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Comparative study on identification and pathogenicity of fungal pathogens associated with post-harvest rot of tomatoes (*solanum lycopersicum* l.) in Umuahia and Okigwe

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Abstract

Tomato fruits sold in the market and at home present symptoms during storage, but the disease causal agents must be better documented. This study aimed to identify the fungal pathogens associated with tomato rot bought in markets at Umuahia and Okigwe and to evaluate its pathogenicity and disease prevalence. A total of 24 and 16 fungal isolates were recorded, respectively, and were microscopically identified and morphologically to specific fungal isolates. The identified isolates were Alternaria solani, *Althelia rolfsii*, *Colletotrichium phlomoides*, *Phytophthora nicotinae*, *Sclerotinia sclerotiorum*, and *Sclerotium rolfsii*. The percentage frequency of isolation of samples from Umuahia ranged from 6.3% - 31%, respectively. *Alternaria solani* had the highest frequency of 31%, with the lowest percentage of 6.3% recorded in Sclerotium rolfsii from samples obtained from Umuahia. The same trend was also recorded on isolated samples from okigwe with a percentage frequency of isolation of 29% for Alternaria solani and 8.3% for *Sclerotium rolfsii*. The high percentage frequency of isolation of Alternaria solani indicates a high chance of these tomato fruits being contaminated with mycotoxins since Alternaria solani is a significant mycotoxigenic fungal genus with notable toxicity. The prevalence of disease incidence (PDI) was conducted to ascertain which locations had the highest rate of fungal rot, and there was a higher PDI of 50% in Umuahia against 33% recorded in Okigwe. The highest disease prevalence recorded in Umuahia could result from poor sanitation, poor storage, overcrowding, and unhygienic practices by fruit handlers in this location.

Keywords: Pathogenicity, Fungal rot, Disease prevalence, Percentage incidence, Rot rating

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INTRODUCTION

Tomatoes are an important crop due to their sensory properties and abundant bioactive compounds. Literature reviews show that tomatoes contain essential nutrients that benefit human health. These include vitamins, minerals and trace minerals that are easily absorbed and help the body strengthen immunity and prevent infections (Ali et al., 2021). However, their susceptibility to contamination to rot by fungus and other pests has increased over the years (Furlong and Rodrigues, 2022). Globally, tomatoes are significant vegetables valued for their nutritional benefits and culinary versatility (Bapary et al., 2024). It is extensively consumed in Nigeria and ranked second most valuable vegetable fruit crop (Yusuf et al., 2020). On average, it is consumed daily at a rate of approximately 18%. The crop is highly perishable, resulting in losses of up to 50% from cultivation to consumption. In 2021, FAO reported that Africa imported around 520,000 tons of tomato puree, representing 15% of the global volume worth US$500 million. However, the susceptibility of tomatoes to postharvest rot and handling processes poses a

significant challenge to maintaining their quality and extending their shelf life (Spricigo et al. 2021). This difficulty is particularly pronounced in underdeveloped nations with limited storage and transportation facilities. Postharvest fungal decay can result in significant financial losses and diminish the accessibility of this crucial food resource (Enyiukwu et al., 2014). A study by Gwa and Lum (2023) showed that postharvest losses in tomatoes vary across locations. These damages disrupt the fruit's physicochemical properties, leading to postharvest losses, which account for 25–42 % of losses globally (Qasim et al., 2022; Roy et al., 2024). Nigeria's tropical climate, with its elevated temperatures and humidity, fosters a favourable setting for the proliferation of fungi and the rapid decay of harvested tomatoes. Due to their significant influence on nutrition, functionality, and economy, multiple cultivars have been genetically modified to thrive in semi-tropical and temperate regions. These cultivars exhibit great production and tolerance to the challenges posed by the field environment, distribution, and processing (Siddique et al., 2015).

Fungal contamination of tomatoes, including species such as Penicillium, Aspergillus, Fusarium, and Alternaria, has been identified in screenings throughout several locations, resulting in frequent fruit loss both in the field and during processing; within this group are species that produce toxins, although investigating these poisons in tomatoes has been limited (Rodrigues & Furlong, 2022). Among them are toxigenic species, but their mycotoxins have been little studied in tomatoes. Alternaria sp. synthesizes approximately seventy secondary metabolites, most of which have been identified as having the ability to function as mycotoxins (Sedighi & Mohammadi, 2024). Moreso are *Collectrotrichumtruncatum*, *Phytophthora infestans*, *Pythium aphanidermatum*, *Dipodascus geotrichum*, *Fusarium curvularia spicifera*, *Cladosporium sp.*, *Penicillum chrysogenum*, *Mucor mucedo*, *Botrytis cinerea*, *etc.*, causing different diseases with distinct symptoms (Tolupe & Odebode, 2021). Alternaria, Aspergillus, Fusarium, Mucor species, Penicillium, Rhizopus, and Trichoderma are pathogenic fungi linked to some types of agricultural deterioration. Fungi that cause tomatoes to deteriorate have not been the subject of many investigations in Nigeria. To determine whether fungal diseases cause tomato fruit to rot after harvest, this study was conducted in Nigeria to isolate, identify and check the pathogenicity of these fungal pathogens associated with fungal rot in tomatoes.

