Scientific Research Publishing

American Journal of Plant Sciences, 2026, 17(9), 956-969
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Molecular Identification of Organisms from Tomato (*Solanum lycopersicum*) Collected from Choba Market, Rivers State, Nigeria

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How to cite this paper: Akiti, F.O., Chuku, S., Jude, K., Uzoma, M., Bakpo, E. and Numbere, A. (2026) Molecular Identification of Organisms from Tomato (*Solanum lycopersicum*) Collected from Choba Market, Rivers State, Nigeria. *American Journal of Plant Sciences*, **17**, 956-969.
https://doi.org/10.4236/ajps.2026.179058

Received: June 10, 2026
Accepted: September 26, 2026
Published: September 29, 2026

Abstract

Tomatoes (*Solanum lycopersicum*) are highly nutritious but prone to microbial contamination due to their high moisture content. This study investigated fungal species associated with tomatoes sold in three markets in Choba (TO1 - TO3), evaluated potential health risks, and provided molecular data relevant to food safety. Tomato samples from three different markets (n = 3 × 10) were cultured on Potato Dextrose Agar (PDA) and examined using morphological, microscopic, and molecular techniques. Distinct colonies were characterized, and DNA was extracted for amplification of the Internal Transcribed Spacer (ITS) region using Polymerase Chain Reaction (PCR) followed by Sanger sequencing. Phylogenetic analysis was used to determine evolutionary relationships among isolates. Results indicated a high fungal load, with colony counts ranging from 50 to too numerous to count (TNTC). Morphological and microscopic observations revealed predominant fungi as *Aspergillus niger*, *Mucor spp.*, and yeast-like species. Molecular identification revealed that isolates were closely related to *Fusarium verticillioides*, *Geotrichum candidum*, and *Puccinia brachypodii*, with sequence similarities of 87.7%, 99.6%, and 88.8%, respectively. Phylogenetic tree analysis showed these identifications and highlighted potential spoilage and health risks associated with the isolates. The low percentages derived may be due to small sample size and limited sequencing. This study demonstrates that tomatoes from Port Harcourt markets may harbor diverse fungal species capable of reducing fruit quality and posing health risks to consumers. Next study will consider increasing the sample size, conducting the experiments in a more aseptic condition and conducting more sequencing.

Keywords

Bacteria, DNA Sequencing, Fungal Infection, Phylogenetic Tree, Spoilage, Tomatoes

1. Introduction

Tomato (*Solanum lycopersicum*), a member of the Solanaceae family, is one of the most widely cultivated and consumed vegetable crops globally and serves as a major component of human diets due to its high nutritional content [1]. In Nigeria, tomato remains a staple vegetable frequently consumed across various regions and socio-economic classes, often eaten raw in salads, cooked in stews, or processed into pastes and sauces [2]. Its popularity stems from its health benefits, which include being a rich source of antioxidants such as lycopene, vitamins A, C, and E, flavonoids, folic acid, potassium, and dietary fiber—all of which contribute to reducing the risk of chronic illnesses like cardiovascular diseases, cancer, and diabetes [3].

Despite its importance, tomato is highly perishable and susceptible to microbial spoilage, especially in tropical environments like Nigeria where high humidity and temperatures provide ideal conditions for microbial growth [4]. The short shelf-life of tomatoes is attributed to both physical injuries during harvest and post-harvest handling as well as microbial contamination from various sources including soil, irrigation water, human handling, storage equipment, and transport systems [5]. These sources often introduce a wide array of spoilage and pathogenic microorganisms onto the surface and internal tissues of tomatoes, potentially leading to foodborne illnesses when consumed raw or undercooked [6].

The global burden of foodborne illnesses remains a significant public health concern, and the World Health Organization (WHO) has consistently emphasized the importance of improving food safety, particularly in developing countries where regulatory frameworks are weak and hygienic standards are often poorly enforced [7]. In Nigeria, the microbial safety of fresh produce such as tomatoes is of growing concern due to the frequent detection of pathogenic organisms like *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Fusarium spp.*, and *Aspergillus spp.* on tomatoes sold in open markets [8]. These organisms can cause serious diseases ranging from gastrointestinal infections to more severe complications such as hemolytic uremic syndrome, especially in children, the elderly, and immunocompromised individuals [9].

