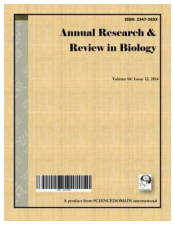

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Determination of Pathogenic Effect of Fungi on Fresh Healthy Tomatoes in Jos North Local Government Area, Plateau State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAO designed the study. Author NCJA wrote the protocol and interpreted the data. Authors OAO and WCJ anchored the field study, gathered the initial data and performed preliminary data analysis. Author NCJA managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the rot (pathogenic) effect of fungi isolated from diseased tomatoes on fresh healthy tomatoes obtained from Jos North LGA markets.
Place and Duration of Study: Jos North Local Government Area market, Plateau State; Microbiology laboratory, Federal College of Forestry, Jos, Plateau State between May 2015 and August 2015.
Materials and Methods: A total of thirty (30) fresh healthy and thirty (30) infected tomatoes (*Lycopersicon esculentum)* were obtained randomly from different market in Jos North LGA between May and August 2015. Three types of fungi were isolated from the infected tomatoes and morphologically identified. Fresh and healthy tomatoes were use for pathogenicity testing.

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Results: Percentage distributions of the isolated fungi showed *Geotrichum candidum* (45.16%), *Rhizopus stolonifer* (22.58%) and *Fusarium oxysporum* (32.26%). All the isolates caused spoilage of fresh and healthy tomatoes with *Rhizopus stolonifer* having the highest percentage severity of rot (70.88%), followed by *Geotrichum candidum* (38.73%) and *Fusarium oxysporum* (32.08%) after 96hours of incubation. The percentage severity results shows significant difference among the three fungi tested (*P*<001).

Conclusion: This study revealed that tomatoes rot are caused by different types of fungi and their activities, therefore proper handling procedure especially during post harvest should be ensured to avoid spoilage.

Keywords: Lycopersicon esculentum; Fusarium oxysporum; Geotrichum candidum; Rhizopus stolonifer; fungi; Jos.

1. INTRODUCTION

Tomato is one of the widely consumed fresh fruit worldwide since it contributes to a healthy well balanced diet which is rich in vitamins such as vitamin A, B, C and E. Carbohydrates such as fructose and glucose; Minerals which include phosphorus, sodium, potassium, calcium, magnesium and trace elements like iron, copper, zinc and dietary fibres [1]. The deep red colouration of ripened tomato is due to the presence of lycopene, a form of B-carotenoid pigment and a powerful antioxidant that help to protect against prostate cancer, cardiovascular disease and diabetes [2], thus there is an appeal and demands of the fruits by consumers as a result of their knowledge that they are healthy, tasty, convenient and fresh [3]. According to Mofeke et al. [4] vegetable crops constitute 30 to 50% of iron and vitamin A in resource poor diet. Vegetable crops including tomatoes are widely cultivated in most parts of Sub Sahara Africa, particularly by small scale farmers in most states of Nigeria [5,6]. Generally, global production of fruits and vegetables tripled from 396 million metric tonnes in 1961 to 1.34 billion metric tonnes in 2003 [7]. Global production of tomatoes is about 89.8 million metric tonnes from an area of about 3,170.000 ha [8]. Adegbola et al. [9] stated that Nigeria is undeniably the 14th largest producer of tomatoes, second to Egypt in Africa at 1.51 million metric tonnes valued at N87.0 billion with a cultivated area of 254,430 ha being the biggest producer in Sub-Sahara Africa.

Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic, these changes may be accompanied by alteration in taste, smell, appearance or texture [10]. Numerous microbial defects (Signs and Symptoms) of tomatoes are characterized by the type of microorganism responsible for the deterioration, in the process of infection in the case of fungal invasion follows the development of fungal penetrating structure. The colonization process involves the ability of the microorganism to establish itself within the produce [11]. Susceptibility of tomato to microbial colonization is due to its differential chemical composition such as high level of sugar, low pH (4.9-6.5) and its high water activity (p>0.99) which favours the growth of microorganism. Pathogenic microorganism in tomato is recognized as a source of potential health hazard to man and animals following ingestion, this is due to their production of toxins which are capable of causing diseases like respiratory infection, meningitis, gastroenteritis, diarrhoea in man [12]. The contamination of tomato by microorganism could be as a result of poor handling practices in the tomato production chain, storage condition, distribution, marketing practices and transportation [13].