Table1. Fungi and mycotoxins in tomatoes and their products.

Product	Fungus	Mycotoxin	Occurrence (µg.kg− 1)	Reference
Ketchup	*A. alternata*	ALT	21.3	Pavon et al., 2012
Dry tomato	*A. alternata*	ALT	280.0	Pavon et al., 2012
Dry tomato	*A. alternata*	AOH	376.0	Pavon et al., 2012
Dry tomato	*A. alternata*	AME	72.0	Pavon et al., 2012
Tomato sauce	*A. alternata*	ALT	20.0	Pavon et al., 2012
Fresh tomato	*P. expansum*	PAT	n.d	Cunha et al., 2014

AOH - Alternariol, AME- Alternariol Monomethyl Ether, ALT- Altenuene, PAT – Patulin. n.d – Not detected.

The European Food Safety Authority as reported by Arcella et al. (2016) states that the primary toxins generated by Alternaria sp. include alternariol (AOH), alternariol monomethyl ether (AME), tentoxine (TeA), and tenuazonic acid (TEN). The primary mycotoxin detected in both dried and fresh tomatoes is TeA. *A. alternata* can infiltrate the fruit surface by exploiting micro-cracks or wounds, swiftly establishing itself in the tissue and resulting in substantial harm. Additionally, research demonstrates that this fungus can generate mycotoxins, which could endanger consumers' health. Tomatoes' vulnerability to fungal infection is affected by multiple factors. This study aimed to identify the fungal pathogens associated with tomato rot bought in markets at Umuahia and Okigwe, in the eastern part of Nigeria, and to evaluate its pathogenicity and disease prevalence.

MATERIALS AND METHODS

Collection of Samples

Eight (8) tomato fruits were collected from different locations (Umuahia and Okigwe). They were wrapped in a sterile zip-lock polyethylene bag and sent to the Mycology Laboratory of the Department of Plant Science and Biotechnology, Abia State University.

Sterilization of Glass wares

All the glassware, including beakers, conical flasks, test tubes, measuring cylinders, a spatula, and a test tube rack, was sterilized in a hot air oven for one hour at 121 degrees centigrade and kept in a laminar airflow chamber.

Sample Preparation

The tomato fruits were washed with sterile water and kept at room temperature (22- 25 degrees Centigrade) for five days to allow fungal rot to develop.

Media Preparation

According to the Manufacturer's instructions, 10.53g of Potato dextrose agar (PDA) was dissolved in 270 ml of distilled water, autoclaved at 121 degrees centigrade for 15 minutes, and allowed to cool. It was supplemented

with 0.1 mg of chloramphenicol antibiotics and dispensed into eighteen (18) 9cm diameter Petri dishes, with two plates serving as controls.