Traditionally, microbial identification has been conducted using culture-dependent techniques involving the growth of organisms on selective and differential media followed by biochemical tests [10]. Although these methods are inexpensive and accessible, they are often limited in scope, time-consuming, and incapable of detecting viable but non-culturable (VBNC) organisms or distinguishing closely related microbial species [11]. Moreover, culture-based techniques often fail to provide sufficient resolution to characterize the genetic diversity and

phylogenetic relationships among microorganisms, which are essential for effective microbial surveillance and control [9].

To overcome these limitations, molecular techniques have been increasingly adopted in microbial studies due to their speed, sensitivity, specificity, and ability to detect a broader range of microbial taxa, including unculturable organisms [12]. These techniques rely on the analysis of nucleic acids (DNA or RNA) to identify and classify organisms based on their genetic signatures. Polymerase Chain Reaction (PCR), quantitative PCR (qPCR), and DNA sequencing of conserved regions such as the 16S rRNA gene for bacteria and Internal Transcribed Spacer (ITS) regions for fungi have revolutionized microbial taxonomy and diagnostics [13]. These tools allow for the accurate detection and identification of microbial communities at species and even strain levels, offering better insights into the microbial ecology of food systems.

The aim of this study is to molecularly identify the microorganisms associated with tomatoes sold in markets within Port Harcourt, Nigeria. The study focuses on identifying fungal species responsible for spoilage and contamination.

The objectives of the study include the following:

1) To carry out morphological identification of fungal species in spoilt tomatoes collected from the market;

2) To conduct a molecular identification of fungal species in spoilt tomatoes collected from the market;

3) To assess the safety risk of spoilage of tomatoes retrieved from the market.

2. Materials and Method

2.1. Study Design

This research adopted an experimental laboratory design. The study was conducted in two major phases: the morphological and microscopic characterization phase, and the molecular analysis phase. The laboratory investigations were carried out at the Microbiology Laboratory of the University of Port Harcourt, while molecular characterization was conducted at MyAfroDNA Laboratory, Port Harcourt. The design of this study was selected to ensure accurate identification of spoilage fungi through both traditional and molecular approaches.

2.2. Sample Collection and Preparation

All the tomatoes samples (n = 30) used were obtained in their fresh form at Choba. Each sample was placed in sterile polythene bags and transported to the Microbiology Laboratory within two hours of collection. In the laboratory, the tomato samples were first washed with sterile distilled water to remove dirt and debris. The surface of each tomato was then sterilized with 70% ethanol for two minutes and rinsed with sterile distilled water to eliminate transient surface microorganisms [14]. After sterilization, the samples were aseptically sliced using sterile scalpels and crushed with sterile mortar and pestle to obtain a homogenized pulp, which was later used for fungal isolation before carrying out further Laboratory

analysis following the example of Senadheera *et al.* [15]. Each tomato sample was appropriately labeled to ensure proper identification throughout the experiment. The isolates were designated as TO1, TO2, and TO3, representing samples obtained from the different markets in Choba.

2.3. Reagents and Materials

All reagents and materials used for this study were obtained from Sigma-Aldrich, USA, and BDH Chemicals, England. Materials used included sterile bowls, calibrated test tubes, Petri dishes, inoculating loops, Bunsen burner, test tube racks, spreader, weighing balance, volumetric flasks, and syringes. Reagents and chemicals included Potato Dextrose Agar (PDA), normal saline, ethanol (70%), distilled water, aluminium foil, cotton plugs, filter papers, and lactophenol cotton blue stain. A light microscope (Olympus CX23) was used for microscopic observation of fungal structures. Other essential equipment included an autoclave (TOMY SX-700, Japan) for sterilization and a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) for DNA quantification during molecular analysis.

