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Fungi Associated with Deterioration of Onion Variety (Colour)

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Abstract:

The incidence of fungi associated with deterioration of three varieties of onion bulbs was investigated in Usmanu Danfodiyo University, Sokoto. The bulbs were purposively collected in sterile polyethene bags through random sampling from kasuwan daji in Sokoto and brought to Mycology Laboratory, Department of Biological Sciences for analysis. Samples were prepared using standard procedures for Isolation and Inoculation of fungal organisms. Isolates were identified based on Morphological and cultural identification using mycological atlas. Pathogenicity test was out carried out using standard procedures. The result obtained revealed the presence of *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus and Rhyzopus oryzae. R. oryzae* had the highest percentage of occurrence (47.4%) in Red onion, *A.niger* and *A. fumigatus* had the highest occurrence (39.1%) in white onion, and in the purple onion *A. niger* and *A. flavus* had the highest percentage of occurrence with (42.8%). The pathogenicity test indicated that all the isolates were found to facilitate spoilage of the bulbs. *R. oryzae* was found to be more pronounced on white onion, *A. niger* was prominent in purple onion, *A. flavus* on red onion. Therefore, fungal spoilage of onion could be determined base on the type of fungal organisms and the type of onion variety. The study could help formers to detect the type of organisms that are responsible for spoilage on the different onion varieties thus, determine the appropriate type of pesticides to apply either in the field or on storage.

Key words: Onion bulb, Isolates, deterioration, storage, onion varieties

1. Introduction

Allium cepa is an important vegetable crop in Nigeria based on its consumption and economic value to farmers. The crop is grown mostly for its bulb which is used daily in every home for seasoning and flavouring of foods. Onion is a valuable ingredient in diet due to its content of sugars, vitamins and minerals (Ole *et al*., 2004). Skin variations are considered as skin colour, which may be white, yellow, brown, red or purple (Ross, 2001). Onion bulb rot caused by fungal pathogens is largely responsible for storage loss.

Fungi are a large group of heterogeneous eukaryotic, spore bearing, achlorophyllous organisms that generally reproduces asexually and sexually (Khalid *et al*., 2006). They are disease causing organisms in plants and animals while others are saprophytic (Prince and Prabakaran, 2011). Due to their competitive parasitic and saprophytic abilities, which are expressed by fast mycelial growth, spore production, presence of efficient and extensive system of powerful enzymes, fungi are able to utilise complex polysaccharides and proteins as their carbon and nitrogen sources (San-Blas and Calderone, 2008). Fungi which affect onions have adapted to the temperature range in which the crop grow. Thus, in temperate lands, the fungi which affect onions grow optimally at about 21^{o}C whereas in hot climate, they grow best at 30^{o}C (Maude, 1992).

Spoilage refers to the deterioration of raw materials or products which could result into a physical change on the components (Paparizas, 1987). The most common or predominant types of spoilage varies not only with the kind of fruit or vegetable, but also to some extent with the variety of microbial organisms. Spoilage may be due to plant pathogens acting on the stems, leaves and other special plant parts used as food (Maude, 1984). It is estimated that bulb rot contributes to 10 – 50 percent of losses of different varieties during 3 months' storage period under local conditions (Methananda, 1992).

To maximize profit and minimize loss, farmers and marketers of onions need to know some of the pathogens causing onions deterioration and effective ways of preserving the product for a considerable period. Most of the diseases are caused by fungi and bacteria whereas disorders may be caused by adverse weather, air pollutant, soil conditions, nutritional imbalances and pest control products (Chaput, 1995). Sometimes, several diseases and disorder can be present at the same time. Accurate disease diagnosis is an important part of an integrated best management programme. It is therefore necessary to identify onion diseases and disorders that occur during storage. Knowledge of fungal pathogens causing deterioration and rot will go a long way in solving the problem of deterioration of onions. Hence this study attempts to identify the fungi responsible for onion spoilage to enable farmers utilise that in managing deterioration caused by the organisms.

