ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 4 No. 2 December 2018*

Isolation of Fungi associated with Spoilage of Selected Vegetables sold in Dutse-Ultra Modern Market, Jigawa State, Nigeria

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Abstract
A study to isolate fungal contaminants particularly those responsible for spoilage of tomatoes, onions and cabbage sold at Dutse-Ultra Modern Market in Dutse Metropolis, Jigawa State, Nigeria was conducted. The spoilt samples of tomato, onion and cabbage were cut into pieces each with a sterile razor blade. The samples were then cultured on PDA and incubated at room temperature for 5 days after which the fungal growths were observed. The isolates were purified on Sabouroud's Dextrose Agar plates. A total of thirty (30) fungal isolates were obtained from the three samples. The fungi isolated and the frequency of occurrence included: A. niger (36.36%), R. stolonifer (27.27%), A. flavus (18.18%), Mucor Spp (9.10%) and Penicilllium Spp. (9.10%) were found to be associated with contamination of tomato. A.niger (55.55%), R. stolonifer (22.22%), A. flavus (11.11%) and Penicillium spp. (11.10%) were found to be associated with contamination of onion. A. niger (30%), R. stolonifer (30%), A. flavus (20%), Mucor spp. (10%) and Penicillium spp. (10%) were found to be associated with contamination of cabbage. Based on these findings, it was observed that perishable food such as tomato, onion and cabbage are susceptible to spoilage by fungi probably because the spores of these organisms are easily transmitted via the air whichcould lead to spoilage of these vegetables. The study recommends that thorough washing and storage of the vegetablesat the appropriate temperatures should be carried out to minimize the level of contamination.

Keywords: Spoilage, Perishable, Fungi, Agar, Microorganisms

INTRODUCTION
Fruit and vegetables constitute commercially and nutritionally important indispensable food commodity. Fruit and vegetables play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet that can

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help to keep a good and normal Health (Al-Hindi *et al*., 2011). Fruits and vegetables are widely distributed in nature. One of the limiting factors that influence the fruits and vegetables economic value is the relatively short shelf-life period caused by pathogens attack (Al-Hindi *et al*., 2011).

It was estimated that about 20-25% of the harvested fruit and vegetables got decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006). In developing countries, post-harvest losses are often more severe due to inadequate storage and transportation facilities. Fungal fruit and vegetables infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer (Al-Hindi *et al*., 2011). Fruits and vegetables contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007).

Micro-organisms are associated in a variety of ways with all the food we eat. They may influence the quality, availability, and quantity of our food. Naturally occurring foods such as fruits and vegetables normally contain some micro-organisms and may be contaminated with additional organisms during handling.

Consumption of fruit and vegetable products has dramatically increased in the world by more than 30% during the past few decades (Barth *et al*., 2009). During the period 1970–2004, US per capital consumption of fruit and vegetables increased by 19.9%, to 694.3 pounds per capita per year (ERS, 2007). Fresh fruit and vegetables consumption increased by 25.8% and 32.6%, respectively, and far exceeded the increase for processed fruit and vegetable products (Barth *et al*., 2009).It is also estimated that about 20% of all fruit and vegetables is lost each year due to contamination (Barth *et al*., 2009).

Fruits and vegetables are vital sources of nutrients to human beings. They give the body the necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development (Bajuka *et al*., 2014.).Fruits and vegetables however, have serious challenges to their existence; these include changes in climatic condition, pests and microbial attack (Bajuka *et al*., 2014). Over the years, there has been an increase in the need to identify and isolate the fungi associated with the contamination, as a way of finding a means of control (Akinyele and Akinkunmi, 2012).

Susceptibility of fruits and vegetables islargely due to differential chemicalcomposition such as pH and moisture contents are associated with greaterpredisposition to microbial spoilage. Theoccurrence of fungal spoilage of fruits isalso recognized as a source of potential health hazard to man and animal. This isdue to their production of mycotoxins (naturally occurring toxic chemical oftenof aromatic structure) which are capable of producing aflatoxin in man, following ingestion or inhalation (Bajuka *et al*., 2014).

These fruits and vegetables are usually displayed onbenches and in baskets for prospectivecustomers in the open markets until sold, thereby exposing them to further microbial infections beside those associatewith these whole fruit and vegetables surface and thosefrom adjacent infected fruits (Baiyewu *et al*., 2007).Fruit and vegetables like Tomato, Onion, and Cabbage are cultivated worldwide and have the greatest nutritional value

(Thompson, 1998). Because they are seasonal food, their mycological study is necessary so as to identify the organisms associated with their contamination.

