Original Research Article

Nigerian Journal of Biotechnology and Life Sciences
ISSN: 2814-0281
Vol. 3 No 2b (2026) p. 415 - 421
http://www.njbls.fud.edu.ng/

Isolation and Identification of Fungi Associated with Spoilage of Tomato Samples Obtained from Kubwa, Iddo and Adelabu Markets in Abuja, Nigeria.

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https://doi.org/10.67601/njbls.v3i2b.66

Article into
Date received
13/08/2026
Date accepted
08/09/2026
Available online
18/09/2026

Keywords
Lycopersicon esculentum, Tomatoes, Fungi, Spoilage, *Aspergillus* species

Abstract

Food spoilage is an alteration in the sensory qualities of food caused primarily by biological, chemical, and physical factors, resulting in the food becoming unsuitable and unsafe for human consumption. This research was carried out to isolate and identify the fungi associated with tomatoes samples obtained from Kubwa, Iddo and Adelabu markets. Tomato samples were collected from specific market locations in Kubwa, Iddo and Adelabu markets. Potato dextrose agar was prepared in accordance with the instructions provided by the manufacturer. Spread plate technique was adopted for the culture and isolation of the isolates. The plates were then placed at room temperature and allowed to incubate for a period of 72 hours. The result obtained showed a fungal count range between 1.7 x 10^5 cfu/ml to 2.5 x 10^5 cfu/ml, while the fungal species isolated were *Aspergillus niger, Geotrichum candidum, Aspergillus flavus,* and *Penicillium chrysogenum. Aspergillus niger* had the highest percentage occurrence of 44.4% while the lowest percentage occurrence was observed in *Geotrichum candidum* (11.1 %.) The result of the pathogenicity test revealed that the fungi inoculated into the healthy tomato, *Aspergillus niger* had the highest decay diameter of 40 mm while *Penicillium chrysogenum* had the lowest which was 12 mm. Tomatoes are usually transported using locally woven baskets and sack under conditions that favour the growth of these organisms therefore, good quality control measures and proper storage methods should be adopted by farmers, marketers and consumers during the harvesting, transportation, handling distribution and processing tomatoes.

Introduction

Lycopersicon esculentum is an important plant primarily cultivated for its consumable fruits and occasionally its foliage. It is a popular vegetable crop in many countries (Adams et al., 2001). It was probably introduced into Nigeria through early trade missions of the Portuguese to Africa and freed slaves from the West Indies (Lawal et al., 2007). However, it has over the years assumed a prominent position in farming systems and diets of Nigerians. The *Lycopersicon* genus is modest in size, comprising just over ten species. These plants are originally from South America but are extensively grown and adapted to warm temperate and tropical regions across the globe (Naika et al., 2005).

Tomato is very essential mainly for its dietary value, as it can be consumed in different ways. It may be cooked as a vegetable, used as an ingredient in many dishes and sauces, in the preparation of stews, or eaten fresh in salads (Ali et al., 2021). Tomatoes and tomato-based dishes provide an accessible source of essential nutrients and bioactive compounds. They are rich in folate, vitamin C, and potassium, and are particularly abundant in carotenoids, with lycopene being the most prominent, followed by beta-carotene, gamma-carotene, phytoene, and several minor carotenoids. The strong antioxidant activity of lycopene, together with other carotenoids present in tomatoes, makes these foods excellent dietary sources of antioxidants (Beecher, 1988; Ali et al., 2021).

Tomato spoilage refers to adverse changes caused mainly by biological and physical factors, which render the food unfit for human consumption. Spoiled tomatoes often show variations in flavor, appearance, aroma, or texture, and this deterioration commonly occurs during storage, transportation, or while awaiting processing. Microbial spoilage reduces both the market and nutritional value of tomatoes. Contamination with mycotoxin-producing fungi, such as those generating aflatoxins, can make tomato fruits unsafe for consumption, as ingestion or inhalation of these toxins may result in food poisoning (Liu et al., 2020; Kumar et al., 2021). According to Snyder et al. (2024), microbial spoilage contributes significantly to food scarcity, which remains one of the major global challenges alongside poverty.

Many developing and developed countries experience food scarcity, which remains one of the major global problems, particularly in Nigeria. It has been reported that about one billion people face severe starvation in these nations, with approximately 10% dying from hunger-related complications (FAO et al., 2019). This challenge arises largely from inadequate agricultural storage, packaging, handling, transportation, and insufficient preservation against microbe-induced spoilage. Tomato fruits, in particular, are often harvested and transported under conditions that expose them to microbial infections. For example, they are commonly carried in locally made baskets, which are prone to fungal contamination. At the markets, tomatoes are typically displayed on benches or in baskets, awaiting customers, during which time they are vulnerable to further microbial contamination from their surfaces and from contact with nearby infected fruits (Baiyewu et al., 2007).