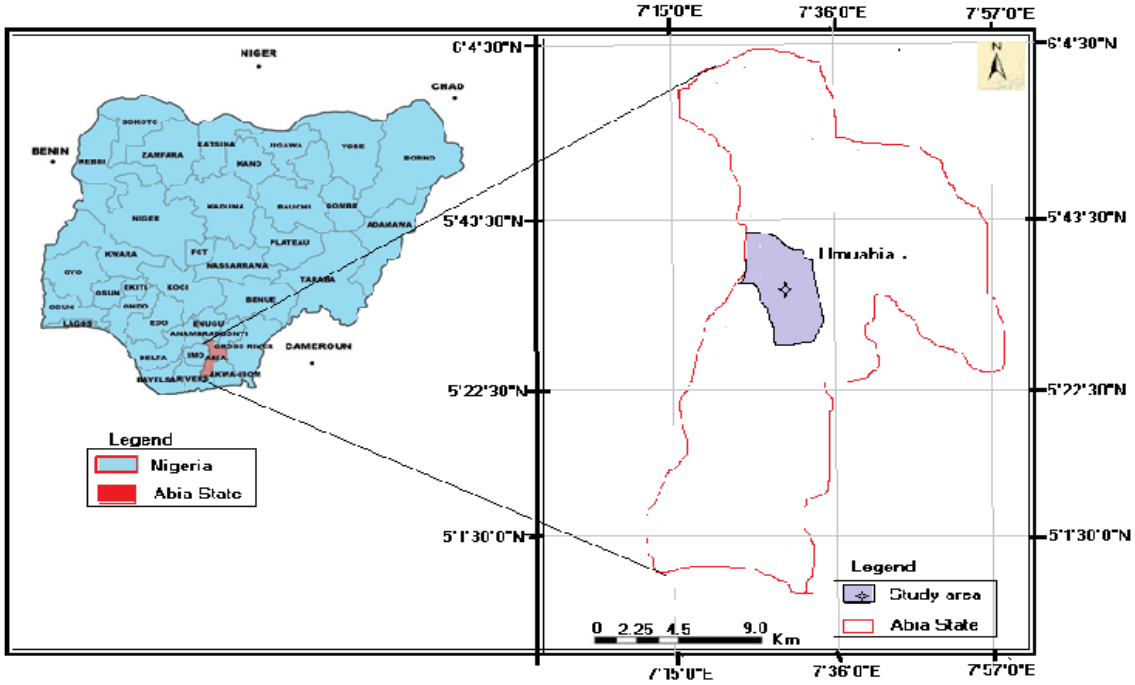

Figure1. Map of Nigeria showing the study location; Umuahia

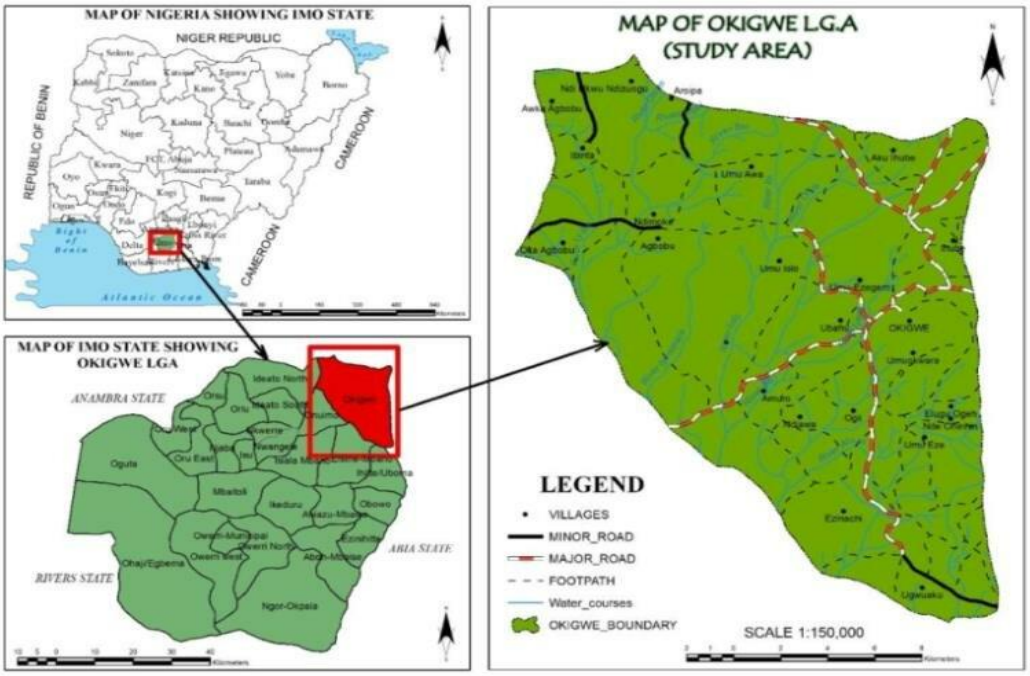

Figure 2. Map of Nigeria showing the study location; Okigwe

Peptone Water Preparation

According to the Manufacturer's instruction, 1.4g of peptone water granules were dissolved in 100 ml of distilled water, autoclaved at 121 degrees Centigrade for 15 minutes, and dispensed into ten (10) beakers (eight for Samples, two for controls). This served as the sample diluent.

Sample Inoculation

A sterile swab stick was used in a randomized control format to swab the tomato fruits with visible fungal rot. Each swab stick was soaked in 10ml aliquots of sterile buffered peptone water for about 120 minutes (2 hours) to dislodge the cells into suspension at ambient temperature. The swab sticks were removed, and the resulting peptone water solution was the test sample. The peptone water was serially diluted (10–¹ to 10–6), and each suspension's

10^{-3} dilution (0.1ml) was inoculated on the already prepared molten potato dextrose agar using the pour plate method. The plates were incubated at 28 degrees Celsius for five days.

