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Mycological evaluation of spoilt onion bulbs sold in Lapai town

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Abstract

Mycological investigation on spoilage fungi on apparently infected onion bulbs collected from Lapai, Niger state, Nigeria was conducted using both direct plating method and dilution of milled onion paste. Total of 15 onion bulbs (3 healthy bulbs and 12 bulbs showing symptoms of rotting and discolorations) were collected; four spoilt and one healthy bulb from each of three different locations: Lapai central market, Baddegi market and Onion Retail Outlet within Lapai town. The onion bulbs were found to be infected with eight species of fungi: *Aspergillus niger*, *A. flavus*, *Fusarium* spp, *Mucor* spp and *Penicillium* spp of which, *A. niger* and *A. flavus* were the most frequent isolated fungi. *Penicillium* spp was the least encountered fungus. The pH of the onion bulbs were also determined, Samples from Onion retail outlet within the town has a mean pH of 8.00 while the control has pH value of 5.83, samples from Baddegi Market has mean pH value of 6.66 and samples from Central Market had the lowest mean pH value of 5.37. Pathogenicity tests revealed that all the isolated fungi were pathogenic to healthy onion bulbs however, *Fusarium* spp and *Mucor* spp were the most pathogenic leading to rapid disintegration of infected bulbs within 4 days of inoculation. Contaminated onion bulbs should be sorted and eliminated to avoid re-infection while washing the onion bulbs with clean water prior to consumption should be strongly encouraged by appropriate authorities with the view to checking the spread of these fungi.

Key words: Fungi, infected, inoculated, pathogenicity, symptoms

Introduction

Onion (*Allium cepa*) is a biennial herb with a surficial root system, orbicular fruit, long and linear leaves, and short flattened stem which increase in diameter as it grows. Like garlic, leeks, chives, scallions and shallots onion is a member of *Amaryllidaceace* family. There are over 600 known varieties of *Allium*; distributed all over Europe, North American, Northern African and Asia. Onion is used as ornamentals, vegetable spices, and as medicine. It is known to produce a strong spicy odor when crushed. The bulb is normally formed as a result of thickening of the base leaves when the plant attains certain stage of it life cycle. Varieties grown in West Africa include early cape, yellow flat, Granex white, Texas grano and yellow Bermuda (Roopa *et al.*, 2014; TDRI, 1986). Purple skinned onion bulbs are cultivated in Nigeria with three cultivars: Goalmi, Malaoua and Scumarana (TDRI, 1986). In Nigeria, the red skinned onion bulb is the main variety grown in Kano state with cultivars like red Creole Texas, grown (Amstel, 1983; Norman, 1992). Generally, people preferred the red purple skinned onion to the white skinned because the red skinned onion can be stored better for a long period of time and more suitable for hot

area (Amstel, 1983; Norman, 1992; Abdel salam *et al.,* 2014).

In another development Onion is an essential crop with a relatively constant all year round consumer demand and economic value to farmers (Metthananda, 2000). It is a valuable ingredient in diet due to its content of sugars, vitamins and minerals (Ole *et al., 2004).* Production of onion is limited to a specific season and as such results in a market glut during this period. Therefore, storage is important to meet up with all year round regular consumers demand at reasonable price especially during off season and to provide producers with an opportunity to receive a higher price (Metthananda, 2000). The importance of storage rots also includes reduction in the quality and quantity of onion which affects the market value (Dogondaji *et al.,* 2005). Other important consequence often overlooked, is mycotoxin development as a result of contamination of the stored onion (Muhammad *et al.,* 2004). Fungi, especially moulds have been identified to be important pathogens of fruits and vegetables particularly under tropical and sub-tropical conditions (Adebayo and Diyaolu, 2003, Roopa, 2014, Gashua *et al.,* 2014). It has also been estimated that bulb rot account for 10% -15% of storage losses of different varieties during three months storage period under local condition (Metthananda, 2000).

A great number of research and development have been devoted to storage fungi as well as their control strategies. Onions are prone to spoilage by fungi during harvesting, handling, storing and marketing processes. These microbes might multiply under favorable condition and make them inedible; it can also serve as reservoir for the transmission of infectious diseases. This present work is a mycological study of spoilt onion samples collected from lapai town. The study focuses on isolation of possible fungi contaminants involved in onion spoilage. Pathogenicity study was also conducted on the isolates.