Various types of fungi, such as *Alternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus* and *Trichoderma* species, are capable of contaminating different food items. The contamination of tomatoes by fungi represents a major health hazard to both humans and livestock, as these organisms produce toxic secondary metabolites known as mycotoxins. When ingested or inhaled, mycotoxins can lead to serious health complications, and importantly, their effects are not confined to the areas where the fungal infections initially occur (Baker, 2006). It is therefore crucial to understand the potential risks posed by the contamination of organically cultivated fruits and vegetables with molds that produce harmful mycotoxins.

Materials and Methods

Study Area

The study was carried out at Kubwa, Iddo and Adelabu Market, all located in the Federal Capital Territory. The laboratory analysis was carried out at the University of Abuja's Microbiology Department Laboratory located on latitude 9°32N and longitude 50° 10E. Abuja possesses rich soil for cultivation and a climate of about 22°C to 35°C all year round (Erondu & Solomon, 2017).

Materials

Tomato samples, Potato dextrose agar (PDA), test tubes, petri dishes, lactophenol cotton blue, cork borers, incubator, autoclave, spirit lamp, microscope, slide, inoculating needle, petroleum jelly, aluminum foil, cover slip, pipette, weigh balance, 70% ethanol, latex gloves, and conical flask.

Sample collection

Ten samples of tomatoes with apparent signs of spoilage were procured from specific retail stands within Kubwa, Iddo and Adelabu market respectively. The spoilt tomatoes were collected in sterile polyethene bags and transported to the Microbiology Laboratory of the University of Abuja for analysis.

Sterilization

All laboratory glassware used were washed using detergent, sterilized and allowed to dry. The work bench was disinfected with a cotton wool dipped in 70% ethanol by wiping the work bench surfaces. The peptone water and distilled water used for the serial dilution was sterilized in the autoclave set at 121°C for 15 minutes.

Media Preparation

Potato Dextrose Agar (PDA) used was prepared according to the manufacturer's instructions with incorporation of commercial antibiotics to prevent the growth of bacteria.

Isolation of Fungi from Spoilt Tomatoes

The isolation was done using the methods as described by Cappuccino and Welsh (2017). Each tomato samples were pulverized into liquid paste. One gram (1 g) of the liquid sample was diluted in a series of test-tubes containing 9 ml of sterile water, a fixed volume of sample, 1.0 ml was taken from the fourth test tube using laboratory hand pippete and transferred onto the surface of the agar. A sterile glass rod was used to evenly spread the sample on the agar surface before incubation. After 72 hrs, the dispersed cells grew into separate colonies. The number of colonies on the plate was counted and multiplied by the dilution factor to determine the total number of viable organisms initially present in the sample. Pure culture of each colonies observed was prepared resulting in the formation of distinct colonies.

Characterization and Identification of Fungal Isolates

Macroscopic Examination

The isolates were recognized and categorized using visual examination at both a macroscopic and microscopic level. The macroscopic examination was carried out by meticulously observing the colonial characteristics particularly the colour formation of both front and reverse sides of the culture plates.

Microscopic Examination

The method of Oyeleke & Manga (2008) was adopted for the identification of the isolated fungi using lactophenol in cotton blue stain. The identification was carried out by picking a small portion of the aerial mycelia from the representative fungi cultures. The sample of the isolates were placed on a clean, grease-free slide with the aid of a sterile wire loop, onto which a drop of lactophenol was added. The aerial mycelia and the lactophenol in cotton blue stain were well spread on the slide. A cover slip was carefully placed with little pressure applied to eliminate air bubbles. The slide was then mounted on the stage of a light microscope and viewed with 10x and 40x objective lenses. The physical traits and visual aspects of the fungal organisms were examined.

Pathogenicity Test

The method of Onuorah & Orji (2015) was adopted. Five healthy tomatoes were washed and rinsed with distilled water and the surfaces were disinfected with 70% ethanol. A sterile cork borer was used to bore holes in each of the tomato fruits. Each of the isolated fungi were afterwards inoculated into the fruits after which the cores of the fruits were replaced. Petroleum jelly was used to seal the holes of the fruits to prevent contamination. The inoculated tomato fruits were placed in sterile polythene bags. Each of the fruits were moistened with wet balls of absorbent cotton wool to create a humid condition. The fruits were later incubated at 28 ^{0}C for 3 days after which they were observed for spoilage and the decay diameter was measured. A set of healthy tomatoes were also washed and rinsed with distilled water and the surfaces were disinfected with 70% ethanol and incubated likewise as control. All sample examinations were done in duplicates.