Fungi are the most important and prevalent pathogens, infecting a wide range of fruit and causing destructive and economically important losses of fruits during storage, transportation and marketing [14]. Different fungal species such as *Alternaria*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus*, *Aspergillus*, *Geotrichum*, *Phytophthora* and *Botrytis* have been implicated as spoilage organisms [14], *Aspergillus niger*, *Rhizopus stolonifer* and *Mucor sp*. have been isolated from spoilt tomatoes [15]. *Aspergillus niger* have also been reported to cause a disease called black mold on certain fruits and vegetables such as grapes, tomato, onions and peanuts [16]. Tomatoes are ready-to-eat food, since they are minimally processed and many people take tomatoes raw directly or via meals of salad usually served cold. They have serious challenges to their existence, these include; climatic condition, pest, bacterial and fungal attack and over the years, there has been an increase in the need to identify and isolate the microorganism associated with their spoilage, especially fungi due to high measure of spoilage

they cause. Microbial spoilage and contaminating pathogens on this product poses a serious problem in food safety and national economy at large [12].

As consumers, the average population need to recognize that food safety is important for fresh fruits and vegetables. Also individuals of the population especially those in developing countries who usually use spoilt and slightly decaying tomatoes as a result of their cheaper prices should be educated that these spoilages are often not due to mechanical damages but microbial colonization and physiological decays.

This study was therefore aimed at determining the rot effect of fungi isolated from diseased tomatoes on fresh, healthy tomatoes obtained from Jos North LGA markets, with the specific purpose of creating awareness on the existing and much overlooked dangers of microbial spoilage, as well as buttressing the need to observe and emphasize on the measures to be taken to prevent further microbial contamination of fresh vegetables in the area.

2. MATERIALS AND METHODS

2.1 Study

This study was carried out in Jos North Local Government Area, Plateau State. Jos North L.G.A. is located on latitude 9°55´N and longitude 8°54´E, at an altitude of 1200 m above sea level. The area falls under Natural Region II of Nigeria's agro-ecological zones, the climate of the area is humid with an average annual rainfall and temperature between 140-1480 mm and 10°-32°C respectively.

2.2 Samples Collection

Infected tomato fruits with symptoms of softness were randomly procured locally (using simple random sampling technique) from Farin gada market, Terminus market and Jarawa Tomato market in Jos North LGA. Thirty (30) samples were collected randomly from three different sellers at the different markets (a total of thirty), placed in sterile polythene bags and conveyed into the laboratory for fungal isolation and subsequent identification.

2.3 Isolation of Fungal Organisms

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instructions and following the techniques described by Arora and Arora [17]. 80 mg of Gentamycin, an antibiotic was added to each 500 ml preparation of the agar to inhibit probable bacteria growth.

Diseased portion of the tomato fruits were cut under aseptic conditions into small bits into a sterile dish with the aid of scissors which was flamed over a Bunsen burner flame and dipped inside methylated spirit [18]. The cut diseased and sterilized bits with 70% ethanol were placed on Petri dishes containing solidified potato dextrose agar (PDA). The solidified plates were incubated at room temperature (28 plus or minus 2°C) in the dark until visible growths were seen on the plates. The fungal colonies grown from the incubated plates were sub-cultured into fresh medium until pure cultures were obtained.

2.4 Identification of Fungal Organisms

Microscopic examination was used for examining the colony characteristics. A sterile needle was used in taking a little portion of the colony, placed on the sterile glass slide, stained with lactophenol cotton blue and examined under the microscope for morphology and culture characteristics of fungal structures.