Materials and Methods

Study Area and Sample Collection

Rot and healthy onion bulbs were collected from different locations in Lapai town. Lapai is located in north central region of Nigeria. Based on 2006 population census, the population of people stood at 110,127 (NPC-2006). A total of 15 onion bulbs (3 healthy bulbs and 12 bulbs showing symptoms of rotting and discoloration) were collected; four spoilt and one healthy bulb from each of Lapai central market, Badeggi Market and Onion Retail outlets within Lapai town and aseptically transported to laboratory for microbial evaluation

pH Measurement

The pH measurement was carried out on onion bulbs. The unspoilt onion bulbs were milled and used as the control, then the spoilt onion bulbs was kept for 4 days and milled before the pH was measured. Both the spoilt and control onion bulbs (10g) was measured and diluted on 10ml of distilled water respectively before the pH was measured with the aid of pH meter (Jenway, model 3510).

Isolation and Enumeration of Fungi

Two processes were used in microbial inoculation of onion bulb.

(i) Direct plating method
(ii) Dilution of milled onion paste.

Direct Plating Method

About 3mm piece of each onion sample was aseptically cut with sterile blade and inoculated directly in Sabouraud Dextrose Agar and incubated for 5 days at room temperature after which the growth was checked. Sub-culturing was done when more than one type of colonies were noticed on plates.

Dilution of Milled Onion Paste

For the purpose of isolation and enumeration of fungi 10ml of milled onion paste was introduced into sterile test tube labeled 10^{o} dilution. One mili-liter was pipette and added to fresh 9ml of sterile distilled water in another sterile test tube and was labeled 10^{-1}. This was done respectively to 10^{-9} dilution .One mili-liter 10^{-6} dilution was used as inoculums.

Identification of Associated Fungi

Thin film of each mycelia colony were aseptically taken with the aid of sterile inoculating needle and placed on a clean grease-free slide. Before picking the isolate, a drop of lacto phenol was placed on the slide thereafter the mycelium was spread very well on the slide with the aid of the needle. A cover

slip was gently applied with pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lens. The species encountered were identified in accordance with Cheesbrough (2000).

Pathogenicity Test

Pathogenicity of the isolated fungi was established by testing for their ability to initiate rot in healthy onion at room temperature. The outer scales of the bulbs were removed and the inner tissues swabbed with cotton wool presoaked in 1% sodium hypochlorite (NaOCL) and subsequently rinsed in 3 stages of sterile distilled water following the techniques demonstrated by Shehu and Muhammad 2011. This was done to remove any contaminant on the surface as it could give false result. The onion sample was then dried by cleaning with a sterile cotton wool. Inoculating needle was sterilized by flaming till it was red hot, allowed to cool and used to wound the samples at 3 different positions. A (2cm) in diameter cork borer was sterilized by flaming and allowed to cool then it was used to introduce the mycelia of the isolate to wound on the onion. The wound was sealed with Vaseline to prevent the entrance of contaminant. Sterile distilled water (20ml) was introduced into a sterile desiccator to provide humidity. The inoculated samples were placed inside the desiccators and incubated at 28 ± 2^0C. They were observed daily for 5 days and the rotten portion from the point of inoculation was measured.

Results

pH Values of Onion Samples

Table 1 shows the pH of the onion bulbs from different locations in Lapai town. Onion bulbs obtained from Retail Outlet within the town has mean value of 8.00 while the control has pH of 5.83. Samples from central market had the lowest mean pH value of 5.37. Samples from Baddegi market has mean value of 6.66.

Cultural and Microscopic Characteristics of Fungal Isolates from Spoilt Onion

Table 2 shows the major fungal isolate from the 12 different onion bulbs. A total of 5 fungi were isolated from the bulbs, *Aspergillus niger*, *Aspergillus flavus*, *Mucor* spp, *Penicillium* and *Fusarium* spp.

Table 1: pH value of onion bulbs.

Location	Sample 1	Sample 2	Sample 3	Sample 4	Mean	Control
C.M	4.70	5.57	4.99	6.74	5.37	5.83
B.M	5.30	5.05	7.98	7.77	6.66	5.80
R.O	5.93	8.41	8.95	8.72	8.00	5.81

Key:C.M ; central market B.M ; Baddegi market R.O; retailer outlet

Table 2: Cultural and microscopic characteristics of fungal isolates from spoilt onion

Isolate	Colony colour	Reverse colour	Conidia shape
Fusarium spp	White at early stage, purple when colonies get older	purple	Sickle shape microconidia
A. niger	Zonation on colony surface	white	Splitting in to columns
A. flavus	Green moulds	Dark-brown	Conidial head radiate
Mucor spp	White	Grey with numerous black dot	Spherical at end, sporangia conidia is elliptical in size
Penicillium spp	Green		Conidia were seen to arise from phalides in strings. Vesicles absent

Fungal Count

The fungal count from the onion bulbs are summarized in table 3. Samples from Central market had mean fungal of 51.5 $x10^{-6}$ cfu /ml. Baddegi market samples had mean fungal of 32.5 $x10^{-6}$cfu /ml while samples from Onion retail outlets within Lapai town had mean fungal of 41 $x10^{-6}$ cfu /ml.