2. Materials and Methods

The Study Area:

The study was conducted in Usmanu Danfodiyo University Sokoto, Department of Biological Sciences. Sokoto metropolis is located in the extreme northwest of Nigeria near the confluence of Sokoto River and Rima River. It lays on latitude 11°30′ to 14° 00′N, longitude 4^0 00″ to 6^0 40″ and altitude 351 above sea level.

Collection of samples:

Infected onion bulbs (White, red, purple) showing symptoms of rotting and discolouration were randomly selected from five different sellers at kasuwar daji market in Sokoto. Fresh samples were also randomly collected from the same market. Each sample was collected separately in a sterile polythene bag. The samples were labelled and brought to Mycology laboratory for the study.

Media preparation:

Potato Dextrose Agar (PDA) media was used. Thirty nine grams (39g) of dehydrated powder (PDA) was weighed and suspended in 1litre of distilled water in a conical flask and capped with cotton wool and aluminium foil; heated on a hot plate to dissolve completely. It was autoclaved at 121^0 C for 15minute to sterilize the medium. The media was allowed to cool and 20ml was dispensed in sterilized (90mm) Petri dishes and allowed to solidify.

Isolation of fungi:

Each onion bulbs sample was surface-sterilized with cotton wool dipped in ethanol (Chinoko and Naqvi; 1989). The infected part of onions were then cut into 3 mm pieces with sterile razor blade, then placed on Potato Dextrose Agar (PDA) using sterile inoculating needle and incubated at room temperature (35 ± 3^0 C) for 5 days. The inoculation was done in triplicates, observations were made daily for emergence of colonies. The colonies obtainedwere sub-cultured 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 (35±2°C) for growth of the organisms. Pure cultures of the fungal isolates were used for identification.Some parts of the pure isolates were kept as stock cultures. Stock cultures were prepared using slant bottles of PDA in McCartney bottles and stored in a refrigerator at 4°C.

Identification of Isolated Fungi:

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 x40 magnification, Model-XSZ-21 for microscopic identification.

Pathogenicity test:

The method described by Anukwuorji *et al*. (2013) was adopted. Fresh bulbs were stripped off their scales. The inner tissues were swabbed with cotton wool soaked in 70% ethanol and washed thrice in running water. The bulbs were bored using 5mm diameter cork- borer and the plug was pulled and exchanged with 3mm diameter mycelia disc inserted into bulb. The plug was carefully placed and the wounded area sealed with Vaseline to prevent extra infection. The inoculated bulbs were incubated for 4 weeks at 30^0 C. Three replications were prepared for each treatment. Control consisted of sterilized 3mm PDA disc placed in the holes of the healthy bulbs. Inoculated onion bulbs were subsequently observed for rot development at the end of the incubation period. Each bulb was cut longitudinally into two parts and the length of rot in the tissue was measured. The degree of pathogenicity of each isolate was determined by measuring the extent of rot in millimeters on the infected bulbs.

Data Analysis

The data obtained from the study were statistically analysed using descriptive statistics.

3. Results

Frequency of Occurrence

The result obtained indicated that fungi isolated from the three types of onion bulbs, red, white and purple were *A. niger, A. fumigatus, A. flavus and R. Oryzae* as presented in the plates below. The percentage of occurrence revealed that *R. oryzae* has the highest percentage of occurrence in Red onion with 47.4%, and *A. flavus* and *A. fumigatus* have the least percentages (table 1). In White onion, *A. niger* and *A. fumigatus* have the highest percentage of occurrence (39.1%) and *R. oryzae* the least (8.7%) (table 2). In the purple onion *A. niger* and *A. flavus* have the highest percentage of occurrence with 42.8% and *A. fumigatus* has the least with 4.8% (Table 3). The result pathogenicity test indicated all the isolated fungi were found to be pathogenic to the

onion bulbs. However, *R. oryzae and A. fumigatus* were found to be more pathogenic on white onion, *A. Niger* was more pathogenic to purple onion, and *A. flavus* was more pathogenic to red onion (table 4).

Identification of Isolates: Morphological and cultural Description.

Plate I: *A. fumigatus* (Colonies showed typical blue-green surface pigmentation)

Plate II: *A. niger* (Colonies appeared black and spongy. The reverse of plate is creamy).

Plate III: Microscopic characteristics of *A. niger* (Conidial heads are large, globose, dark brown.)

