

# Fungi Associated with the Spoilage of Post-harvest Tomato Fruits Sold in Major Markets in Awka, Nigeria

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**Abstract** Tomato contains large amount of water which makes it more susceptible to spoilage by fungi. These fungi that spoil tomatoes produce mycotoxins that are detrimental to human health. This study was therefore carried out to isolate, characterize and identify the fungi associated with the spoilage of tomato fruits sold in Okpuno, Amaudo, Eke-Awka, Nodu and Amaikwo Markets in Awka, Nigeria. The average fungal counts ranged between  $1.3 \times 10^3$  and  $2.0 \times 10^3$  cfu/ml, while the fungal isolates were *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Alternaria alternata*, *Penicillium digitatum* and *Geotrichum candidum*. The percentage occurrence of the isolates in the fruits from Eke Awka was the highest (32.73%) while that of the fruits from Nodu market was the least (12.73%). *Aspergillus niger* had the highest percentage occurrence (47.27%) in the fruits studied, with *Saccharomyces cerevisiae* and *Geotrichum candidum* having the lowest percentage occurrence (3.64%). Pathogenicity test result showed that *Aspergillus niger* also had the highest decay diameter of 30mm in the healthy tomato fruits while *Geotrichum candidum* had the lowest decay diameter. Proper handling and adequate storage facilities must therefore be employed to prolong the shelf life of tomato fruits.

**Keywords** Fungi, Spoilage, Post-Harvest, Tomato Fruits, Major Markets

## 1. Introduction

Tomato is a widely consumed fruit eaten in both raw and processed forms [1]. It has the botanical name *Lycopersicon Esculentum* and belongs to the plant family *solanaceae*. It is rich in vitamins including vitamin A and vitamin C, carbohydrates, proteins, fats, fibres and potassium [2]. It is rich in lycopene which has many beneficial health effects. It contains large amount of water which makes it more susceptible to spoilage by the action of microorganisms [3]. Tomato has a much lower sugar content than other fruits and

is therefore not as sweet.

Tomatoes may be pear-shaped, elongated, flattened and heart shaped. They are edible, fleshy and reddish when ripe and vary in their acid composition, with white and yellow ones being less acidic. Tomatoes can be used as savoury or flavouring in soups and cooked foods or can be eaten as fruits. It is used in many dishes, salads, sauces and drinks and can also be dried and ground into pancakes [4].

The consumption of tomatoes throughout the world is believed to benefit the heart and other organs. The richest source of lycopene is tomato and tomato-based products [5]. Lycopene has been found to prevent prostate cancer, improve the skin's ability to protect itself against the harmful ultra violet rays, decrease the risk of breast, lung, stomach, bladder, uterine, head and neck cancers, protect against neurodegenerative diseases, lower urinary tract infections and reduce the cardiovascular risk associated with type 2 diabetes [6-10]. Tomatoes have serious challenges to their existence. These include changes in climate conditions, pests, inadequate rainfall and microorganisms particularly fungi. One of the limiting factors that influence tomato economical value is its relatively short shelf life caused by pathogen attack.

Spoilage of tomatoes are those adverse changes in the quality of tomatoes that are brought about by the action of predominantly biological and physical factors. These may be changes in taste, smell, appearance or texture of the fruits. Ghosh [11] reported that fungi were the source of spoilage of most of the tomato samples accessed than bacteria. Fungi affecting tomatoes include *Aspergillus phoenicis*, *Absidia* spp, *Trichoderma* spp, *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium moniliformis*, *Aspergillus niger*, *Mucor* spp, *Rhizopus stolonifer*, *Penicillium* spp, *Geotrichum* spp and *Phytophthora* spp [12-19].

Fungal spoilage of tomatoes has been recognized as a source of potential health hazard to humans and animals due to the fact that they produce mycotoxins which are capable of causing mycotoxicoses in man following ingestion or inhalation [18]. The mycotoxins are not limited to their areas of infections. Since tomatoes contain large amount of fluid,

these mycotoxins diffuse rapidly throughout them, contaminating all parts and making the fruits unfit for consumption. The importance of tomato in the food industry and its nutritional benefits cannot be overemphasized. Routine microbiological examination of tomatoes is very crucial as it contributes to a large extent to economic development.

Tomatoes are consumed either raw or cooked worldwide. Numerous reports on their spoilage organisms in the developed countries are available but there is dearth of information on the spoilage organisms of tomatoes in a developing city such as Awka, Nigeria. There is therefore a need to evaluate the fungi associated with their spoilage, hence in this work, the fungi associated with the spoilage of post-harvest tomatoes sold in major markets in Awka, Nigeria were isolated, characterized and identified.