Frequency of Fungi Isolates

Table 4 shows that almost all the 5 fungi isolate were found in the onion sample collected. However, *Aspergillus niger* and *Aspergillus flavus* occur mostly. *Mucor* species was not isolated in any of the samples collected from Retail Outlet within Lapai Town.

Establishment of Pathogenicity

Figure 1 show all isolates obtained were pathogenic but with varying degree of infection. The *Fusarium* spp (20mm) is observed to be most pathogenic that is which produce the highest rot formation.while *Mucor* spp (18mm), *Aspergillus niger* (12mm), *A. flavus* (11mm), resulting in moderate infection and *Penicillium* spp (10mm) had the lowest growth rate after 5 days of inoculation with an offensive odour.

Table 3: Fungal counts ($x10^{-6}$cfu /ml) from spoilt onion bulbs.

Sample/location Plate	1	2	3	4	(Mean) Cfu/ml
Central Market	59	47	60	40	51.5
Baddegi Market	34	20	27	49	32.5
Retail Outlets	39	40	45	40	41

Table 4 : Frequency of Fungi Isolates

Sample Location	No. of samples	*Aspergillus niger*	*Penicillium* spp	*Fusarium* spp	*Aspergillus flavus*	*Mucor* spp
C.M	4	2(50)	1(25)	1(25)	3(75)	2(50)
B.M	4	3(75)	2(50)	2(50)	2(50)	1(25)
R.O	4	2(50)	1(25)	2(50)	2(50)	0(00)
Total	12	7(58.3)	4(33.3)	5(41.6)	7(58.3)	3(25)

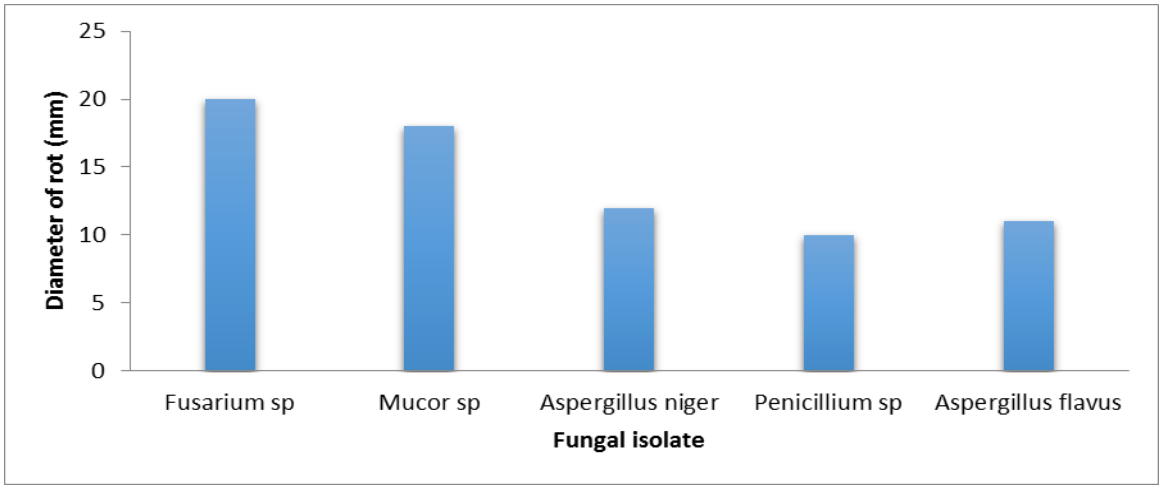

Fig 1: Pathogenicity of the isolated fungi on onion bulbs

Discussion

Deterioration of food items is caused mainly by the activity of microorganisms that thrive in the product. Subsequently, the cumulative deterioration renders the food items undesirable to the consumer. Fungal spoilage of commercially purchased onion bulb is clearly a course for concern. Five fungal isolates belonging to different genera were found to be responsible for the spoilage on onion bulb. These genera include *Penicillium* spp, *Aspergillus flavus*, *Fusarium* spp, *Aspergillus niger* and *Mucor* spp. The initial fungal contamination of the onion bulbs may have been derived from air, soil, water, insects, animals, workers and harvesting and transportation equipments (FDA, 2002). Shehu and Muhammad (2011) isolated *Rhizopus stolonifer* and *Alternaria porri* from rot onion bulbs in sokoto, Nigeria in addition to *A. niger, Fusarium spp, A. flavus, penicillum spp* isolated in this study. They however used Pathogenicity test to show *Rhizopus stolonifer, P. citrinum,* and *Alternaria porri* to be considerably pathogenic on the onion bulbs.

