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# PATHOGENIC FUNGI OF SOME SELECTED VEGETABLES IN SOKOTO METROPOLIS

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## *ABSTRACT*

*A study on fugal pathogens associated with vegetable crops was carried out in Sokoto metropolis, Sokoto State. Market and fresh field vegetable samples were randomly and purposively collected from three different locations using standard procedures for sample collections. All the samples were brought to mycology laboratory of Usmanu Danfodiyo University, Sokoto for analysis. Samples were separately cultured on Potato Dextrose Agar (PDA) medium for fungal growth, isolation and identification. Pathogenicity test of isolates was carried out. The result obtained revealed that thirty three (33) fungal isolates were identified based on cultural and microscopic characteristics. The isolates were Rhizopus oryzae, Scopuriolupsis candida, Aspergillus niger, Scoporiolupsis flava, Rhizopus stolonifer, Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata and Absidia ramosa. Pathogenicity test result indicated that all the isolates except S. candida, S. flava and M. racemosus were able to cause rot on fresh vegetable samples within one week of inoculation at room temperature of (32 ± 2$^0$C). The most virulent among the test organisms were R. Oryzae and R. Stolonifer with rot incidence of 80% and the least were M. Racemosus and A. Alternata with rot incidence of 20%. The study suggested that pathogenic fungi cause rot of vegetables within few days of incubation. Thus, this could lead to heavy loss of the crops for farmers and marketers due to deterioration. This research could help in educating farmers and sellers of vegetables on the effects of types of fungal pathogens on the produce in order to ease ways of the managing and controlling of such vegetables' pathogens.*

***KEYWORDS:*** *Fungal Pathogens; Vegetables; Pathogenicity; Infection; Incubation*

## INTRODUCTION

Fungi are a large group of heterogeneous eukaryotic, spore bearing, achlorophyllous organisms that generally reproduce asexually and sexually (Khalid *et al*., 2006). Some are agents of diseases in plants and animals while others are saprophytic (Prince and Prabakaran, 2011). Fungal plant pathogens cause serious losses in world food crops, particularly postharvest (Fisher *et al*., 2012). Among different species of fungi *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp cause heavy losses to grains, fruits, vegetables and other plant products during picking, transit and storage rendering them unfit for human consumption by producing mycotoxins, hence affecting their nutritive value (Miller, 2002; Janardhana *et al*., 1998; Galvano *et al*., 2001). Vegetable diseases generate serious economic losses and increase production costs (Claudia *et al*., 2010) for growers. Fresh fruits, vegetables and flowers may be infected with various pathogens which are not visible prior to storage but will cause decay and rot during storage and transportation. There is an increasing demand for vegetables as food and this is largely so because of the increase in the level of awareness of the importance of having such crops to be included in our diet, particularly for the diabetic and hypertensive patients as well as obese and ageing members of the population. Most pathogenic fungi infect crops on the field before harvesting and storage. However, most of these diseases

manifest during storage, thereby causing deterioration and damages leading to great loss to farmers. Most farmers, especially peasant farmers, lack knowledge of the pathogens that are responsible for deteriorations, spoilages and diseases of the vegetables. Therefore, this research is aimed at determining pathogenic fungi of some vegetables in Sokoto metropolis and making the findings available for farmers and other vegetable growers.

## MATERIALS AND METHODS

### Sampling Size

### Field Samples

Field samples of selected vegetables were purposively collected from the cultivation areas. At each farm, 120 square feet was marked and samples were collected at an interval of 25 feet. For each location, five samples of each selected vegetable type were picked and a total of 30 samples per selected vegetable types collected. These were separately placed in sterile bags and brought to mycology Laboratory, Usmanu Danfodiyo University, Sokoto for the research. Selected vegetable samples collected were fruits of *Solanum lycopersicum* L. (Tomatoes), *Capsicum annuum* L. (Chilli pepper), *Solanum melongena* L. (eggplant) and leaves of *Brassica oleracea* L. (cabbage) and tuber of *Daucus carota* L (carrot).