2.4. Sterilization Procedures

All glassware, reagents, and materials were sterilized before use to prevent contamination. Petri dishes, conical flasks, and test tubes were wrapped in aluminium foil and sterilized using an autoclave at a temperature of 121˚C and a pressure of 15 psi for 15 minutes. The working surfaces of the laboratory benches were wiped thoroughly with 70% ethanol before and after use to maintain aseptic conditions. All inoculating loops and needles were flame-sterilized using a Bunsen burner before and after each inoculation. The culture medium was sterilized separately in the autoclave and dispensed aseptically into Petri dishes within a laminar flow cabinet to avoid contamination [16].

2.5. Serial Dilution

The fresh tomatoes were allowed to stay for a period of 3 - 5 days, when spoilage was noticed. A tenfold serial dilution technique was used to reduce the microbial population for effective isolation of distinct fungal colonies. One gram of homogenized tomato pulp was transferred into 9 mL of sterile normal saline to obtain a 10^{-1} dilution. From this suspension, 1 mL was transferred into another 9 mL of sterile saline to produce a 10^{-2} dilution, and the process was repeated serially up to 10^{-4}. Aliquots of 0.1 mL from 10^{-2}, 10^{-3}, and 10^{-4} dilutions were then plated on freshly prepared Potato Dextrose Agar plates using the spread plate method to ensure uniform distribution of spores and mycelia fragments, while streaking method was used alongside the spread plate to isolate the microbial type in the samples as described by Murgia *et al.* [17].

2.6. Media Preparation

Potato Dextrose Agar (PDA) was used for the cultivation and isolation of fungi.

A total of 39 grams of PDA powder was weighed and dissolved in 1 liter of distilled water in a conical flask. The mixture was stirred continuously and gently heated until it completely dissolved. The prepared medium was then sterilized in an autoclave at 121˚C for 15 minutes. After sterilization, the medium was allowed to cool to approximately 45˚C before being poured aseptically into sterile Petri dishes (about 20 mL per plate) and left to solidify under sterile conditions [17].

2.7. Culturing and Isolation of Fungi

Each plate was inoculated with 0.1 mL of the appropriate dilution and spread evenly using a sterile glass spreader. The plates were then incubated in an inverted position at 28˚C for five to seven days. Fungal colonies began to appear after 48 hours and were observed daily for changes in morphology, colour, texture, and sporulation. Distinct colonies were picked and sub-cultured on freshly prepared PDA plates to obtain pure cultures. These pure isolates were used for both morphological identification and subsequent molecular studies [17].

2.8. Sub-Culturing and Preservation of Pure Cultures

Sub-culturing was done to obtain pure fungal cultures. Individual colonies with distinct characteristics were picked using a sterile inoculating loop and streaked onto fresh PDA plates. The plates were incubated at 28˚C for 3 - 5 days to allow sufficient growth. Pure cultures were maintained on PDA slants in universal bottles, covered with cotton plugs, and stored at 4˚C for preservation until further analysis [18].

2.9. Morphological and Microscopic Identification

The fungal isolates were characterized based on both macroscopic and microscopic features. Macroscopic examination involved observation of colony colour, surface texture, margin shape, and spore formation on PDA plates. Microscopic examination was conducted using the lactophenol cotton blue staining technique. A small portion of the fungal mycelium was placed on a clean glass slide, stained with a drop of lactophenol cotton blue, and covered with a cover slip. The prepared slides were observed under the microscope at ×40 magnification. The arrangement of hyphae, shape of conidia, and other diagnostic features were recorded and compared with standard descriptions in fungal identification manuals.

2.10. Molecular Analysis

The molecular characterization of the isolates involved DNA extraction, PCR amplification, gel electrophoresis, sequencing, and phylogenetic analysis. DNA was extracted using the ZymoBIOMICS DNA Microprep Kit following the manufacturer's protocol. Fungal cells were lysed mechanically using ZR Bashing Beads, and DNA was isolated through a series of lysis, washing, and elution steps. The extracted DNA was quantified using a NanoDrop spectrophotometer, and purity was determined by measuring absorbance at 260 nm and 280 nm. PCR amplifica-

tion of the Internal Transcribed Spacer (ITS) region was performed using universal primers ITS1 and ITS2. The reaction mixture contained template DNA, primers, and FirePol Master Mix in a total volume of 20 µL. The amplification conditions consisted of initial denaturation at 95˚C for 5 minutes, followed by 35 cycles of denaturation at 95˚C for 30 seconds, annealing at 55˚C for 30 seconds, and extension at 72˚C for one minute, with a final extension at 72˚C for 5 minutes. The amplified DNA fragments were separated on 2% agarose gel prepared in 1 × TBE buffer containing Safe Green stain, and the results were visualized under a UV transilluminator.

