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Studies on Periodic Fungal Isolation from Freshly Harvested and Stored Sweet Potato (*Ipomoea batatas* (L) Lam) Root Tubers in A Constructed Storage Barn

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ABSTRACT

Sweet Potato (*Ipomoea batatas* (L) Lam) is a good source of staple food on the Jos Plateau but farm propagules constituting of fungi and yeast species cause decay of the freshly harvested and stored produce. Freshly harvested and stored sweet potato varieties: CIP4400168, Ex-Igbariam, Tanzania, TIS 8164 and TIS 87/0087 in an In-door storage barn were sampled. The cultivars were identified, evaluated and characterized at the National Root Crops Research Institute (NRCRI) Umudike, Nigeria. The sampled root tubers in the barn were picked prior to storage and every fort-night for a period of 8 weeks. Samples were washed, peeled, pulverized into slurry and the homogenous mixture was used to plate out fungal and yeast species using Malt Extract Agar (MEA) and Sabouraud glucose agar (SGA) culture media respectively. Also the aerial mycoflora of the storage environment was examined. Seventeen (17) species were isolated from the freshly harvested cultivars prior to storage, originating from the experimental farm propagules. Two (2) weeks after storage the number of the isolates increased to 19 species, all the initial fungal isolates on the surface of the cultivars were still present with the emergence of *Volutela ciliate*; *Hanselula* sp and *Schizosaccharomyces pombe*. There was no increase in isolates after 4 weeks of storage but *Chaetomium funicola* emerged while *Volutella ciliata* exited. There were further build-up of fungi and yeast species after 6 weeks that rose to 24, at 8 weeks of storage, leading to continuous succession, either originating from the farm propagules or the aerial environment of the barn. Two (2) weeks of the cultivars storage encouraged succession and to avoid this menace the produce should be processed into secondary products with extensive shelf-life.

Keywords: Sweet potato, aerial environment, exited species, emergent species, soil propagules, succession.

Introduction

Sweet potato (*Ipomoea batatas* (L) Lam) root tubers are a great source of staple food on the Jos Plateau but the loss of this commodity is attributed to microorganisms especially fungi and yeast species. These organisms are distributed in the air, soil and on decaying materials during storage to constitute storage fungi (Oduola *et al*., 2018).

Different authors have shown that a wide range of fungal species deteriorate the root tubers. Some of the studies indicated that the fungi were encountered during storage and within the storage environment at different favourable conditions (Charles and Wisdom, 2010; You *et al*., 2014; Harichandra and Surekha, 2016).

The harvesting of the tubers on the Jos Plateau is a manual process and the poor handling of the commodity increases root damage (Rupsa *et al*., 2017; Sila *et al*., 2017).

Physiological changes in the root tubers increase their susceptibility to decay as fungi invade root tissues more readily through wounds (Lewthwaite *et al*., 2011; Sila *et al*. 2020). Decay makes the tubers unattractive and unmarketable (Agu *et al*., 2015; Sila *et al*., 2017; Prathiksha and Ramachadra, 2019) resulting in economic losses. The losses of the root tubers also emanates from enzymes such as amylases, cellulases, zylanases, polygalactunases and pectin-mythly esterases which degrade different components of the root tubers leading to emission of foul odour and water (Issah *et al*., 2017). Enzyme activities reduce quality, quantity and market values.

During storage some of the fungal species produce mycotoxins, secondary metabolites that can be lethal in small quantities (Bankole and Adebanjo, 2003; Shukla *et al*., 2012). The poisoning of humans can result from oral, dermal or exposure through inhalation of mycotoxin-contaminated tubers (Amri and Lanoi, 2016).

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Aero-microorganisms play very important role in the biodeterioration of stored products. Fisk *et al*. (2007) reported that fungal spores are spread through air but land and survive on stored root tubers and develop into biodeteriogens. A diverse group of amylolytic fungi in the genera *Aspergillus*, *Chaetomium*, *Fusarium, Trichoderma* utilize sugar in the peels of the cultivars for their energy source (Olaitan, 2012; Sila *et al*., 2018).