### Market Samples

Market samples of the selected vegetables were randomly collected from different stalls from vegetable sellers, at the Sokoto Meat and Vegetable Market. Three stalls were selected at random for the collection through ballot. A total of 10 samples of each fruit of tomatoes, chilli peppers and eggplants, cabbage leaves and tubers of carrot were collected. A total of 30 pieces of each vegetable type were collected. The samples were separately placed in sterile bags and brought to the mycology laboratory, Usmanu Danfodiyo University, Sokoto, for isolation and identification of fungi.

### Media Preparation

Potato Dextrose Agar (PDA) was used. The agar was prepared according to the manufacturer's specification. Thirty nine grams (39 g) of dehydrated powder (PDA) was weighed and suspended in 1 litre of distilled water that is 1000 ml in a conical flask and was heated on a hot plate ($40^{o}$C) to dissolve completely. Cotton wool and aluminium foil was used to wrap the mouth of the conical flask, which was then autoclaved (Model-YX-28OA) at $121^{0}$C for 15 minutes to sterilize the medium. The medium was allowed to cool and 20 ml was dispensed in sterilized (90 mm) Petri dishes and allowed to solidify. One milligram (1 mg) of streptomycin was added to suppress bacterial growth (BAM, 2018).

### Isolation of Fungi from Diseased Vegetable Samples

The method of Okigbo and Emeka (2010) was adopted for isolation of fungi from diseased rotted samples. Rotted samples were rinsed in sterilized water, surface sterilized with 70% ethanol and cut open. About three pieces (3 mm diameter) of the diseased tissues were picked with a flame-sterilized forceps and inoculated on the solidified PDA medium in different plates. The inoculated plates were incubated at room temperature (35±2°C) and observations were made daily for emergence of colonies. The colonies obtained were subcultured by aseptically isolating and transferring the colonies from the culture to a fresh medium in order to obtain pure isolates. The fresh medium was then incubated at room temperature (33 ± 2°C) for growth of the organisms. Pure cultures of the fungal isolates were used for identification. While some parts of the pure isolates were kept as stock cultures, stock cultures were prepared using slants of PDA in McCartney bottles and stored in a refrigerator at 4°C.

**Identification of the Fungal Isolates**

Fungi were identified based on morphological and cultural characteristics as outlined in mycological atlas and Description of Medical fungi (David *et al*., 2007). The fungal isolates was mounted on slides and viewed under a light microscope at x 40 magnification, Model-XSZ-21 for microscopic identification. The pure cultures obtained were then transferred into slant bottles, labeled and kept for later use. Some of the identified pure isolates were used as test organisms for further investigations.

**Test for Pathogenicity of the Fungal Isolates**

The method described by Anukwuorji *et al*. (2013) was used for the pathogenicity test. Each of the test fungi was tested for its ability to cause disease (rot) condition in healthy, fresh sampled vegetable. Healthy vegetables were washed with tap water, rinsed in five changes of distilled water to remove dirt and debris from their surfaces, then surface sterilized with 70% ethanol. Each sample was bored to a depth of 1 cm, using flame sterilized 5-mm diameter cork borer, and the bored tissues were removed aseptically. A disc of seven days' old PDA pure cultures of the test isolates were inoculated inside the hole made on the test vegetable sample. A portion of the vegetable flesh removed earlier was cut off to compensate for the thickness of the agar-inoculum and then replaced core. The points of inoculation were sealed with Vaseline to prevent entry of external contaminants. The control was set up in the same manner except that sterilized agar disc was used instead of the inoculum. The inoculated samples were placed in three replicates and incubated for seven days at a room temperature (in August). Each preparation was observed daily for signs of rot or establishment of rot. Data were expressed in percentage. The formula in calculating the rot incidence is (Anon, 2008):

$$\% \text{ rot incidence} = \frac{\text{Number of vegetable samples infected} \times 100}{\text{Total number of vegetable samples}}$$

## RESULTS

Table 1 indicated that significant differences exist between the field and market samples of tomato, cabbage and chilli pepper in terms of the number of fungal isolates obtained from the samples. However, there was no significant difference between the field and market samples of carrot and eggplant with regards to the number of fungal isolates obtained.