Purified PCR products were sent for sequencing using the BigDye™ Direct Cycle Sequencing Kit. The resulting sequences were analyzed using ChromasLite and BioEdit software. Species identification was achieved through sequence comparison with existing data in the NCBI BLAST database. Multiple sequence alignment was performed using ClustalW, while phylogenetic relationships were established using MEGA11 software through the Neighbor-Joining method with 500 bootstrap replications.

2.11. Data Analysis

All data obtained from the morphological, microscopic, and molecular analyses were analyzed using descriptive statistics. DNA concentration and purity values were expressed as mean ± standard deviation, while fungal identity was determined based on percentage similarity from BLAST results (**Appendix**). Comparative analysis was also made between the morphological characteristics observed in this study and those reported in previous research to establish species confirmation. The data were presented in the form of tables, figures, and phylogenetic trees to illustrate the relationships between the isolates obtained.

3. Results

3.1. Culturing Observations

The culturing of tomato samples on Potato Dextrose Agar (PDA) revealed significant fungal growth after 72 hours of incubation at 28˚C. Distinct colonies were observed across different dilution plates (10^2, 10^3, and 10^4). The plate count results showed that colonies were *Too Numerous To Count* (*TNTC*) on the 10^2 dilution plate, while the 10^3 dilution recorded 75 and 85 colonies for the first and second replicates respectively (Table 1). The 10^4 dilution yielded fewer colonies, with counts of 50 and 80 for the replicates. This means the higher the dilutions, the fewer the colony counts. This result indicates that the samples harbored a high fungal load, especially in the lower dilutions, reflecting substantial microbial contamination in the tomato samples obtained from Choba Markets.

The macroscopic observation of the fungal colonies revealed different cultural characteristics that facilitated preliminary identification. Isolate TO1 developed black colonies with a cream reverse, a cream-colored margin, and dense black spores without surface cracks. Isolate TO2 showed a white colony with a cream reverse and dry

powdery surface. Isolate TO3 produced white colonies with cream margins, non-cracked surfaces, and a yeast-like texture (Figure 1).

Table 1. Plate count results on PDA medium.

Dilution	Replicate 1	Replicate 2
10^2	TNTC	TNTC
10^3	75	85
10^4	50	80

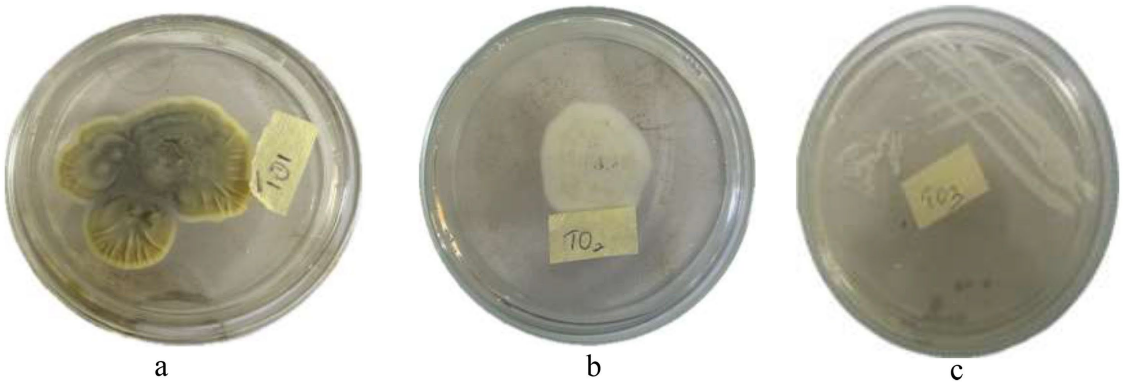

Figure 1. Colony Morphology of Fungal Isolates on PDA after 72 Hours of Incubation. (a) TO1: Presence of black spores in septate hyphae; (b) TO2: Presence of oval-shaped spores with powdery surface) TO3: showed oval yeast cells that stained purple.