Sila *et al.* (2024) has demonstrated that when the root tuber's moisture content values became low at the peels its dry matter became concentrated with sugars mobilized from its circumference due to directional moisture content loss. Nutrients extracted from the root tubers peels by these organisms are utilized in the formation of macromolecules resulting in its deterioration.

Food security and sustainability is one of the ways of addressing food scarcity and shortage due to the activities of fungi (Rakesh *et al*., 2017). On the Jos Plateau the tuber is an effective and economic source of energy, antioxidants and anthocyanins, root protein and vitamin A (Oduola *et al.*, 2018). However the shelf-life of the tuber is seriously hampered by the activity of fungi an impediment to the drives for food security on the Jos Plateau (Sila *et al.*, 2024). Therefore the objective of this study was to examine the fungi and yeasts of the freshly harvested and stored root tubers to determine their keeping quality as practiced by the farmers, who harvest and store them on the floor and withdraw from the storage lot periodically for sale in the local organised markets and the more distant towns of Jos.

Materials and Methods

Location of the Experimental Plot for Propagation of Cultivars

The experimental plot for the cultivation of the cultivars was located in Rayfied. The plot was carved out from an ancestral farm land. The selected sweet potato cultivars were propagated on an experimental plot measuring 45m×25m at Rayfield an environ of Jos town, Plateau State, Nigeria; 9.20 North Latitude, 8.90 East Longitude and 1208 meters elevation above sea level. The geolocation of Jos is on the North and East hemisphere with a cool temperature which fluctuates between 34.50 – 13^{0}C (GPS Coordinates of Jos and environs, August 2023). The cool temperature encourages the production of both temperate and tropical crops.

The experimental plot is an agricultural farm which receives adequate rays of sunshine with soil that is adequately drained with a pH of 5, organic content of 4%, and moisture content of 50%.

The preparation of the piece of land (tilling and ridging) was done manually after an early rainfall in the month of June 2023. Each ridge measured 9 meters in length, 45cm high and 60cm apart. Sweet potato cultivars (vines) were collected from the farmers in guided random selection of 9 Local Government Areas on the Jos Plateau. The vines were collected and put in moistened jute bags, labelled and taken to the experimental plot. The vine cuttings were planted and were allowed to grow to maturity then harvested and sent to National Root Crops Research Institute (NRCRI) Umudike for proper identification.

Identification of the Sweet Potato Cultivars

A preliminary experiment was conducted at the research station to raise fresh vines from the sweet potato root tubers of Jos (sweet potato root tubers harvested from the experimental plot at Rayfield). A second experiment was conducted with the vines from the preliminary experiment and sent to the National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria for identification, evaluation and characterization of the genotypes.

The sweet potato cultivars harvested from the experimental farm in Rayfield, Jos, Plateau State were genotypes as planted, amongst which were improved varieties: CIP4400168, Ex-Igbariam, Tanzania, TIS 8164 and TIS 87/0087 and land-racers (Genotypes that had lost their identifying characters). These improved varieties identified were then replanted on the farm in Rayfield, in Jos, harvested and used as the experimental root tubers (samples).

Studies on the Freshly Harvested Sweet Potato Root Tubers

The experimental root tubers (Samples) were harvested and stored as practised by the local farmers before selling them at the local organized markets and the towns of Jos. An In-door storage barn method of Okwuowulu and Asiegbu (2002) was modified and adopted for the storage of the root tubers. This was done in order to determine the shelf – life of the commodity in storage. The method was adopted in order to simulate the local method of sweet potato storage by the farmers. In this method the root tubers are stored on the floor of locally constructed huts before the commodity is sold periodically in the local organized markets and surrounding towns of Jos.