Sub-Culturing and Preservation of Isolates

Based on their colonial characteristics, the fungal representative colonies were picked and sub-cultured on potato dextrose agar supplemented with 0.1ml of chloramphenicol antibiotics aseptically using the streaking technique and incubated at 28 degrees Celsius. The isolates were preserved in slants of potato dextrose agar in a bottle, which served as a stock for assessing their morphological and biochemical characteristics. The test identification of isolates at the genus level based on cultural morphological and biochemical characteristics was carried out according to Cheesbrough (2000).

Identification of Fungi

After five days, the growth of fungi on potato dextrose agar was examined critically using prepared microscopic slides. The prepared specimens were mounted on KOH Preparation and observed with the microscope's X40 objective lenses.

Pathogenicity Test

The various isolates were subjected to a pathogenicity test to establish Koch's postulates. Healthy tomato fruits were purchased from the market. The fruits were washed with clean tap water to remove any soil debris. The fruits were surface-sterilized in 75% ethanol for about three minutes, rinsed with distilled water, and then air-dried. Each of the isolates was subjected to two methods of inoculation. A sterile cork borer (5mm diameter) was used to wound freshly procured tomato fruits. Mycelial discs of the equivalent diameter obtained from the edge of actively growing pure cultures were placed on the wound concerning the number of isolated pathogens. Two wounded fruits were inoculated with sterile discs and served as controls (Nizamani *et al*., 2021). The fruits were kept at room temperature of 24 degrees Celsius for five days for possible rot development. The isolates were re-isolated from the new host and compared morphologically to the original isolates. An evaluation was done after five days by cutting the fruits longitudinally and rating the post-harvest fungi rot on a 0-4 rating scale as follows.

0= no visible rot
Rot 1 = 1-25%
Rot 2 = 25-50%
Rot 3 = 50-75%
Rot 4 > 75%

RESULTS

Identification and characterization isolates of fungal

A total of five fungal isolates were recorded, namely *Alternaria solani, Althelia rolfsii, Colletotrichium phlomoides, Phytophthora nicotinae, Sclerotinia sclerotiorum, and Sclerotium rolfsii.* Various parameters, which include the percentage frequency of isolation pathogenicity test, rot ratings, percentage rot range, pathogenicity prevalence, and prevalence of disease incidence, were recorded to ascertain the rot and deterioration effect of the five fungi. Table 2 shows the identification and characterization of the various fungus. The morphology of *Alternaria solani* showed a straight, flexuous brown mycelium on the PDA. Viewing it under the microscope, oblong conidia were observed. *Althelia rolfsii* showed smooth, white fruiting bodies with ribbon-like hyphae and clamp connections. *Colletotrichium phlomoides* showed white to grey pasty colonies with rod-shaped hyphae and single budding cells. The *Phytophthora nicotinae* showed a dense cottony mycelium that is slightly petaloid in a pattern. *Sclerotinia rolfsii* showed small tufts of white mycelium that covered the plate in a fan-like pattern. A microscopic view of it showed hyaline thin cell walls. There were sparse, fluffy, creamy white brown to brown colonies observed on *Sclerotinia sclerotiorum* with spherical to oval hyaline hyphae.

Table 2. Identification and characterization isolates of fungal

Isolates	Morphology	Microscopy	Identified Fungus
A	Straight, flexuous; Brown mycelium on PDA	oblong conidia	*Alternaria solani*
B	Smooth and white fruiting bodies	ribbon-like hyphae with clamp connection	*Athelia rolfsii*
C	White to grey pasty colonies	rod-shaped hyphae with single budding cells	*Colletotrichium phomides*
D	Dense cottony mycelium with slightly petaloid pattern	Papillate ovoid sporangia with oospores	*Phytophthora nicotanae*
E	Small tuffs of white mycelium that covers the plate in fan pattern	Hyaline thin cell walls with spare cross walls	*Sclerotium rolfsii*
F	Sparse fluffy creamy white to brown colonies	Spherical to oval Hyaline hypahe	*Sclerotinia sclerotiorum*

Percentage frequency of isolation for samples from Umuahia

The result in Table 3 shows the percentage frequency of isolation of samples from Umuahia, which ranged from 6.3% to 31%, respectively. *Alternaria solani* had the highest frequency of 31%, with the least percentage of 6.3% recorded in *Sclerotium rolfsii* from samples obtained at Umuahia.