## 2. Materials and Methods

### 2.1. Samples Collection

Thirty tomato samples were purchased from Okpuno, Amaudo, Eke Awka, Nodu and Amaikwo markets in Awka, Anambra State, Nigeria. They were transported to the microbiology laboratory of Nnamdi Azikiwe University Awka in sterile polythene bags for fungal isolation. The samples were left for one week for spoilage to occur. The thirty spoilt tomatoes were used for the study.

### 2.2. Materials Sterilization

All the glass wares were properly washed, dried and sterilized in the oven at 160°C for one hour. The entire working surfaces were also disinfected with ethanol to reduce contaminants.

### 2.3. Samples Processing

One gram of each of the spoilt tomatoes was carefully cut with the aid of a sterile scalpel and enriched in sterile sabouraud dextrose broth for twenty four hours. Ten fold serial dilutions of the samples were thereafter carried out.

### 2.4. Isolation of Fungi

The pour plate method was used. One milliliter of the serially-diluted sample ( $10^3$ ) was dispensed into a conical flask containing sterile sabouraud dextrose agar (SDA) and two percent chloramphenicol to inhibit bacterial growth. The contents were properly mixed and dispensed aseptically into sterile petri-dishes. Incubation was carried out in an inverted position at 28°C for five days. The colonies that developed were counted and subcultured repeatedly on sabouraud dextrose agar plates to obtain pure cultures. They were later stored on SDA slants for characterization and identification.

### 2.5. Characterization and Identification of the Isolates

The pure cultures of the moulds were identified on the basis of their colony growth pattern, conidial morphology and pigmentation using the slide culture technique and microscopic examination. The yeasts were characterized and identified using the Gram stain, Chlamyospores formation, Germ tube, sugar assimilation and motility tests. The identity of each fungus was confirmed with the aid of a mycological atlas.

#### 2.5.1. Slide Culture Technique

A fragment of the aerial mycelia was picked with a sterile inoculating needle and inoculated on a slide containing prepared sabouraud dextrose agar. The slide was thereafter incubated at room temperature for twenty four hours after which it was examined under the microscope.

#### 2.5.2. Microscopic Examination

Lactophenol cotton blue solution was used. A drop of the solution was placed on a clean grease-free slide. A fragment of the mould was emulsified in the solution after which the slide was covered with a cover slip, avoiding bubbles. The slide was thereafter viewed under the microscope.

#### 2.5.3. Gram Stain

A smear of the test yeast cell was made on a slide and stained, using the Gram stain procedure. The slide was thereafter viewed under the microscope for the presence of gram positive yeast cells.

#### 2.5.4. Chlamyospore Formation Test

The procedure employed by Menza et al [20] was used. The isolate was inoculated on corn meal agar containing tween 80 and incubated at 25°C for seventy two hours. The isolate was thereafter introduced on a slide and stained with lactophenol cotton blue solution. The slide was thereafter viewed under the microscope for chlamyospore formation.

#### 2.5.5. Germ Tube Test

The procedure of Menza et al [20] was employed in the test. A colony of the test yeast was inoculated into human serum and incubated at 37°C for three hours. A drop of the incubated serum was placed on a microscope slide and covered with a coverslip. The slide was thereafter examined under the microscope for the presence of germ tube.

#### 2.5.6. Sugar Assimilation Test

The test was performed using the procedure of Menza et al [20]. Carbohydrate-impregnated filter paper discs were placed on carbohydrate-free yeast nitrogen base agar contained in petri-dishes and incubated at 30°C for eighteen hours. Growth around the paper discs was thereafter observed. Growth in the medium indicated the ability of the isolate to assimilate a sugar. The sugars used were glucose, sucrose, lactose, raffinose and galactose.

2.5.7. Motility Test

Hanging drop slides were used. Vaseline was spread with a sterile toothpick on the corners of a clean coverslip. A drop of the yeast suspension was aseptically placed on the centre of a coverslip using a sterile inoculating loop. The depression slide was lowered on the coverslip so that the drop protruded into the centre of the concavity of the slide. The hanging drop slide was turned over and placed on the stage of the microscope so that the drop was on the light hole. The drop was thereafter examined under the microscope.