**Table 1: Number of Fungal Organisms Isolated from Vegetables Based on the Sample Source**

| Vegetable | Sample Source | | SEM | Sig. |
|---|---|---|---|---|
| | Fresh Field | Market | | |
| Tomato | 1 | 6 | 0.138 | ** |
| Carrot | 4 | 4 | 0.000 | - |
| Cabbage | 2 | 4 | 0.151 | ** |
| Egg Plant | 3 | 3 | 0.000 | - |
| Chilli Pepper | 1 | 5 | 0.122 | ** |

**** =** There is significant difference

SEM = standard error mean

Sig = significant difference

In the field samples, *R. oryzae* (Plate I) was a common isolate of tomato, carrot and cabbage while *R. stolonifer* (Plate II) was commonly associated with carrot, eggplant and chilli pepper. *S. candida* (Plate III) was observed to be an organism associated with carrot, cabbage and eggplant. *S. flava* (Plate IV) was isolated from carrot only while *A. ramosa* was found to be associated with eggplant alone. Isolates from vegetable samples from market in Sokoto metropolis include: *R. oryzae* which was a common isolate of tomato, carrot and cabbage. *R. stolonifer* was found to be a common isolate of carrot, eggplant and chilli pepper. *A. fumigatus* (Plate V) was commonly associated with tomato, carrot, cabbage and chilli pepper while *A. niger* (Plate VI) was observed to be associated with tomato, cabbage, egg plant and chilli pepper. *A. flavus* (Plate VII) was isolated from tomato, cabbage and chilli pepper. *M. racemosus* (Plate VIII) was a common isolate of tomato and chilli pepper. *A. alternata* (Plate IX) was found to be associated with tomato and carrot. *S. flava* was isolated from eggplant alone.


**Figure 1: *Rhizopus Oryzae* (Colonies have White Cottony at First then Changed to Brownish Grey and Blackish-Grey).**

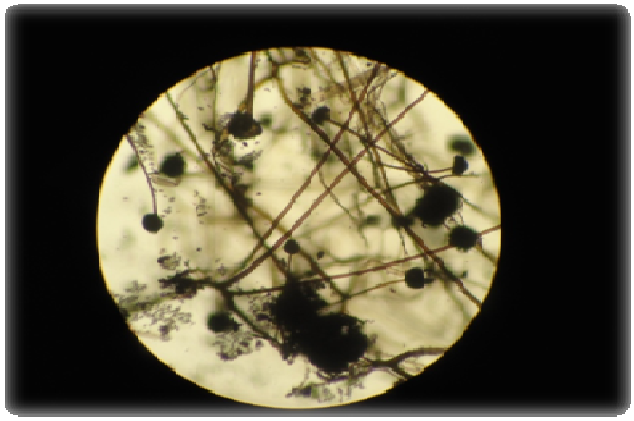

**Figure 2: Microscopic Characteristics of *R. Oryzae* (Sporangia were Globose with a Flattened Base, Grayish Black, Powdery Appearance).**


**Figure 3: *R. Stolonifer* (Colonies were Whitish but Later Changed Grayish-Brown).**

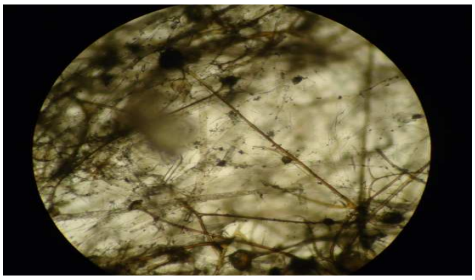

**Figure 4: Microscopic Characteristics Of *R. Stolonifer* (Sporangia Appeared To Be Brown-Black. Sporangiophores Were Brownish And Irregular In Shape)**