Table 3. Percentage frequency of isolation for samples from Umuahia

Fungal Isolates	Specific fungal isolates	Total number of fungal isolates	% Isolation Frequency
Alternaria solani	5	16	31%
Athelia rolfsii	3	16	19%
Colletotrichium phomoides	3	16	19%
Phytophthora nicotianae	2	16	13%
Sclerotinia sclerotiorum	2	16	13%
Sclerotium rolfsii	1	16	6.3%

$$\% \text{ Isolation} = \frac{\text{no. of specific fungal isolate}}{\text{Total no. of isolates}} \times \frac{100}{1}$$

Percentage values were rounded off to the nearest whole numbers.

Percentage frequency of isolation for samples from Okigwe

Table 4 shows the percentage frequency of isolation of Okigwe samples ranging from 8.3% - 29%. The highest percentage frequency of isolation of 29% was recorded on *Alternaria solani*, with the lowest percentage of 8.3% recorded on *Sclerotium rolfsii*

Table 4. Percentage frequency of isolation for samples from Okigwe

Fungal Isolates	**Specific fungal isolates**	**Total number of fungal isolates**	**% Isolation Frequency**
Alternaria solani	7	24	29%
Athelia rolfsii	5	24	21%
Colletotrichium phomoides	4	24	17%
Phytophthora nicotianae	3	24	13%
Sclerotinia sclerotiorum	3	24	13%
Sclerotium rolfsii	2	24	8.3%

$$\% \text{ Isolation} = \frac{\text{no. of specific fungal isolate}}{\text{Total no. of isolates fungal isolates}} \times \frac{100}{1}$$

Percentage values were rounded off to the nearest whole numbers

Pathogenicity test, rot ratings and percentage rot range

Results from Table 5 show the pathogenicity test, rot ratings, and percentage rot range of the various fungi. The rot ratings ranged from 1 – 4. The highest rot rating of 4 was recorded on *Alternaria solani* with a percentage rot range of >75%, followed by *Althelia rolfsii* with a rot rating of 3 and a percentage rot range of 50 -75%. The least rot rating of 1 and percentage rot range of 1-25% was recorded on *Sclerotium rolfsii*

Table 5. Pathogenicity test, rot ratings and percentage rot range

Isolates	Rot Ratings	% Range
Alternaria solani	4	> 75%
Athelia rolfsii	3	50-75%
Colletotrichium phomoides	2	25- 50%
Phytophthora nicotinae	2	25-50%
Sclerotinia sclerotiorum	2	25-50%
Sclerotium rolfsii	1	1-25%.

LEGEND:
Rot 1= 1-25%
Rot 2= 25-50%
Rot 3= 50-75%
Rot 4> 75%

Pathogenicity prevalence

Table 6 shows the pathogenicity prevalence of the isolated fungi on eight samples of the tomato. A pathogenicity prevalence of 13% - 50% was recorded, with the highest prevalence of 50% on *Alternaria solani*, with a rot rating of 4. A pathogenicity prevalence of 25% each was recorded on *Colletotrichum phlomoides, Phytophthora nicotinae, and Sclerotinia sclerotiorum.* The lowest pathogenicity prevalence, 13%, was recorded in *Sclerotium rolfsii*.

Table 6. Pathogenicity prevalence

Isolates	Rot Ratings	Sample Size	Pathogenicity Prevalence (%)
Alternaria solani	4	8	50%
Athelia rolfsii	3	8	38%
Colletotrichium phomoides	2	8	25%
Phytophthora nicotianae	2	8	25%
Sclerotinia sclerotiorum	2	8	25%
Sclerotium rolfsii	1	8	13%

$$\text{Pathogenicity prevalence} = \frac{\text{Rot ratings}}{\text{Sample size}} \times \frac{100}{1}$$

Percentage values were rounded off to the nearest whole numbers.

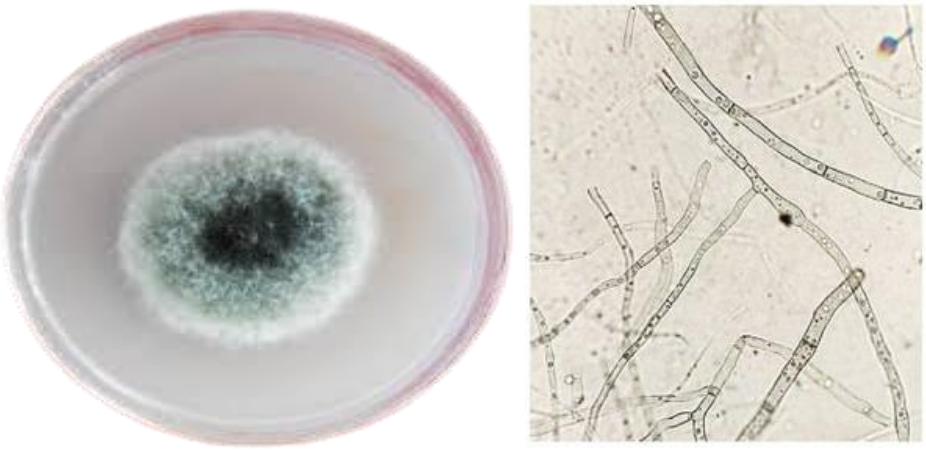

Figure 3. (a) Micrograph of *Culture of A. solani (b):* Micrograph of *Culture of A. Solani*