Data Analysis

Data obtained in this study was analyzed statistically using Analysis of Variance (ANOVA) from Ms Excel Statistics (Window 10 version) and the test applied was F-test statistic at $P < 0.05$.

Results

Colonial Count of the Samples

Table 1 shows the total colony forming unit for each spoilt tomato and it ranges from 1.7 x 10^5 to 2.5 x 10^5 cfu/ml.

Table 1: Total Colony Count

Sample	Total Colony Forming Unit (cfu/ml)		
	Iddo	**Kubwa**	**Adelabu**
T1	2.5x10^5	1.8x10^5	2.3x10^5
T2	1.9x10^5	1.8x10^5	2.1x10^5
T3	2.2x10^5	2.0x10^5	1.7x10^5
T4	2.5x10^5	2.3x10^5	1.8x10^5
T5	1.8x10^5	2.1x10^5	2.1x10^5
T6	2.1x10^5	2.5x10^5	2.0x10^5
T7	2.2x10^5	2.3x10^5	2.4x10^5
T8	2.3x10^5	1.9x10^5	2.2x10^5
T9	1.9x10^5	1.8x10^5	2.1x10^5
T10	2.4x10^5	2.3x10^5	2.0x10^5
Mean ± SD	2.18±0.25 x10^5	2.08±0.26 x10^5	2.07±0.21 x10^5

Values are expressed as Mean ± Standard Deviation

Key; T1 to T10 = Tomato Samples; Iddo, Kubwa and Adelabu = Market Locations

Macroscopic Characteristics of Fungal Isolates

Fungal isolates were identified based on macroscopic and microscopic morphology. The following were noted for the macroscopic examination; shape, pigments and pattern of growth. Table 2 portrays the macroscopic morphology of the isolates.

Table 2: Macroscopic Morphology of the fungal Isolates

Isolate	Macroscopic Observation
Isolate 1	Black tip with white mycelia
Isolate 2	Bright yellowish green colony with powdery appearance
Isolate 3	Blue-green (cyan) colony appearing with a band of white margin
Isolate 4	Cloudy white cotton-like colonies

Microscopic features of the fungal isolates

Table 3 represents the microscopic features of the isolates, they were observed under the microscope after staining with the appropriate fungal stain.

Percentage occurrence of fungal isolates

Table 4; depicts the number of isolates and the percentage occurrence of each isolate. *Aspergillus niger* (44.4%) had the highest percentage occurrence while *Geotrichum candidum* (11.1%) had the lowest percentage occurrence.

Pathogenicity of Fungal Isolate

The result of the pathogenicity test is represented in Table 5. The fungal isolate *Aspergillus niger* inoculated into healthy tomato had the highest decay diameter of 40 mm and *Penicillium chrysogenum* had the lowest decay diameter of 12 mm.

Discussion

Tomato fruits have very high nutritional and dietary value. The high-water contents makes it prone to fungal spoilage. Spoilage of tomatoes caused by fungi can lead to foodborne diseases and intoxications (Onuorah & Orji, 2015). In this study, fungi associated with the spoilage of tomatoes sold in Kubwa, Iddo and Adelabu markets were carried out. The fungal colony counts ranged from 1.7 x 10^5 cfu/ml to 2.5 x 10^5 cfu/ml which was higher compared to the report of Onuorah & Orji (2015) who studied similar case and obtained results which ranged from 1.3 x 10^3 cfu/ml to 2.0 x 10^3 cfu/ml. The disparity in results could be attributed to duration of exposure before sales and handling practices of sellers in Kubwa, Iddo and Adelabu markets.

The isolates identified from spoilt tomatoes in Kubwa, Iddo and Adelabu markets were *Aspergillus flavus, Geotrichum candidum, Aspergillus niger, Penicillium chrysogenum. Aspergillus niger* was the most frequently encountered fungus, with a mean occurrence of 2.67 ± 1.15, and was particularly prevalent in Iddo, where four isolates were recorded.

Table 3: Microscopic Morphology of the Fungal Isolates

Isolate	Microscopic Observation	Fungi
Isolate 1	Smooth, thin conidiophores with radial conidial heads and septate hyphae	*Aspergillus niger*
Isolate 2	Long conidiophores with chains of conidia dispersed around vesicle with branching septate hyphae	*Aspergillus flavus*
Isolate 3	Slender, tubular and branched septate hyphae, thick conidiophores and a chain of conidia dispersed like paintbrush	*Penicillium chrysogenum*
Isolate 4	Septate hyphae, branched and break into chains or segments	*Geotrichum candidum*