Among the fungi isolated from rotten bulbs, *Fusarium species* are responsible for rotten of the onion bulbs (Klich, 2002, Moharam *et al*., 2013). *Aspergillus* spp grow on the surface of onion bulbs but did not cause rotting when inoculated artificially. Major storage fungal pathogens which were reported as causal agents of rot of onion bulbs in other countries are *Aspergillus niger*, *Botrytis alli*, *Fusarium oxysporium*, *Follelotrichum circinans*, *Alternaria poori*, *Sclerotium copivorum*, *Perenospora destructor*, *Colletotrichum gloeosporodes* and *Penicillium species* (Fang and Yunfei, 2006; Shehu and Muhammad 2011)). This indicates that common fungal genera are involved in rotting of onion bulbs during storage in different countries.

The Pathogenicity test shows *Fusarium* spp (4.4 mm) to be most pathogenic showing visible signs of infection and deterioration after 24 hours of incubation. The virulence of *Fusarium* spp is due to the fact that they have extremely wide range of hosts (Moharam *et al*., 2013). Others include *Aspergillus niger* (3.6mm), *Mucor* sp (3.2mm) and *Penicillium* sp (2.8mm), which shows visible signs of infection and deterioration after 5 days of incubation. This implies that fungal isolates obtained from this study were capable of causing disease under suitable condition. The characteristic symptoms originally observed in the spoilt onions were also noticed. Contrally to our findings Okereke *et al*. (2010) indicated that the fungi species isolated from the infected mangoes included *A. niger, Fusarium* sp and *A. Flavus* and that *Fusarium* sp and *A. Flavus.* However, they could not prove their pathogenicity when inoculated into healthy mango fruits.

Several fruit spoilage fungi from different region has been isolated and identified (Al-Hindi *et al*., 2011, Patel *et al.*, 2013). The most common fungi found in a study by Akintobi *et al*. (2011) were *Aspergillus flavus, A. niger , Fusarium solani, Pencillium digitatum,Rhizopus stonlonfer* and yeast. Baiyewu *et al*., (2007) had also reported that *Rhizopus nigricans, Aspergillus flavus, Aspergillus niger* and *Fusarium moniliforme* among others, were responsible for post-harvest losses in Pawpaw in South Western Nigeria. Chukwuka *et al.*, (2010) had earlier reported *A. flavus, A. niger, Fusarium* spp and *Mucor* spp to be responsible for the spoilage of Pawpaw fruit in a farm in Oyo State, South Western Nigeria. Some yeast genera which bring about fermentation in fruits with the production of alcohol and carbondioxide are found associated with fruits.

Generally, spoilage fungi are known to be toxigenic or pathogenic under favorable conditions (Adebayo *et al.*, 2012, Dacruz *et al*., 2013, Tafinta, 2013). Al-Hindi *et al*., (2011) has isolated toxigenic fungi from spoiling fruits. Pathogenic fungi are capable of causing infections or allergies. *Aspergillus* spp. are known to produce several toxic metabolites including malformins, naphthopyrones (Adebayo *et al*., 2012; Al-Hindi *et al*., 2011; Wells *et al*., 1975) as well as Ochratoxins (OTA), a mycotoxin which is considered to be a dangerous toxin to human and animal health worldwide (Petzinger and Weidenbach, 2002) and other mycotoxins that are known to be heptocarcinogenic and nephrogenic in nature (Ajay *et al*., 2011). During refrigeration some moulds may also produce mycotoxins. There is therefore need for extra care during handling of onion bulbs such as harvesting, cleaning, transport and storage and marketing

Conclusion and Recommendation

Bulb rotting caused by storage fungi is one of the major reasons for storage losses of onions in Nigeria as well as other countries in the world. The field survey revealed that different fungal genera were associated with rotten bulbs collected from onion stores in different places in Lapai. Five fungal genera were found to be associated with rotting of onion bulbs. Among them, major causual agents of rotting onion bulbs were found to be *Fusarium* spp, *Mucor* spp, *Penicillium* spp *Aspergillus niger*, and *Aspergillus flavus*, while *Aspergillus niger*, and *Aspergillus flavus* grew on surface of onion bulbs but did not cause rotten.

It is recommended that both the farmer who harvests the vegetable (onion) into bags for transportation, the marketers and consumers take necessary and appropriate precautions in preventing contamination and consuming of contaminated onion. This will however reduce the risk of mycotoxins associated with fungi contamination which are deleterious to human health. From this study, some of the isolated fungi have serious public health risk while others fasten spoilage of the vegetables. High numbers of these microorganisms in raw consumed and onion produce would lead to the consumer's illness with attendant symptoms and consequences of the particular or combined microbial presence. Reduction of risk for human illness associated with raw product can be better achieved through controlling points of potential contamination in the field during harvesting, during processing or shipment, storage or distribution in the retail markets, food services facilities or home. There is need for further identification of isolating fungi using molecular techniques in order to ascertain the organisms responsible for this spoilage.

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