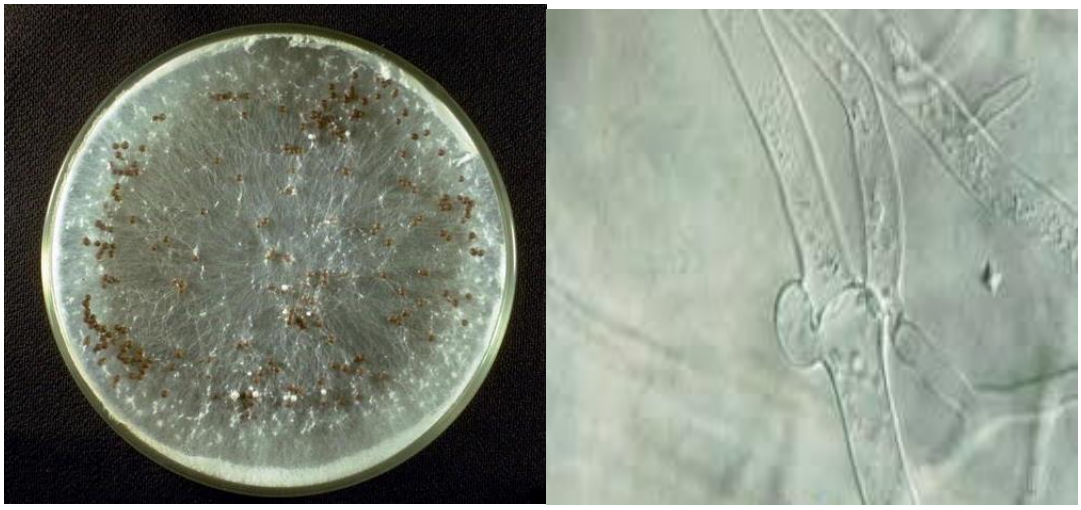

Figure 4. (a): Culture plate of *Athelia rolfsii* (b): Micrograph of *Athelia rolfsii*

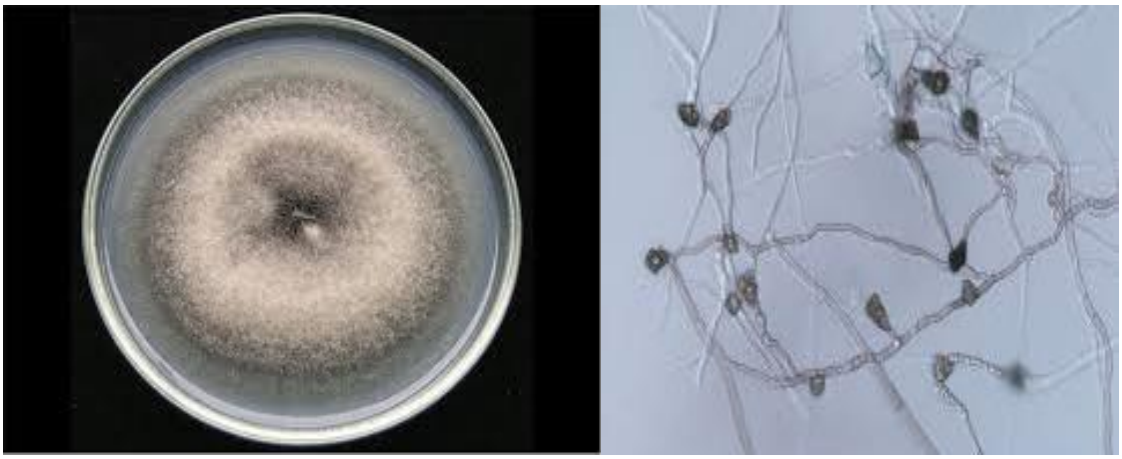

Figure 5. (a): Culture of *Colletotrichium phomoides* (b): Micrograph of *C. phomoides*

Figure 6. (a): Culture of *Phytophthora nicotianae* (b): Micrograph of *Phytophthora nicotianae*