Table 4: Number of Fungi per Location

S/N	Fungi	Iddo	Kubwa	Adelabu	Mean ± SD
1	*Aspergillus niger*	4	2	2	2.67±1.15
2	*Aspergillus flavus*	2	1	0	1.00±1.00
3	*Penicillium chrysogenum*	2	2	1	1.67±0.58
4	*Geotrichum candidum*	1	1	0	0.67±0.58
	Mean ± SD	2.25±1.26	1.50±0.58	0.75±0.96	

Key; S/N= Serial Number

Table 5 Percentage Occurrence of Fungal Isolates

S/N	Fungi	No of Isolate	Occurrence (%)
1	*Aspergillus niger*	8	44.4
2	*Aspergillus flavus*	3	16.7
3	*Penicillium chrysogenum*	5	27.8
4	*Geotrichum candidum*	2	11.1
	Total	18	100

Key; S/N= Serial Number

Table 6 Decay diameter of Fungi in healthy tomato

S/N	Fungi	Rot Diameter (mm)
1	*Aspergillus niger*	40±0.11
2	*Aspergillus flavus*	30±1.01
3	*Penicillium chrysogenum*	12±0.21
4	*Geotrichum candidum*	20±0.10

Key; S/N= Serial Number

Penicillium chrysogenum was the second most frequently encountered organism (1.67 ± 0.58), followed by *Aspergillus flavus* **(**1.00 ± 1.00**)** and *Geotrichum candidum* **(**0.67 ± 0.58). The fungal occurrence was highest in Iddo (9 isolates**)**, followed by Kubwa (6 isolates) and Adelabu (3 isolates**).** This indicates that Iddo had the greatest fungal abundance among the three sampling locations, while Adelabu had the lowest. This report aligns with the work of Muhammad et al. (2004) who carried out a study on the survey of the market diseases and aflatoxin contamination of tomato (*Lycopersicon esculentum* Mill.) fruits in Sokoto, north-western Nigeria and found out part of the fungi species associated with the spoilage were *Aspergillus flavus, Geotrichum candidum* and *Penicillium chrysogenum.*

Percentage occurrence of the fungi shows that *Aspergillus niger* has the highest percentage occurrence of 44.4% and *Geotrichum candidum* has the lowest percentage occurrence of 11.1%. This study concurs with Onuorah & Orji. (2015) who carried out a research on fungi associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka, Nigeria and reported that *Aspergillus niger* had the highest percentage occurrence among the fungal isolates recovered from the tomato samples. The predominance of *A. niger* may be associated with its widespread distribution and ability to survive and grow under diverse environmental conditions. Differences in geographical location, climatic conditions, post-harvest handling, storage conditions and modes of distribution may also influence the prevalence and distribution of fungal species associated with tomato spoilage. The result of the pathogenicity test showed that the fungi inoculated into the healthy tomato, *Aspergillus niger* has the highest decay diameter while *Penicillium chrysogenum* has the

lowest. This could be attributed to the fact that *Aspergillus niger* has stronger spoilage capacity and greater production of extracellular hydrolytic enzymes.

The fungi identified in this research are rich in powerful mycotoxins that can cause serious harm if accidentally consumed. *Aspergillus flavus* is a type of pathogenic fungus that takes advantage of crops. It is capable of generating aflatoxin, a powerful cancer-causing agent, as a byproduct in the seeds of various crops, both pre and post-harvest. Aflatoxin is a potent carcinogen subject to stringent regulations in the majority of nations (Klich, 2007). Infections caused by *Aspergillus flavus* may occur in the field before harvest, yet typically remain symptomless until the storage or transportation stage after harvest. Apart from initiating infections both before and after harvest, numerous strains of this fungus generate substantial amounts of harmful substances called mycotoxins. These mycotoxins, when ingested, can be poisonous to mammals (Amaike & Keller, 2011).

Conclusion

This study revealed the presence of *Aspergillus niger, Geotrichum candidum, Aspergillus flavus*, and *Penicillium chrysogenum* as associated with the spoilage of tomato in Iddo, Kubwa and Adelabu markets. These fungi and their metabolites are capable of causing health hazards and result in food shortages and economic loss. The tomatoes are usually transported using locally woven baskets and sack under conditions that favour the growth of these organisms therefore, good quality control measures and proper storage methods should be adopted by farmers, marketers and consumers involved in the various stages of acquiring, moving, managing, delivering, and transforming tomatoes.

Conflict of Interest

No conflict of interest

Acknowledgement

Special appreciation to Prof. S.C. Ugoh for being a guide to us through the research work.

Author Contribution

The authors confirm contribution to the paper as follows: study design: Idama, J.O-P; Oladapo, Z.A; data collection: Idama, J.O-P; analysis and interpretation of results: Idama, J.O-P; Oladapo, Z.A; draft manuscript preparation: Oladapo, Z.A. All authors reviewed the results and approved the final version of the manuscript.

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