2.5 Pathogenicity Test

Healthy tomato fruits were surface sterilized with ethanol for 1 minute and washed in five changes of distilled water. Each fruit was weighed and readings were recorded. A 5 ml cork borer was punched to a depth of 4 mm into the healthy tomato fruits and the bored tissues were removed. A 5 mm diameter disc from the pure culture was placed back into the bored hole on the tomato fruits. The wounds were sealed with prepared candle wax according to the method of Fawole and Oso [18]. Control experiment was set up in the same manner except that the sterile agar disc was used instead of inoculums. The inoculated tomato fruits were placed at room temperature (28 plus or minus 2°C) under sterile condition. After 96 hours, the healthy fruits with inoculum introduction showed signs of rotten and the rotten part were scooped off, and then re-weighed. The control experiment showed no sign of rotten. Three (3) replicates were carried out for each inoculum. The pathogens obtained from the fruits were re-inoculated and identified using the same procedures described earlier.

2.6 Determination of Fungi Rots Severity

To determine the severity of rots caused by these organisms, the formula illustrated by Chuku et al. [19] was used based on the weight

values recorded from pathogenicity test procedure.

$$\% \text{ Severity} = \frac{W-w \times 100}{w}\%$$

Where W = Initial weight of healthy tomato
w = final weight of rotted fruit.

2.7 Statistical Analysis

One way Analysis of Variance (ANOVA) was used to analyze the results obtained to determine if the significant effect of the different fungi isolate on fresh healthy tomatoes. Each test was conducted at 95% confidence interval, $P<0.05$ at the appropriate degree of freedom (d.f.). A P-value of $P<0.05$ was considered significant. The data were analyzed using the program IBM SPSS version 22.

3. RESULTS

Table 1 illustrates the culture and morphological characteristics of the identified fungi. These were *Geotrichum candidum*, *Rhizopus stolonifer* and *Fusarium oxysporum*. Table 2 shows the percentage distribution of the fungal organisms isolated from infected tomatoes in the different markets. The percentage distributions were estimated; *G. candidum* (45.16%), *R. stolonifer* (22.58%) and *F. oxysporum* (32.26%). The result of the pathogenicity tests shown in Table 3 reveals that all the isolated fungi were pathogenic on tomato fruits. The percentage rot severity caused by *Rhizopus stolonifer* was 70.88%, *Geotrichum candidum* was 38.73% and *Fusaarium oxysporum* was 32.08%, indicative that *Rhizopus stolonifer* cause more rot severity than the other isolates within four days of incubation.

Table 1. Culture, morphological characteristics and identification of fungal species

Culture characteristics	Microscopic characteristics	Identification
Clustered growth, appears creamy on the surface	Macro-conidia sparse in some strains, borne on phialides on branched conidiospores, septate, fusiform, more or less curved, pointed at both ends with a pedicellate basal cell	*Fusarium oxysporum*
Distinct colonies, whitish became grayish brown	Columnella globose, subglobose or ovoid. Zygospores brownish-black, warted, with unequal suspensors	*Rhizopus stolonifer*
Mouldy surface covered the plate	Advancing hyphae septate, dichotomously branched (forked). Conidia cylindrical, barrel-shaped or ellipsoidal, formed by breaking up fertile hyphae, chains mostly aerial, erect or decumbent.	*Geotrichum candidum*