3.2. Microscopy

Microscopic examination using lactophenol cotton blue staining revealed distinct structural features that revealed a preliminary identification of the fungal isolates. Isolate TO1 showed septate hyphae bearing conidiophores and black spores characteristic of *Aspergillus niger*. Isolate TO2 exhibited oval-shaped conidia arranged singly, corresponding to *Mucor* morphology. Isolate TO3 showed oval yeast cells that stained purple, suggesting *Geotrichum* or *Candida*-like yeast (Figure 2). These preliminary microscopic findings complemented the macroscopic characteristics and provided the basis for subsequent molecular identification.

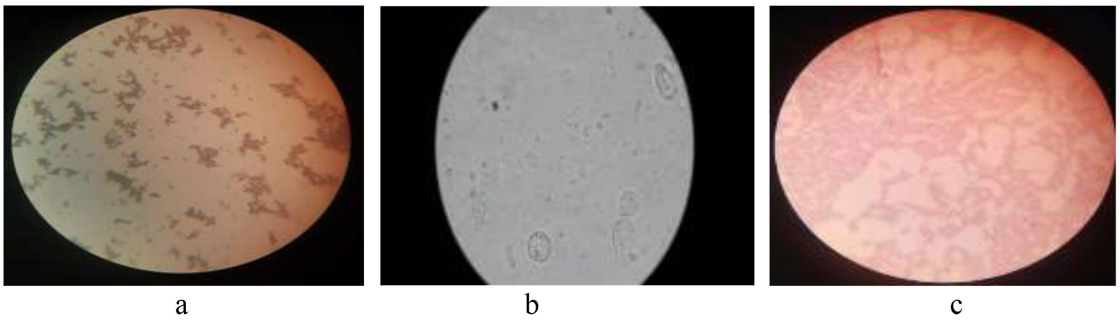

Figure 2. Microscopic View of Fungal Isolates using Lactophenol Cotton Blue Stain. (a) TO1 showing septate hyphae with black spores; (b) TO2 showing oval conidia; (c) TO3 showing oval yeast cells.

3.3. DNA Isolation

High-quality genomic DNA was successfully extracted from all three fungal isolates (TO1, TO2, and TO3) (Table 2). The DNA samples showed distinct and sharp bands when subjected to agarose gel electrophoresis, confirming their integrity and absence of degradation. Spectrophotometric quantification revealed DNA concentrations of 28 ng/μL, 42 ng/μL, and 59 ng/μL for TO1, TO2, and TO3, respectively. The purity ratios (A260/A280) ranged between 1.84 and 1.88, indicating minimal protein contamination and suitability for downstream PCR analysis.

Table 2. DNA concentration and purity levels of fungal isolates.

S/N	Code	PCR Target	DNA Concentration (ng/μL)	Duplicate	Mean	Purity (A260/A280)	Duplicate	Means
1	TO1	ITS	28	30	29	1.86	1.89	1.88
2	TO2	ITS	42	44	43	1.84	1.82	1.83
3	TO3	ITS	59	60	59.5	1.88	1.85	1.87

These values suggest that the DNA extraction process was efficient across all isolates. The relatively higher concentration observed in TO3 indicates a more robust cellular structure or a denser mycelial mass, while the consistent purity across samples confirms that the DNA was of sufficient quality for PCR and sequencing.

3.4. Fungal DNA Successfully Detected via PCR

PCR amplification of the ITS region was successful across all three isolates, producing distinct bands of approximately 600 bp in size (Figure 3). The sharp and uniform bands observed in the agarose gel indicated strong and specific amplification of the fungal ITS gene. The results validated the effectiveness of the primers used and confirmed that the extracted DNA was suitable for molecular analysis.