**Figure 5: *S. Candida* (Colonies Appeared White and Powdery with a Central Tuft. the Color became Dark after Two Weeks of Growth. the Reverse of Plate was Creamy).**

**Figure 6: Microscopic Characteristics of *S. Candida* (Conidia are Subglobose with More or Less Rounded Apex and Smooth Walled Which is Whitish in Mass).**


**Figure 7: *S. Flava* (Colonies Appeared Creamy, Funiculose and then became Powdery with a Prominent Central Tuft. the Reverse of the Plate was also Creamy)**

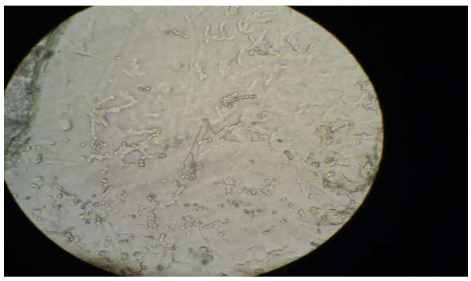

**Figure 8: S. Flava Microscopic Characteristics (Conidia are Subglobose with Distinctly Truncate base, with Pointed Apex and Rough Walled)**

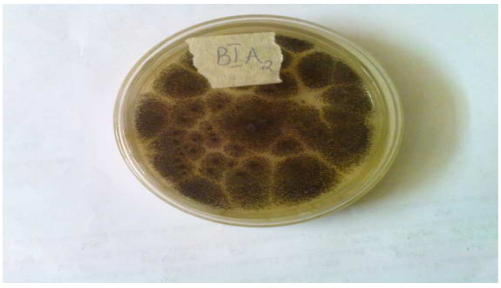


**Figure 9: *A. Fumigatus* (Colonies Showed Typical Blue–Green Surface Pigmentation)**

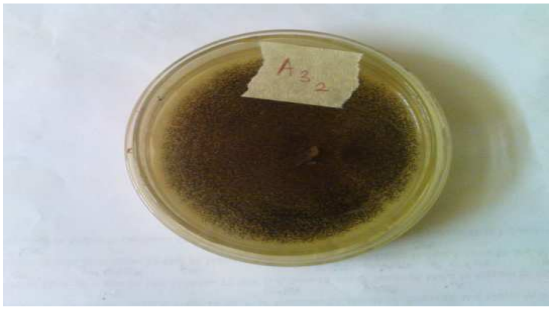


**Figure 10: *A. Niger* (Colonies Appeared Black and Spongy. the Reverse of Plate is Creamy).**

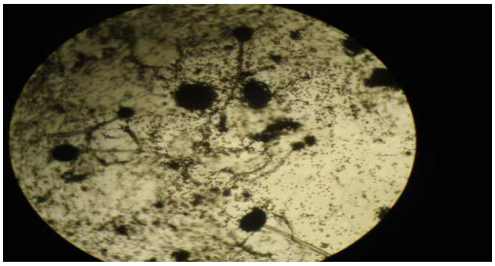

**Figure 11: Microscopic Characteristics of *a. Niger* (Conidial Heads are Large, Globose and Dark Brown.)**


**Figure 12: *A. Flavus* (Appeared Yellow–Green at First but become Dark Yellow–Green with Age)**


**Figure 13: *M. Racemosus* (Colonies were White, Later became Brownish–Grey).**

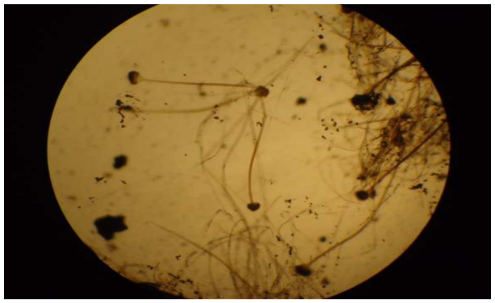

**Figure 14: Microscopic Appearance of *M. Racemosus* (the Sporangiophores were Branched. Sporangia Hyaline Later became Brownish.**