Plate IV: *A. flavus* (appeared yellow-green at first but become dark yellow-green with age)

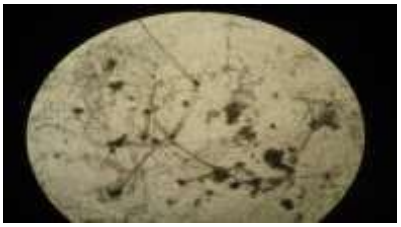

Plate V**:** microscopic appearance of *A. flavus* (Conidia were globose, pale green and conspicuously echinulate).

Plate VI: *Rhizopus oryzae* (Colonies have white cottony at first then changed to brownish grey and blackish-grey}.

Plate VII: Microscopic characteristics of *R. oryzae.* (Sporangia were globose, with a flattened base, greyish black, powdery appearance).

Table 1: Frequency of occurrence in red onion

Organism	**Frequency**	**Percentage of occurrence**
Aspergillus niger	4	21
Aspergillus flavus	3	15.8
Aspergillus fumigatus	3	15.8
Rhizopus oryzae	9	47
Total	19	100

Table 2: Frequency of occurrence in white onion

Organism	**Frequency**	**Percentage of occurrence**
Aspergillus niger	9	39.1
Aspergillus flavus	3	13.0
Aspergillus fumigatus	9	13.9
Rhizopus oryzae	2	8.7
Total	23	100

Table 3: Frequency of occurrence in purple onion

Organism	Frequency	Percentage of occurrence
Aspergillus niger	9	42.8
Aspergillus flavus	9	42.8
Aspergillus fumigatus	1	4.8
Rhizopus oryzae	2	9.5
Total	21	100%

Pathogenicity

The result of pathogenicity test indicated that all the organisms were able to cause rot on the onion samples.

Table 4: Mean rot extension in millimetre (mm)

ORGANISM	ONION TYPE		
	RED	WHITE	PURPLE
R. oryzae	24.00	33.33	29.67
Niger	36.67	35.00	39.00

Flavus	43.00	26.00	30.67
fumigatus	31.33	41.67	34.00

4. Discussion

The variation in the frequency of occurrence of fungi isolated from bulbs could be due to the ability of the organisms to utilise 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 favouring their growth. The extents of infection vary with the fungal species and physical environment. 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. The ability of the test fungi to cause rot and deterioration of the onion bulbs 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 the abilility of the organisms to cause rot could be because onion contains 84-90% moisture which made it a suitable flora for fungal growth. More so, different organisms have different ability to respond to physiological activity such as growth and development under the same environmental conditions.

Aspergillus spp were found to be the dominant on the all the onions sample and were able to cause rot on the samples as indicated by the pathogenicity test. Most *Aspergillus* spp have been reported to survive between onion crops as soil saprophytes in or on bulbs in field or storage and are found everywhere in nature. They invade bulbs of onion in the field on storage whenever they find injured tissues by producing enzymes of toxins (Samuel and Ifeanyi, 2015).Some researchers have previously reported the predominant occurrence of *Aspergillus* spp in stored onion bulbs (Brice *et al*., 1997). For the types of onions, it was also noted that *R. oryzae* dominant in red onion while *A. niger* and *A. fumigatus* showed high manifestation on white onion. On the other hand, *A. niger* and *A. flavus* have the highest percentage of occurrence in the purple onion. The difference in percentages could have attributed to the difference in the nutritional and anti-nutritional composition of each of the onion variety which the fungi utilised as substrates.

5. Conclusion and recommendations

Various fungi are responsible for most spoilage of onion between the period of production in the field, storage and consumption. *Aspergilus* species were found to be the most prevalent in the onion bulbs. *R. oryzae* was found to be predominant in the red onion. *A. flavus* is the most virulent in the red onion while *A. fumigatus* was more pathogenic to white onion than red and purple.

6. Recommendations

Aspergellus species are more predominant in both red and purple onions and are therefore not suitable for storage.

The white onion could be a better candidate for storage.

The findings from this research could serve as a guide for the choice of specific bio-fungicides and synthetic fungicides for the different onion varieties.

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