MATERIALS AND METHODS

Study Area

The study area was Dutse Ultra-Modern market within Dutse metropolis located along Ibrahim Aliyu bye-pass opposite mobile police base at latitude 1142′04″N 920′31″E.It is the major market in the state capital were different kinds of vegetables are sold.

Study Population

A total of thirty samples of tomato, onion and cabbage were randomly collected from different sites of within the study area, where 10 samples of each vegetable were collected.

Sample collection

The samples were aseptically collected using sterile leather bags and disposable sterile hand gloves and taken immediately to the laboratory for analyses.

Sample Preparation and Inoculation

The contaminated portions of the samples were cut into pieceseach with sterilized razor blade, sterilized in 1% sodium hypochlorite solution for 2 minutes and then placed gently on Potato Dextrose Agar plate (PDA) using spread technique as described by Akintobi *et al*., (2011), Dimpka and Onugba (2010), Ebenezer and Seshi (2012) and Kutama *et al*. (2012).

Sterilization

All apparatuses or materials used for the research were sterilized using an autoclave at 121^{o}C for 15 minutes. This was done to avoid contamination during the media preparation as well as the sample processing.

Media Preparation

All the media used were weighed, prepared and sterilized according to the manufacturer's instruction.

Incubation of the Inoculated Plates

The inoculated plates were incubated at room temperature for 5-7 daysunder light to enhance fungal growth and sporulation (Okigbo and Osuinde, 2003).

Purification of the Fungal Isolates

After the appearance of a mixed growth, each colony was sub-cultured in a fresh Sobaroud Dextrose Agar in order to obtain a pure culture by using streak method as described by Cheesbrough (2006).

Identification and Characterization of Fungal Isolates

Identification and characterization of the various isolates were based on macroscopic and microscopic examination outlined in Kutama and Aliyu (2007).

Macroscopic Examination

This was done on the basis of the nature appearance of the colony and morphological characteristics of the isolated fungi using Color Atlas of Diagnostic Microbiology by Luis *e tal*., (1997).

Microscopic Examination

Slides of the mycelium observed from different isolates were prepared as follows: A few drops of lacto phenol cotton blue solution was placed at the centre of clean grease free slides. A small portion of the unidentified fungal isolates were picked with sterile wire loop. The portions were placed in the lacto phenol cotton blue droplet on the slides and emulsify out with a sterile wire loop. Cover slips were placed at the centre of the slides for viewing and examining the structure of the mycelia, spore structure and fruiting bodies were identified with the help of Standard Color Atlas of Diagnostic Microbiology by Louis *et al*. (1997).

Determination of Percentage Frequency of Occurrence

The frequency of the occurrences of different types of isolated fungal contaminants associated with spoilage of tomato, onion and cabbage were determined. The percentage of the occurrence was calculated using the formula (Morsy *et al*., 2009).

$$\% \text{ Frequency} = \frac{Number\ of\ isolated\ fungi}{Total\ number\ of\ isolates} \times 100$$

Results

The fungal contaminants associated with the spoilage of tomato, onion and cabbage were identified based on colony appearance, morphology and cellular characteristics as shown in Table, I. The frequency of occurrence of fungi associated with tomato were shown in Table, 2 where *A. niger* (36.36%), R. *stolonifer* (27.27%), *A. flavus* (18.18%), *Mucor Spp.* (9.10%) and *Penicillium Spp.* (9.10%) were found to be associated with the contamination of tomato. Table, 3 shows the frequency of occurrence of fungi associated with onion where *A. niger* (55.55%), R. *stolonifer* (22.22%), *A. flavus* (11.11%) and *Penicillium Spp*. (11.10%) were found to be associated with the contamination of onion and finally, *A. niger* (30%), *R. stolonifer*(30%), *A. flavus (*20%), *Mucor spp.* (10%) and *Penicillium Spp*. (10%) were found to be associated with the contamination of cabbage as shown in Table, 4. However, all the control experiments of fresh tomato, onion and cabbage showed no any fungal growth.