2.6. Pathogenicity Test of the Isolates

The procedures of Chukwuka et al [21], Baiyewu et al [22] and Onuorah et al [23] were used. Fifteen healthy tomatoes were properly washed with tap water, rinsed with distilled water and surface-disinfected with ethanol. Sterile cork borers were used to bore holes in each of the tomato fruits. Each of the isolated fungi was thereafter inoculated into the fruits after which the cores of the fruits were replaced. Sterile petroleum jelly was used to seal the holes of the fruits to prevent contamination. Fifteen tomato fruits wounded with the cork borers but were not inoculated with the fungi served as controls.

The inoculated tomato fruits and the controls were placed in sterile polythene bags (one fruit per bag). Each of the fruits was moistened with wet balls of absorbent cotton wool to create a humid condition. The fruits were thereafter

incubated at 28°C for five days and observed for spoilage. The fungi were re-isolated from the fruits and compared with the original isolates. The decay rate of each fungus in the healthy fruits was also determined by measuring its rot diameter after two weeks of its inoculation into the healthy tomato fruit.

3. Results

The average fungal counts of the spoilt tomato fruits are presented in Table 1. The fruits from Eke Awka market had the highest count of  $2.0 \times 10^3$  cfu/ml while those from Nodu market had the lowest count of  $1.3 \times 10^3$  cfu/ml.

Table 1. Average fungal counts of the spoilt tomato fruits

Market	Average fungal counts (x 10 <sup>3</sup> cfu/ml)
Okpuno	1.6
Amaudo	1.7
Eke-Awka	2.0
Nodu	1.3
Amaikwo	1.8

The fungal isolates from the spoilt tomato fruits are shown in Table 2. They were *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Alternaria alternata*, *Penicillium digitatum* and *Geotrichum candidum*.

Table 2. Fungal isolates from the spoilt tomato fruits

Market	Fungal isolates
Okpuno	<i>Aspergillus niger</i> and <i>Rhizopus stolonifer</i>
Amaudo	<i>Fusarium oxysporum</i> , <i>Rhizopus stolonifer</i> and <i>Aspergillus niger</i>
Eke Awka	<i>Fusarium oxysporum</i> , <i>Saccharomyces cerevisiae</i> , <i>Alternaria alternata</i> , <i>Penicillium digitatum</i> and <i>Aspergillus niger</i>
Nodu	<i>Geotrichum candidum</i> and <i>Aspergillus niger</i>
Amaikwo	<i>Aspergillus niger</i> , <i>Rhizopus stolonifer</i> and <i>Alternaria alternata</i>

The occurrence of the fungi in relation to the markets is shown in Table 3. The fungi occurred most in the samples from Eke Awka market (32.73%) while they had the lowest occurrence in the fruits from Nodu market (12.73%).

Table 3. Percentage of the fungi in relation to the markets

Markets	<i>Aspergillus niger</i> (n)	<i>Rhizopus stolonifer</i> (n)	<i>Fusarium oxysporum</i> (n)	<i>Saccharomyces Cerevisiae</i> (n)	<i>Alternaria alternata</i> (n)	<i>Penicillium digitatum</i> (n)	<i>Geotrichum candidum</i> (n)	% occurrence
Okpuno	4	4	0	0	0	0	0	14.54
Amaudo	4	3	3	0	0	0	0	18.18
Eke-Awka	7	0	4	2	2	3	0	32.73
Nodu	5	0	0	0	0	0	2	12.73
Amaikwo	6	2	0	0	4	0	0	21.82

n=number of isolates

The percentage occurrence of the fungi from the spoilt tomato fruits is presented in Table 4. *Aspergillus niger* occurred most frequently (47.27%) while *Saccharomyces cerevisiae* and *Geotrichum candidum* had the lowest percentage occurrence (3.64% each).

**Table 4.** Percentage occurrence of the fungal isolates in the spoilt tomato fruits

Fungi	No. of Isolates	% Occurrence
<i>Aspergillus niger</i>	26	47.27
<i>Rhizopus stolonifer</i>	9	16.36
<i>Fusarium oxysporum</i>	7	12.73
<i>Saccharomyces cerevisiae</i>	2	3.64
<i>Alternaria alternata</i>	6	10.91
<i>Penicillium digitatum</i>	3	5.45
<i>Geotrichum candidum</i>	2	3.64

The decay diameter of the fungi in the healthy tomato fruits is presented in Table 5. *Aspergillus niger* had the highest decay diameter of 30mm while *Geotrichum candidum* had the least decay diameter of 10mm.