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Construction of Indoor Storage Barn

An exclusive space, measuring 4m x 1m was marked and cleansed within an empty room with 2 windows which were gauzed with a plastic net. The floor was washed with water, mopped dry, filled first with rain washed sand and ash to a height of 4.00cm as described by Lancaster and Coursey (1984). These served as an insulator preventing the invasion of the root tubers by marauding insects. Sizeable stones of equal dimensions were placed on the corners and middle of the marked area. Three wooden planks of equal length were laid on top of the stones which provided a flat surface area above the floor. Ply-woods of 1.00 metre height were used to fence the demarcated area.

The experimental sweet potato root tubers were harvested 4 months after planting (MAP) from the experimental farm in Rayfield. One kilogram (1kg) of each cultivar without blemish was weighed out using top-loading Balance, Mettler: P2010. They were put in plastic baskets of known weight with wide opening on top, having ample openings on the sides to facilitate air circulation. The baskets of the root tubers were placed on the flat surface of the barn. The entire space between the rows of the baskets and the walls of the ply-wood was filled with *Digitaria* (Acha) straw to prevent rapid moisture content loss of the commodity. The entire room provided the shade which kept the barn cool as well as a wind break. This arrangement was adopted for an in–door storage barn which allowed for air to circulate freely within the commodity without interference of humans and rodents (Okwuowulu and Asiegbu, 2002). The root tubers were examined periodically (fortnightly) for fungal colonisation for a storage period of 8 weeks. This was conducted to simulate the selling habit of the commodity by the local farmers who present them at the local organised markets within this time frame.

Periodic Fungal Isolation from the Peels of the Stored Root Tubers

This was done in order to find out whether there was any relation between the fungal flora of the peels of the stored tubers and the aerial environment where the tubers were stored. The results obtained were compared with the fungal flora obtained from each fortnight for a period of 8 weeks.

Isolation of the Fungal Colonisers

Some of the stored root tubers of each variety were picked after every fortnight and peeled with sterile knife. The resultant peels were then pulverized into slurry and dispensed into a clean crucible. This was stirred with the aid of a sterile spatula in order to obtain a homogenous mixture which was distributed into 30 culture plates using the pour plate method. A volume of 15ml of Malt Extract Agar (MEA) and Sabouraud glucose agar (SGA) was then run into each culture dish. The culture plates were swirled in order to obtain a proper mixing of the slurry and the culture medium and then allowed to solidify.

The solidified culture plates were then divided into two batches. The first batch of 5 plates of each variety of the cultivars totalling 25 plates was incubated at 25^0C for the isolation of fungi species. The second batch also of 25 plates was incubated at 37^0C for the isolation of yeast species. Control plates were also set out for each incubation temperature for comparison. The culture plates were examined after 24 and 48 hours for the development of yeast colonies and after 4 – 7 days for the development of other fungal colonies. All the plates were re-examined after 14 days for the development of additional species.

Dilution Plate Method of Fungi Isolation from the Decaying Root Tubers

Some of the pieces of the root tubers were washed in sterile distilled water. The resultant wash water was then subjected to series of dilutions and plated out employing the microbial dilution method. The pieces of the decaying root tubers were put in sterile glass bowl containing sterile distilled water and gently shaken in order to obtain fungal suspension. The resultant suspension was then serially diluted until the desired final solution was reached.

All the serial dilutions were separately plated out on Malt Extract Agar (MEA) and Sabouraud glucose agar (SGA) using the spread plate method. A total of 25 plates were also employed for each dilution. The drops of suspension on the agar plates were evenly dispersed over the surface of the culture medium with the aid of bent or L – shaped sterile glass rod. The resultant culture plates were incubated as described above. The plates were examined after 24 – 48 hours for the presence of yeasts and after 4 – 7 days for the development of fungal colonies and re-examined after 14 days for the development of additional species.

Gram – Staining and Biochemical Analyses of the Yeast Isolates

The yeast isolates were examined microscopically, Gram stained and subsequently subjected to various biochemical tests: India ink (wet preparation) Test, Urease Test, Fermentation Test and Sugar Assimilation.

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Purification of Isolates

The colonies of fungi that developed from the suspension were sub-cultured several times until pure cultures were purified and obtained. The *Aspergillus* and *Penicillium* species were further cultured on Czapek Dox Agar (CZA) to aid their identification since these organisms produce pigments in such medium.