**Figure 15: *A. Alternata* (Colonies were Black and Reverse of Plate also Appeared Black).**

The results of pathogenicity test (Table 2) revealed that all the test organisms (*A. fumigatus, A. flavus, A. niger, R. oryzae, M. racemosus, A. alternata, R. stolonifer and A. ramosa*) except two (*S. candida and S. flava*) induced rot on the healthy vegetable samples after seven days of inoculation. The isolates differ in their ability to cause rot, hence the most virulent among the test organisms were *R. oryzae* and *R. stolonifer* with rot incidence of 80%, followed by *A. fumigatus, A. flavus* and *A. niger* 60%, the least virulent was *A. ramosa; M. racemosus* and *A. alternata* with rot incidence of 40% and 20% respectively.

**Table 2: Pathogenecity Test of the Identified Fungal Isolates.**

| Host | Isolate | Rot Incidence (%) |
|---|---|---|
| Tomato | *A. fumigatus* | 60 |
| | *A. flavus* | 60 |
| | *A. niger* | 60 |
| | *R. oryzae* | 80 |
| | *M. racemosus* | 20 |
| | *A. alternate* | 40 |
| Carrot | *R. oryzae* | 60 |
| | *R. stolonifer* | 60 |
| | *S. candida* | 00 |
| | *S. flava* | 00 |
| | *A. fumigatus* | 40 |
| | *A. alternate* | 20 |
| Cabbage | *A. fumigatus* | 60 |
| | *A. flavus* | 60 |
| | *A. niger* | 60 |
| | *R. oryzae* | 60 |
| | *S. candida* | 00 |
| Eggplant | *A. niger* | 40 |
| | *R. stolonifer* | 60 |
| | *S. flava* | 00 |
| | *S. candida* | 00 |
| | *Absidia romasa* | 40 |
| Chilli pepper | *R. stolonifer* | 80 |
| | *A. fumigatus* | 60 |
| | *A. flavus* | 60 |
| | *A. niger* | 60 |
| | *M. racemosus* | 00 |

## DISCUSSIONS

Vegetable samples obtained from market have the highest number of isolates (22) compared to field ones (11). This could probably be because infection might have started at the field and then fully developed in the market stalls. This is in accordance with Pawanexh (2008) findings that fresh fruits, vegetables and flowers may be infected with various pathogens, which are not visible prior to storage but will cause decay and rot during transit and at storage. Kader (1986) reported that environmental factors such as physical damage and chilling injury from the field and on transit influenced deterioration of vegetables, thus making them susceptible to decay.

The variation in the frequency of occurrence of fungi isolated from both field and market vegetable samples could be due to the ability of the organisms to utilize the substrate for growth differently. This is in accordance with the findings of Shehu and Bello (2011), who reported that variation in the frequency of occurrence of fungi reflects in the inoculums' density in the area or prevailing environmental conditions favoring their growth. The ability of the test fungi to cause rot and deterioration of vegetables' samples, as indicated from the pathogenicity test could be due to the fact that different fungi have different growth rate depending on temperature, pH and relative humidity, as reported by Agrios (2005). Furthermore, different organisms have different ability to respond to physiological activity such as growth and development under the same environmental conditions. This is in agreement with Rayner and Boddy (1988), who reported that attainment of full growth by pathogenic fungi varies from one species to the other given the same environment condition such as moisture, temperature, pH and appropriate media.

## CONCLUSIONS AND RECOMMENDATIONS

The research revealed that the highest number of fungal pathogens were isolated from market vegetable samples. *R. oryzae* and *R. stolonifer* have the highest frequency of occurrence and the least was *A. ramosa*. The pathogenicity test of fungal isolates confirmed that all the fungi were pathogens except *S. candida* and *S. flava* because they were able to cause rot on healthy fresh samples within a week of inoculation. It was also discovered that the level of deterioration defers amongst the isolates. This research could be used as a guide by vegetable growers on how to manage and control the spread of different fungal pathogens of vegetable crops on the field and in storage.

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