**Table 5.** Decay diameter of the fungi in the healthy tomato fruits

Fungi	Decay diameter (mm)
<i>Aspergillus niger</i>	30
<i>Rhizopus stolonifer</i>	22
<i>Fusarium oxysporum</i>	19
<i>Saccharomyces cerevisiae</i>	11
<i>Alternaria alternata</i>	16
<i>Penicillium digitatum</i>	12
<i>Geotrichum candidum</i>	10

## 4. Discussion

The fungi associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka, Nigeria were studied and the result revealed the presence of a teeming population of fungi. The average fungal counts ranged from  $1.3 \times 10^3$  to  $2.0 \times 10^3$  cfu/ml. The fruits from Eke-Awka market had the highest count of  $2.0 \times 10^3$  cfu/ml while those from Nodu market had the lowest count of  $1.3 \times 10^3$  cfu/ml (Table 1).

The fungal isolates from the fruits were *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Alternaria alternata*, *Penicillium digitatum* and *Geotrichum candidum* (Table 2). Ibrahim et al [15] isolated *Aspergillus niger* as one of the major fungi responsible for the production of volatile compounds in spoilt tomatoes. Baker [18] also isolated *Aspergillus niger* from rotten tomato fruits and reported that they are pathogenic on tomato fruits. Akinmusire [13] reported that *Rhizopus* spp were associated with the spoilage of tomatoes. Wogu and Ofuase [24] isolated *Aspergillus* spp, *Penicillium* spp, *Fusarium* spp and *Saccharomyces* spp from spoilt tomato fruits. Mbajiuika and Enya [25] also isolated *Aspergillus* spp, *Penicillium* spp and *Saccharomyces cerevisiae* from spoilt tomatoes while Fatih et al [19] reported the presence of *Alternaria alternata* and *Fusarium oxysporum* in the spoilt tomato fruits they

studied. Ghosh [11] also isolated *Fusarium oxysporum*, *Aspergillus niger* and *Rhizopus stolonifer* from the spoilt tomato fruits studied.

The percentage occurrence of the fungi in relation to the markets showed that the fungi had the highest percentage occurrence of 32.73% in the samples from Eke Awka market while their percentage occurrence was lowest (12.73%) in the fruits from Nodu market (Table 3). The detection of more fungi in the fruits from Eke-Awka market could be as a result of poor sanitation, overcrowding, poor storage and unhygienic practices by the fruits handlers.

*Aspergillus niger* had the highest percentage occurrence of 47.27% in the spoilt tomato fruits examined while *Saccharomyces cerevisiae* and *Geotrichum candidum* each had the lowest percentage occurrence of 3.64% in the fruits studied in (Table 4). The result agreed with the work of Akinmusire [13] and Ibrahim et al [15] They reported that *Aspergillus niger* had the highest rate of occurrence in the tomato fruits they studied and concluded that the fungus may be the major organism responsible for the spoilage of tomato fruits.

The result of the pathogenicity test showed that the fungi inoculated into the healthy tomato fruits had the same features as the ones re-isolated from them, indicating that the fungi were responsible for the spoilage of the tomato fruits. *Aspergillus niger* produced the highest rot in the tomato fruits, with a rot diameter of 30mm while *Geotrichum candidum* produced the lowest rot diameter of 10mm in the tomato fruits (Table 5).

Fungal spoilage of tomatoes is attributable to the high water content, environmental conditions, state of handling, state of storage facilities, the fungal load of the handlers and the quality of the tomatoes. These fungi isolated in this study are sources of potent mycotoxins which are detrimental to health. *Aspergillus niger* is a source of Ochratoxin which is considered to be a potent Carcinogen, therefore spoilt tomatoes must not be consumed but disposed off, since such consumption could be detrimental to health. Farmers and marketers of the produce are also advised to take appropriate precautions during the harvesting, transportation, storage and sale of tomatoes to reduce the risk of these toxins and other metabolites that are deleterious to health.

## 5. Conclusions

Tomato fruits have high dietary and nutritional qualities. Their spoilage by fungi results in loss of economic resources as well as food poisoning. The fruits are usually transported from areas of production to areas of consumption in locally woven baskets and sacks under conditions that encourage the growth of fungi. Good quality control measures must therefore be employed by the farmers, marketers and consumers during the harvesting, transportation, handling and processing of the fruits. Frequent inspection of the fruits for sale by food inspectors is also recommended. These will

go a long way in preventing the consumption of contaminated tomato fruits thereby reducing the health hazards posed by the mycotoxins produced by these fungi isolated in this study.

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