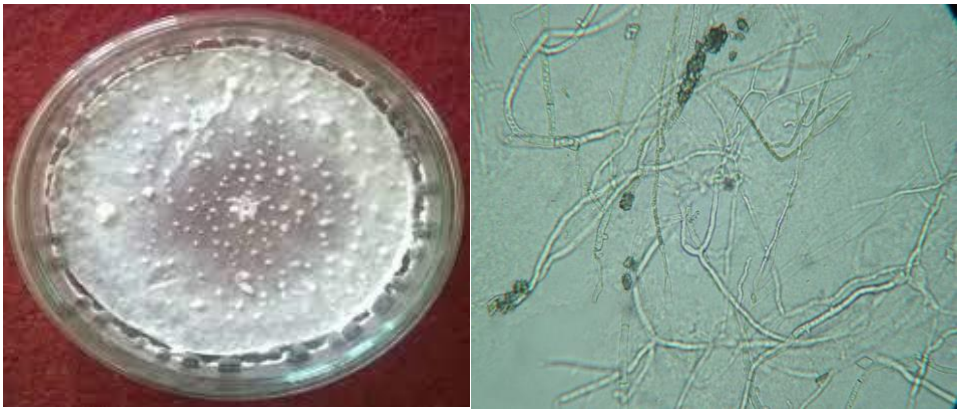

Figure 7. (a): Culture of *Sclerotinia sclerotiorum* (b): Micrograph of *Sclerotinia sclerotiorum*

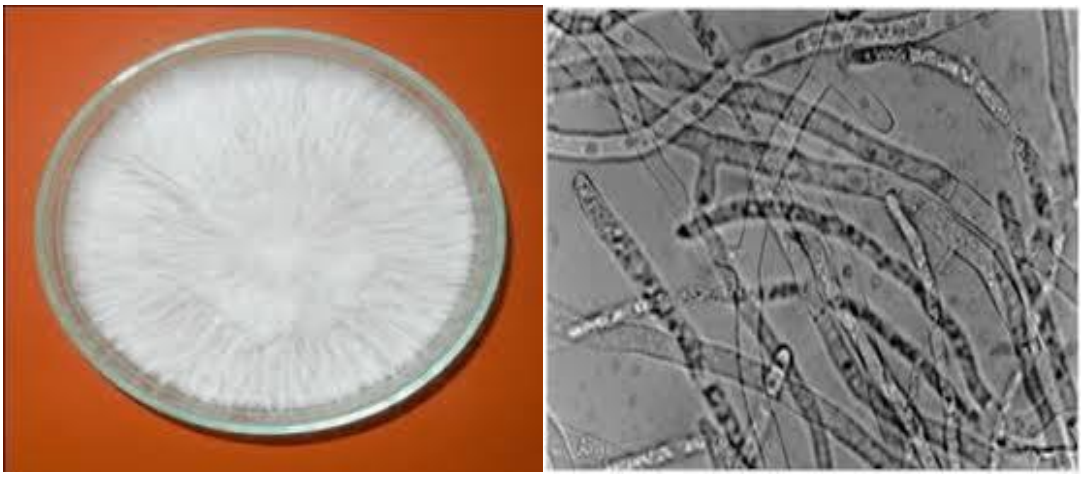

Figure 8. (a): Culture of *Sclerotium rolfsii* (b): Micrograph of *Sclerotium rolfsii*

Prevalence of disease incidence for the different locations (pdi)

Table 7 shows the disease incidence (PDI) prevalence for the different locations to ascertain which locations had the highest prevalence. There was a higher PDI of 50% in Umuahia against 33% recorded in Okigwe.

Table 7. Prevalence of disease incidence for the different locations

Sample Location	Total no. of Isolates	Total no. of samples	%PDI
Umuahia	16	**8**	50
Okigwe	24	8	33

$$\text{Prevalence of disease incidence} = \frac{\text{Sample size}}{\text{No. of Isolates}} \times \frac{100}{1}$$