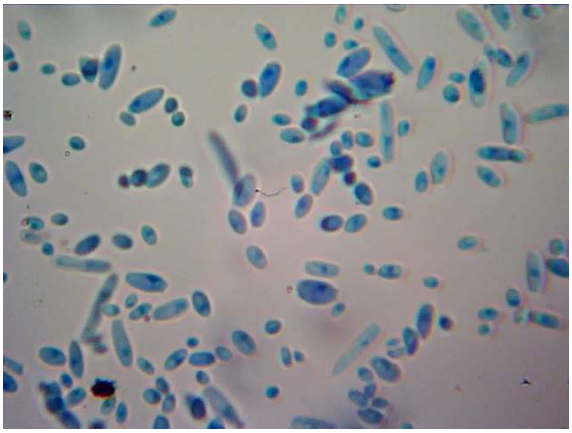

Fig. 1. *Fusarium oxysporum*

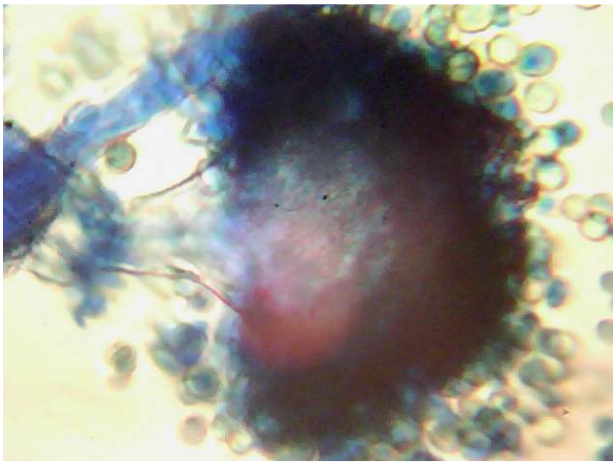

Fig. 2. *Rhizopus stolonifer*

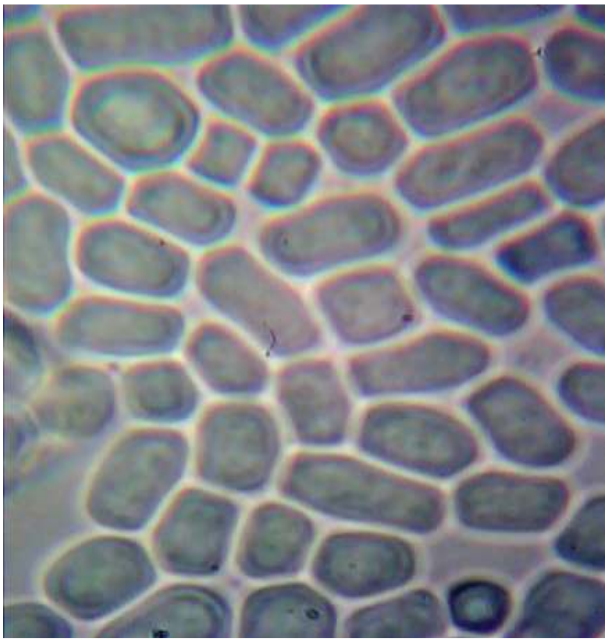

Fig. 3. *Geotrichum candidum*

Table 2. Percentage distribution of fungal organisms isolated from infected tomatoes

S/No	Organisms	Number of organisms isolated	Percentage (%)
1	*G. candidum*	14	45.16
2	*R. stolonifer*	7	22.58
3	*F. oxysporum*	10	32.26
Total		**31**	**100.00**

4. DISCUSSION

Tomato fruits from Farin gada Market were more contaminated with most fungi isolates than the other two markets. This could be as a result of poor handling, packaging, storage and transportation, eventually resulting in decay and increase the growth of microorganisms, which become activated because of the changing physiological state of the produce [20].

Table 3. Mean effect of rot caused by the fungal isolates after four days of incubation

Organism	Severity rot (%)
A	70.88[a]
B	38.73[b]
C	32.08[c]
SE±	2.28

Means in the same column having the same letters are not significantly different (P<0.001)
Keys: A – Rhizopus stolonifer, B – Geotrichum candidum, C – Fusarium oxysporum

Fig. 4. Fungi rot

This study revealed the frequency of occurrence of fungi (*Geotrichum candidum, Rhizopus stolonifer* and *Fusarium oxysporum*) and observed that the fungi were pathogenic to fresh healthy tomatoes in Jos North L.G.A. The study revealed that *Geotrichum candidum* had higher prevalence of contamination, followed by *Fuarium oxysporum*, then *Rhizopus stolonifer* had least prevalence though causes the most severe rot.