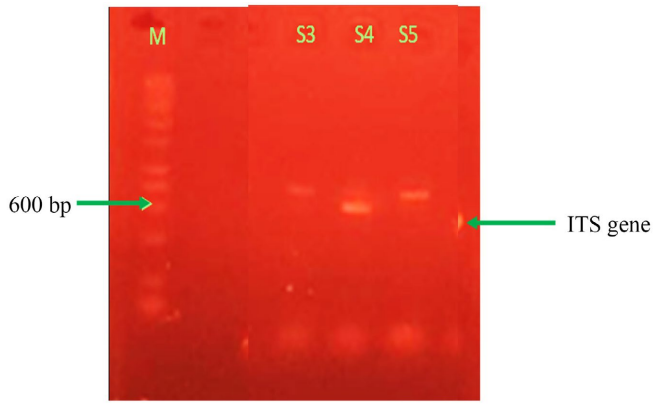

Figure 3. Lane M: 100 bp DNA Ladder; S3, S4, S5: Fungal Isolates TO1, TO2, and TO3 showing distinct ITS amplification bands (~600 bp). This indicates the likely presence of fungal DNA in all isolates, establishing the success of the DNA extraction and amplification stages.

3.5. Fungal Species Identified via Sanger Sequencing

The amplified ITS gene fragments were sequenced using the Sanger sequencing method. The resulting chromatograms exhibited clear and well-defined peaks, indicating high-quality sequences. Subsequent BLAST analysis against the NCBI GenBank database identified the isolates with varying degrees of similarity to known fungal species (Table 3).

Table 3. BLAST results summary for fungal isolates.

S/N	Code	Name of Organism	Closest Gen Bank Match	% Identity
1	TO1	*Fusarium verticillioides*	*Fusarium verticillioides* isolate 42FEB	87.7
2	TO2	*Geotrichum candidum*	*Geotrichum candidum* isolate PD10019	99.6
3	TO3	*Puccinia brachypodii*	*Puccinia brachypodii* isolate OTU0822	88.8

The high similarity (99.6%) of TO2 with *Geotrichum candidum* indicates strong identity confirmation, while TO1 and TO3 showed lower identity percentages, suggesting potential sequence variations or mixed species presence.

3.6. Blast and Phylogenetic Tree Construction

Phylogenetic analysis was performed to establish the evolutionary relationships among the fungal isolates (Figure 4). The sequence alignment of isolate TO1 showed close similarity with members of the genus *Fusarium*, with the highest identity (87.7%) observed with *Fusarium verticillioides*. The constructed phylogenetic tree clustered TO1 within the *Fusarium verticillioides*, *F. oxysporum*, *F. graminearum* lineage, confirming its close association with *Fusarium* species.

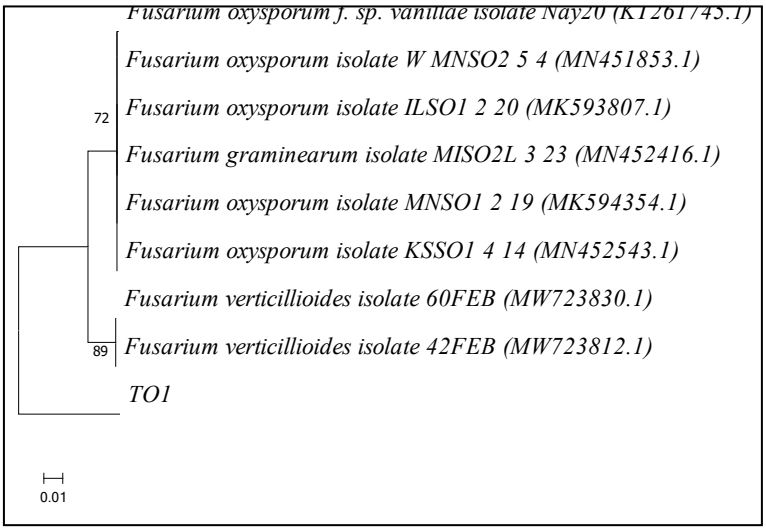

Figure 4. Neighbor-Joining phylogenetic tree based on ITS gene sequences showing the relationship of isolate TO1 with reference sequences. Tree was constructed using the Jukes Cantor model with 500 bootstrap replicates, and bootstrap values are shown at the nodes. The analysis clusters TO1 within the *Fusarium verticillioide*, *oxysporum*, *graminearum* lineage, confirming its close evolutionary relationship with *Fusarium* species.