DISCUSSION

This study was conducted in two markets: Eke Okigwe, Okigwe Imo state, and Isi gate market, Umuahia North, Abia State, Nigeria. The isolation frequency differed across both towns. The fungus was isolated more frequently in Umuahia than in Okigwe. Comparing our report with that of Kabiru & Yusuf (2023), who reported an isolation frequency of 7.7%—100%, we had an isolation frequency of 8.3%—29% in Okigwe and 6.3%—31% in Umuahia, even though the isolated fungus differed. The differences in the frequency of isolation could be attributed to the tomato variety, locations and the population in the various towns. However, this report shows the susceptibility of tomato fruit to fungal rot and deterioration, which complies with our report. The highest prevalence disease incidence (PDI) of 50% was recorded in Umuahia. This high PDI could result from the high population density in Umuahia compared to Okigwe, which has fewer people. The percentage frequency of isolation for samples from Umuahia showed significant variation from that of Okigwe. Isi gate market located in Umuahia had a percentage frequency (PF) of 6.3% - 31% compared to Eke Okigwe, 8.3% - 29%. *Alternaria solani* from both markets had the highest PF except for *Phytophthora nicotianae and Sclerotinia sclerotiorum*, which had 13% PF in both markets. The high PF frequency, as reported in our work, although higher in *Alternaria solani*, conforms with Osemwegie et al., 2019; Kaur and Banyal, 2019 who carried out a study on fungal pathogens associated with the rot of Tomato fruits and reported *Phytophthora nicotianae and Sclerotium rolfsii* as fungal isolates which causes tomato rot.

In our report, *Alternaria solani* had the highest rot ratings of 4, with a 75% rot range and 50% pathogenicity prevalence in both markets, which conforms to the reports of Schmey *et al*. (2023) and Aminuzzaman *et al*. (2021) on the high disease prevalence of *A. solani* as a blight of tomato. 85.6% and 29% for disease incidence and 29% severity, respectively, were reported by Aminuzzaman *et al*. (2021) for *A. solani, which aligns with* the 29% isolation frequency as reported in our work. One of the primary diseases found in most post-harvest products is caused by fungal pathogens, as reported by Koka *et al*. (2022) on the incidence and severity of tomato rot, which conforms with the identified and isolated fungus in our result.

Of the total of six fungal isolates, *Alternaria solani, Athelia rolfsii, Colletotrichium phomoides, Phytophthora nicotianae, Sclerotinia sclerotiorum, and Sclerotium rolfsii, Alternaria solani* had the highest pathogenicity prevalence which is however different from what was reported by many researchers who reported *Aspergillus*

niger as the most frequent in occurrence in their studies (Mailafia et al., 2017). However, reports on *Sclerotinia rot*, caused by *Sclerotinia sclerotiorum*, which affects tomatoes, have also been documented by Laurence *et al*. 2014; McGovern, 2015), which conforms with our report.

Studies on the isolation and identification of fungi associated with spoilt fruits in Gwagwalada market, Abuja, Nigeria, also carried out by Mailafia et al. (2017) in which *Aspergillus niger*, *Fusarium avenaceum*, *Penicillium digitatum* and *Rhizopus stolonifer* were identified against our report. Reports on *Sclerotinia rot*, caused by *Sclerotinia sclerotiorum*, which affects tomatoes, have also been documented (Laurence et al.. 2014; McGovern, 2015), which conforms with our report. In storage conditions, Shakya and Aryal (2020) reported that several species of fungi, such as *Alternaria alternata* and *Collectrotrichum truncatum*, often infect tomatoes. Similar reports by Schmey et al. (2023) were also reported on the Alternaria diseases in potatoes and tomatoes, which conforms with our report.

CONCLUSION

Six fungal isolates were isolated from the tomato sample collected from the two major cities, Umuahia and Okigwe, in Abia and Imo State, respectively—Alternaria *solani*, *Athelia rolfsii*, *Colletotrichium phomoides*, *Phytophthora nicotianae*, *Sclerotinia sclerotiorum*, and *Sclerotium rolfsii.* The highest pathogenicity prevalence was observed in *Alternaria solani* and *Athelia rolfsii*, with the least recorded on *Sclerotium rolfsii*. *Alternaria solani* and *Athelia rolfsii* were also found to be the most pathogenic, with a high percentage rot range. Both fungi also had high percentage isolation frequency, with the highest prevalence of disease incidence recorded in the tomato fruit from Umuahia. Although morphological characteristics, as applied in this report, were used to identify and characterize the fungi isolates, the genus is often tricky due to overlaps in the configuration of morphological features among identical species. Identifying fungi isolates based on morphological characteristics may be deceptive in ascertaining the genus; thus, we recommend combining the morphological characteristics and molecular analysis approach as ideal for accurate identification. There is also the need to develop target fungicides against *Alternaria solani*, *Athelia rolfsii*, *Colletotrichium phomoides*, *Phytophthora nicotianae*, *Sclerotinia sclerotiorum*, and *Sclerotium rolfsii* to reduce post-harvest losses associated with tomatoes in Nigeria.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no known competing conflicting interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Ezeibe Chidi Nwaru: Writing – original draft, Supervision, Methodology, Data curation, Conceptualization; Eke Tobechukwu: Writing – Review and editing; Nkechi P. Onyeabor: Writing – Review and editing; Matthew Chiemerie Ahaiwe: Writing – Review and editing

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