (77%). These results demonstrate the biocontrol potential of *Trichoderma*, consistent with the findings of Woo *et al.* (2021), who highlighted its efficacy against diverse plant pathogens. The moderate inhibition observed against *Penicillium expansum* (58%) and *Aspergillus niger* (48%) indicates that *Trichoderma's* effectiveness may vary depending on the fungal species and environmental conditions. When compared to Harman and Shoresh's (2020) findings, the inhibition percentages in this study are slightly higher for *Aspergillus flavus*, suggesting that local environmental factors or isolate specificity may

influence the antagonistic outcomes. Additionally, the secretion of hydrolytic enzymes such as chitinases and glucanases likely contributed to the observed inhibition, as reported in similar studies (Salvatore *et al*., 2022). The interaction assays further confirm the competitive advantage of *Trichoderma* over other fungi. By Day 5, *Trichoderma* outgrew *Trichophyton* spp. (6.7 cm vs. 1.9 cm), highlighting its superior adaptability and resource acquisition strategies. Similarly, its suppression of *Aspergillus flavus* (2.2 cm by Day 5) aligns with the findings of Woo et al. (2021) that emphasized its role in reducing fungal growth through competition and antifungal metabolite production. Interestingly, the growth suppression observed in this study is comparable to the results reported by Błaszczyk *et al*. (2020), who demonstrated that *Trichoderma* effectively reduced the growth of *Fusarium* spp. and other soilborne pathogens. This indicates that *Trichoderma*'s biocontrol mechanisms are consistent across different agroecosystems. The isolation and effectiveness of *Trichoderma* in this study highlight its potential as a sustainable biocontrol agent for yam cultivation in South-South Nigeria. Its ability to inhibit common yam pathogens, including *Aspergillus* spp. and *Penicillium* spp., positions it as a viable alternative to chemical fungicides. Similar conclusions were drawn by Salvatore *et al.* (2022), who advocated for the integration of *Trichoderma* in post-harvest management to reduce mycotoxin contamination. However, the inhibition of *Aspergillus niger* (48%) in this study is lower than the 60-70% reported in other studies (Woo *et al*., 2021). This discrepancy may be attributed to isolate specificity or experimental conditions. Further research using molecular characterization and gene expression analysis could provide insights into the variability of *Trichoderma's* antagonistic effects. Overall, the results confirm that *Trichoderma* is a promising biocontrol agent capable of mitigating fungal infections and improving yam quality, consistent with global findings (Harman & Shoresh, 2020; Woo *et al*., 2021).

The amplification of the internal transcribed spacer (ITS) regions and their analysis provided crucial insights into the identity and evolutionary relationships of the fungal isolates. The agarose gel electrophoresis results demonstrated clear ITS bands at approximately 500 bp for the fungal isolates, indicating successful amplification. This is consistent with studies emphasizing the universality of ITS primers in fungal identification, as ITS regions are highly conserved and widely used as genetic markers for fungal taxonomy (White *et al*., 2020). The subsequent BLAST analysis revealed a 100% similarity of the ITS sequences to species within the *Trichoderma* genus, particularly *Trichoderma harzianum*. This result is corroborated by Harman *et al*. (2020), who highlighted *T. harzianum* as a dominant and ecologically significant species with a broad spectrum of biocontrol capabilities. Furthermore, the phylogenetic analysis, computed using the Jukes-Cantor model, confirmed the close evolutionary relationship between the isolates and *T. harzianum*. Similar findings have been reported by Woo *et al*. (2021) that utilized ITS-based phylogenetic analyses to differentiate fungal isolates at the species level. The chromatogram nucleotide sequence confirmed the accuracy and quality of the sequencing results. Distinct and evenly spaced peaks corresponding to the DNA bases (adenine, cytosine, guanine, and thymine) indicated high-quality sequencing data. Such high-resolution chromatograms are essential for downstream applications like phylogenetic analysis and molecular comparisons. The method aligns with reports by Salvatore *et al.* (2022), who emphasized the importance of chromatogram accuracy in detecting sequence variations in fungal DNA.

The identification of *Trichoderma harzianum* in this study aligns with previous research emphasizing its ubiquity in tropical and subtropical soils. Harman and Shoresh (2020) reported its prevalence in diverse ecosystems and its role as an efficient biocontrol agent due to its ability to produce antifungal enzymes and secondary metabolites. The ITS sequence analysis and phylogenetic placement also parallels the findings of Błaszczyk *et al*. (2020), that demonstrated the utility of molecular tools in accurately delineating fungal species. Comparatively, the chromatogram-based approach used here surpasses traditional methods in precision and reliability, as highlighted by Woo *et al*. (2021). The successful identification of *T. harzianum* in yam tubers and soil from South-South Nigeria suggests a potential for its use in integrated pest management strategies, particularly in mitigating yam rot caused by fungal pathogens. The molecular identification of *T. harzianum* provides a foundational step for further investigations into its functional roles in agricultural systems. Given its proven efficacy in suppressing fungal pathogens like *Aspergillus flavus* and *Penicillium expansum*, as demonstrated in this study, it holds significant promise for sustainable yam cultivation in Nigeria. Future research should focus on exploring the genetic mechanisms underlying its biocontrol activity and its adaptability to varying environmental conditions.

CONCLUSION

The study effectively demonstrated the antagonistic potential of *Trichoderma harzianum* against fungal pathogens isolated from yam tubers and soil in South-South Nigeria. Through morphological and molecular analysis, the fungal isolates were identified and characterized, revealing the significant inhibitory effects of *T. harzianum* on pathogens such as *Aspergillus flavus*, *Penicillium expansum*, and *Aspergillus niger*. Agarose gel electrophoresis confirmed the amplification of the ITS regions of the isolates, with sequences showing 100% similarity to *T. harzianum*. The evolutionary analysis further supported the relatedness of these isolates, highlighting the reliability of molecular tools in fungal identification. The chromatogram analysis provided high-quality nucleotide sequences, reinforcing the accuracy of the sequencing results. The observed inhibitory effects and molecular findings underscore the potential of *T. harzianum* as an eco-friendly solution to fungal diseases in yam cultivation, addressing both post-harvest losses and soil-borne infections.

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