Table 1: Colonial and Morphological characteristics of fungal contaminants associated with spoilage of Tomato, Onion and Cabbage

Colony appearance	Morphology and cellular characteristics	Fungi isolated
Yellow fluffy myecilia and some black sporongiospores	Septate hyphae with filamentous structure	*A. flavus*
Colonies with loose white myecilium rapidly becoming dark brown to black on the development of conidia	The conidiospore are large with septate hyphae	*A. niger*
White to grey and fast growing with some black sporongiospore	The sporongiosphores have terminal sporogaia containing round sporongiospores and columella was well developed with non septate hyphae	*Mucorsp.*
Green fluffymyecilia with some white sporongiospore	Septate hyphae with filamentous structure	Penicillium *sp.*
Colonies light grey, growing rapidly and filling the petri dish with dense cottony mycelium, producing mass of sporangia	Large sporangiophores with non septate hyphae were formed.	*R. stolonifer*

Table 2: **Frequency of occurrence of identified fungal contaminants associated with spoilage of Tomato**

Identified Fungal Isolate	Number of isolates	Frequency of occurrence
A. flavus	2	18.18 %
A. niger	4	36.36%
R. stolonifer	3	27.27%
Penicillium spp	1	9.10%
Mucor spp	1	9.10%
Total	**11**	**100%**

Table 3: Frequency of occurrence of identified fungal contaminants associated with spoilage of Onion

Identified Fungal Isolate	Number of isolates	Frequency of occurrence
A. flavus	1	11.11 %
A.niger	5	55.55%
R. stolonifer	2	22.22%
Penicillium spp.	1	11.11%
Total	**9**	**100%**

Table 4: Frequency of occurrence of identified fungal contaminants associated with spoilage of cabbage

Identified Fungal Isolates	Number of isolates	Frequency of occurrence
A. flavus	2	20%
A. niger	3	30%
Mucorspp	1	10%
R. stolonifer	1	10%
Penicillium spp	3	30%
Total	**10**	**100%**

Discussion

Vegetables are important farm produce that can get infected at the point of harvest, during handling, processing, storage or at point of sale. This has caused serious economic impact on the farmers, consumers and the society at large. Environmental conditions such as temperature, humidity, acidic pH and other factors contribute to spoilage of these perishable items. Dutse and indeed Jigawa state has Sahel savannah vegetation with high temperature and humidity that supports the growth and proliferation of these fungal species. The findings of this study showed that several fungi at different frequency of occurrences were found to be associated with contamination of tomato, onion and cabbage commonly sold at Dutse Ultra-Modern market. The most commonly encountered fungi associated with tomato contamination were; *Aspergillus flavus (18.18%)*, *A. niger (36.36%)*, *Mucor spp*. (18.18%), *Penicillium spp*.(9.10%) and *Rhizopus stolinifer (27.27%)*. This is in agreement with the finding of Akintobi *et al*., (2014) who isolated *A. niger*, *R. stolonifer*, *Mucor spp*. and *A. flavus* from microorganisms associated with deterioration of tomato and pawpaw at Umuahia market, Abia State, Nigeria. This could probably be due to mechanical injuries such as cuts that occur during harvesting, post-harvesting or storage periods which could provide infection sites for spoilage fungi, improper handling and lack of good storage facilities, ability of the fungi to produce spores and their ubiquitous nature, environmental conditions such as temperature and relative humidity etc.*A. niger* (55.55%), *R. stolonifer* (22.22%), *A.flavus* (11.11%) and *Penicillium spp*. (11.10%) were found to be associated contamination of onion. This is in contrast with the finding of Samuel and Ifaenyi, (2015) who isolated *A. niger*(34%) *R. stolonifer* (11.43%) and *Penicillium spp.* (11.43%) from onion bulb sold in some markets of Awka, Anambra State, Nigeria. This is closely linked to the fact that spores of these organisms are easily transmitted via the air which could lead to spoilage of these vegetables*A. niger* (30%) *R. stolonifer*(30%), *A. flavus* (20%) *Mucor spp*. (10%) and *Penicillium spp*. (10%) were found to be associated with contamination of cabbage. This is in agreement with the findings of Shu'aibu *et al.,* (2014) who isolated *A. flavus*, *R. stolonifer* and *Mucor spp.* from fungi associated with contamination of cabbage and lettuce sold in Kano metropolis. Some of the fungi isolated from this research have been reported to produce secondary metabolites which are potentially harmful to humans and other animals (Baiyewu *et al*., 2007).

Conclusion

Vegetables sold in Dutse Ultra-modern market were found to be contaminated with different species of fungi which are responsible for the spoilage of these vegetables. Findings from this research revealed that tomato, onions and cabbage had the highest contamination from *A. niger, R. stolonifer and A. Flavus* respectively.

Recommendations

From this study, the following recommendations were made:

- Appropriate control measures should be employed during the harvesting, transportation, handling and processing of tomato, onion, cabbage or any other perishable food items because of their susceptibility to spoilage by microorganisms particularly fungi.

- The vegetable sellers association should collaborate with Government to ensure provision of good storage facilities in the market in addition to vendor - education through sensitization, focused group discussion and workshops among others.
- Further research should be carried out on other species of microorganisms such as bacteria, viruses and protozoa.

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