Isolate TO2 showed 99.6% similarity to *Geotrichum candidum* and clustered tightly with other *Geotrichum* species in the phylogenetic tree (Figure 5). This high similarity and complete query coverage confirm the identity of TO2 as *Geotrichum candidum*, a common spoilage yeast known to affect soft fruits and vegetables.

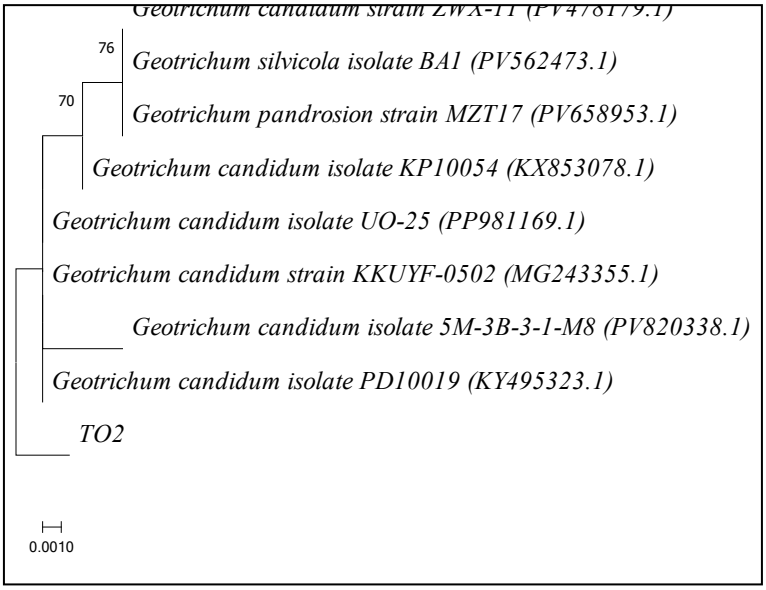

Figure 5. Neighbor-Joining phylogenetic tree based on ITS gene sequences showing the relationship of isolate TO2 with reference sequences. The tree was constructed using the Jukes-Cantor model with 500 bootstrap replicates, and bootstrap values are shown at the nodes. The analysis clusters TO2 within the *Geotrichum verticillioide*, *pandrossion*, *silvicola* lineage, confirming its close evolutionary relationship with *Geotrichum* species.

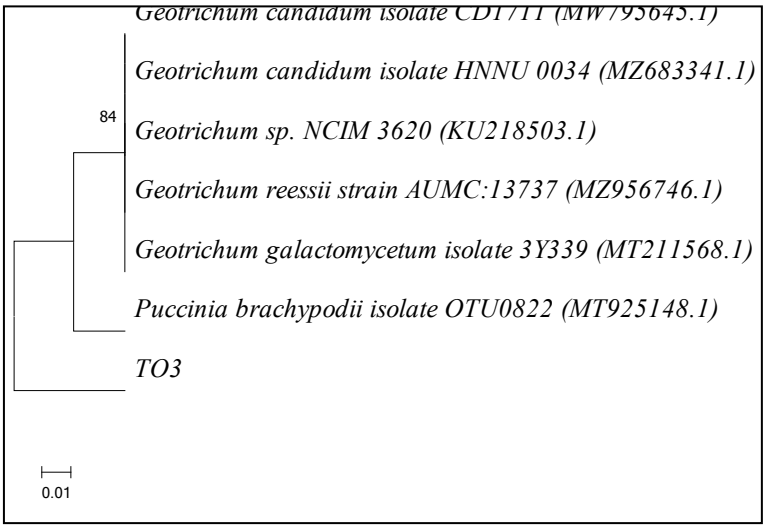

Figure 6. Neighbor-Joining phylogenetic tree based on ITS gene sequences showing the relationship of isolate TO3 with reference sequences. The tree was constructed using the Jukes-Cantor model with 500 bootstrap replicates, and bootstrap values are shown at the nodes. The analysis clusters TO3 within diverse taxa, including *Geotrichum* spp. and *Puccinia brachypodii*.