The prevalence of fungi as the spoilage organism of fruits and vegetables is due to a wide range of factors which are encountered at each stage of handling from pre-harvest to consumption and is related to the physiological and physical conditions of the produce as well as the extrinsic parameters to which they are subjected [21].

Fusarium oxysporum isolated in this study is one of the fungi responsible for spoilage of tomato fruit which agrees with the finding of Ibrahim et al. [22] carried out in North Western Nigeria. Okigbo and Nmeka [23] also identified similar fungi from Yam in their work on Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. Effiuvwevwere [21] reported that *Fusarium* sp, and *Rhizopus stonolifer* are responsible for the soft rot of tomato fruits. Ohr et al. [24] also reported that *Fusarium moloniforme, Aspergillus niger* and *Rhizopus stonolifer* were isolated from rotten tomato fruits. Abhinaba [25] additionally found *Fusarium* as fungi specie responsible for spoilage of tomato.

Geotrichum candidum was encountered more frequently in the infected tomato samples collected, followed by *Fusarium oxysporum,* then *Rhizopus stonolifer* was least encountered. This is similar to the work of Abhinaba [25] who reported high occurrence of *Fusarium sp.* as one of the fungi responsible for spoilage of tomato fruit. In variation with this work he identified *Aspergillus niger* and *Penicillum sp*.

The pathogenicity test result revealed that all the fungi isolate identified (*Geotrichum candidum, Rhizopus stolonifer* and *Fusarium oxysporum*) have the ability to cause infection to healthy tomato fruits, though having various percentage of rot severity. *Geotrichum candidum* have 38.73%, *Rhizopus stolonifer* have 70.88% and *Fusarium oxysporum* have 32.08% rot severity. Statistical analysis showed significant different among rot severity of these organisms at (P<0.001); this agrees with the work of Chuku et al. [19], however differences was revealed in that they recorded *Fusarium moniliforme* instead of *Fusarium oxysporum*.

Rhizopus stolonifer caused the highest tomato rots in this study. This observation is in line with the report of Okoli and Erinle [26] and Chuku et al. [19] which stated that *R. stolonifer* caused the most rapid rotting on stored tomatoes fruits in Nigeria. Chuku [27] also reported that *Rhizopus stolonifer* recorded the highest rot (80%) on Avocado pear in Nigeria. The rot induced by *Geotrichum candidum* exhibited moderate to severe infection 2 days after inoculation. *Fusarium oxysporum* showed a water soaked lesions that spread rapidly covering the fruit which then became covered by white cottony mycelium topped by pinkish mass of fungal spore.

Rot development of the inoculated tomatoes were gradual. In all cases soft rot was induced that became watery after 4 days and accompanied by offensive odour. Several other fungi have been described as potential postharvest pathogens. Such fungi are able to infect tomato fruit in the field and later lead to postharvest decays [28].

Pathogens identified from these fruits are microorganisms and a major characteristic of microorganisms is its ubiquitous nature. Fungi produce spores which can be spread long distances by air, water, or soil borne and also can over-winter or over-summer until the subsequent growing season when condition is favourable for their further development. They also produce mycotoxins which is dangerous to human health. *G. candidum* for instance produces geotrichosis, which affects the oral, bronchial, skin and bronchopulmonary epithelia in human [29].

5. CONCLUSION

This work revealed that several fungi growth on tomatoes result in rots. The result obtained from frequency of occurrence of fungi associated with tomatoes and their rot activities on tomatoes gotten from three markets in Jos North LGA shows a close relationship with works conducted by different researchers in other geographical locations. This suggest that *Rhizopus stolonifer, Fusarium oxysporum* and *Rhizopus stonolifer* are among the most common fungi associated with post hervest tomatoes deterioration. Hence the need for proper handling of farm produce and use of fungicides in the control of pathogenic fungi.

6. RECOMMENDATION

Further research should be explored to determine effects of wide range of fungi on tomatoes and anti-fungal drug should be tested as a means of rot control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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