The sequence for TO3 showed 88.8% similarity to *Puccinia brachypodii* and also aligned with *Geotrichum* species, indicating conserved ITS regions and possible genetic relatedness across genera (**Figure 6**). The phylogenetic tree positioned TO3 in a diverse clade containing both *Puccinia* and *Geotrichum* sequences, suggesting that it shares conserved sequence motifs but could not be resolved precisely to species level.

4. Discussion

The culturing observations revealed significant fungal growth, which agrees with the findings of Jaboro *et al.*, who reported high fungal contamination in Nigeria [19].

Macroscopic observations revealed distinct colony morphologies, which were further corroborated by microscopic examination. These morphological characteristics align with those reported in similar studies on tomato spoilage fungi [20]. This observation is partly supported by the DNA analyses, which shows close relationship to the fungal species.

Furthermore, sequencing results show some similarities to the fungal isolates of TO1, TO2 and TO3 as corroborated by other researchers [20] [21]. The surprising outcome is the identification of *Pucinia brachyopodii* in TO3, which may be a result of contamination that would require more investigation in future studies. Although, *Geotrichum candidum* has high percentage identity, the other isolates were below 90%.

Phylogenetic analysis based on ITS gene sequences confirmed the taxonomic placements of the fungal isolates. TO1 clustered within the *Fusarium* lineage, TO2 grouped with *Geotrichum* species, and TO3 was positioned among diverse taxa, including *Puccinia* and *Geotrichum*. The moderate identity percentages for TO1 and TO3 suggest possible sequence variations or mixed species presence, which is consistent with challenges in fungal identification based solely on the ITS region [21].

The findings of this study are consistent with recent research on tomato spoilage fungi in Nigeria. Kabiru and Yusuf identified *Rhizopus oryzae*, *Rhizopus microsporus*, *Aspergillus flavus*, and *Malassezia furfur* as prevalent fungi in tomatoes from Biu markets [20]. Similarly, Amaechi *et al.* reported *Aspergillus niger* and other fungi associated with tomato spoilage in Port Harcourt markets [19].

Further studies will carry out more investigation on the morphological and molecular aspects of the isolates. Similarly, more sequencing will be done to increase the percentage of species identification.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

>TO1_ITS1

AAAAGTCSTAACAAGRWTCCRAAGSWGAGGAG-
CAAAYGGATCMTTAMSRAK-
TGAAMCCCRGGGGGAAAYTTGCGCASMWAACAATAGTTGTATAGKCG
AAACAAAAATAATCCAAMYTTTTAAMAATGGRWCYCTT-
GGGTCCCCTAAACTCTAAGACTA-
TATGGAACTTCTGAGTAACASGATAAAWAAATCAAAACTTTCAACAAC
GGA

>TO2

ATAATCAAAACTTTTAACAATGGATCTCTTGTTTCTCG-
TATCGATGAAAAACGCAGCGAAAC-
GCGATATTTCTTGTGAATTGCAGAAGTGAATCATCAGTTTTTGAACGC
ACATTGCACTTTGGGGTATCCCCCAAAGTATACTTGTTTGAGCGTT-
GTTTCTCTCTT-
GGAATTGCATTGCTTTTCTAAAATTTCGAATCAAATTCGTTTGAAAAAC
AACACTATTCAACCTCA

>TO3

MTCGAWGACAAMGCAGARAACAAAAATGTTTCCTGAAGAATTGAA-
MATAGTTGG-
GAAAYTTACACAGCAAACAATAATTTTATAGTCAAAACAAAAATAATC
AAAAYTTTTAAMAAYGAATCTCTTGGTCCCCGCACCGATGAAGAAC-
GCATGGRYATTCTGAG-
TTTCTGCATAAATAAATCAAAACTTTCAACAACGGA