Identification of the Isolates

The yeast isolates were finally identified after been subjected to the biochemical analyses. The fungal isolates were microscopically examined; cultural characteristics and morphological parameters were also taken into consideration in order to determine their identities. References were made to stock fungal cultures to aid such identifications and also to different Compendia: Barnett and Hunter 1998 and Nyongesa *et al.*, 2015. Also the keys of Klich (2002) were useful to further identify the *Aspergillus* and *Rhizopus* species.

The Aerial Mycoflora of the Root Tuber Storage Environment

The aerial mycoflora of the sweet potato root tubers storage environment was carried out employing the Petri dish culturing exposure method described by Ogbonna and Pugh (1983).

Freshly prepared culture plates of MEA were exposed at the root tuber storage barn. Fifteen of such plates were closed at one hour interval and incubated at the different temperatures as described above. A total of 5 exposed plates were incubated at each temperature. Control plates that were not exposed were also incubated at the various temperatures. The resultant fungal colonies were purified and identified as described above.

Result

Results showed that, the freshly harvested root tuber peels of sweet potato prior to storage had 13 species of fungi and 4 species of yeast making a total of 17 isolates (Table 1). Fifty three per cent (53%) of the isolates belonged to the Hyphomycetes. Ascomycetes and Phycomycetes had 12% each. while 23% occurrence was of the Yeast. *Aspergillus* species were dominant but *Mucor pusillus*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* occurred more than the other isolates in the freshly harvested root tubers. The isolates must have originated from the experimental farm soil propagules adhesively attached to the root tuber peels before storage in the barn. The isolates were more in association with Ex-Igbariam vatiety (10) and Tanzania variety (9) in comparison with the other freshly harvested cultivars.

Table 1: Fungal Isolates from the Peels of the Root Tubers Prior to Storage

	Sweet Potato Cultivars					
Fungal Isolates	**CIP**	**EX**	**TAN**	**TIS 81**	**TIS 87**	**TOTAL**
Ascomycetes						
Emericella nidulans (Eidam) Vuill.	-	-	-	+	-	**1**
Eurotium herbariorum (Wiggers) Link	-	+	-	-	-	**1**
Hyphomycetes						
Aspergillus candidus Link	-	+	-	-	-	**1**
A. clavatus Desm	+	-	-	-	+	**2**
A. flavus Link ev. Gray	-	+	+	-	-	**2**
A. fumigatus fres	-	+	+	-	+	**3**
A. niger van Tieghem	+	-	-	-	+	**2**
*A. oryzae (*Ahlburg) cohn.	-	+	-	+	-	**2**
A. terreus Ihom	-	+	+	-	-	**2**
Fusarium oxysporum Schlecht	-	-	+	-	-	**1**
Penicillum paraherquei Abe ex.G.Smith	+	-	+	-	-	**2**
Phycomycetes						
Mucor pusillus Lindt	-	+	+	+	+	**4**
Rhizopus stolonifer (Ehrenh) Link	+	+	+	+	-	**4**
Yeasts						
Saccharomyces cerevisiae Hansen	+	+	+	+	-	**4**
Rhodotorula sp.	-	+	-	+	-	**2**
Debaryomyces hansenii Zopt	-	-	-	-	+	**1**
Rhodoturula glutinis (Fres) Harrison	-	-	+	+	-	**2**
Total	**5**	**10**	**9**	**7**	**5**	**36**

Key: +Present - Absent CIP = CIP 4400168, EX = Ex-Igbariam, TAN =Tanzania TIS81 = TIS 8164, TIS87 = TIS 87/0087.

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After 2 weeks of storage in the barn the emergence of the fungus *Volutela ciliate*; the yeasts: *Hanselula* sp and *Schizosaccharomyces pombe* increased the number of the isolates to 19 species (Table 2). The root tubers had fourteen species of fungi and five species of yeast consisting of Ascomycetes (16%), Hyphomycetes (47%), Phycomycetes (11%) and the Yeast (26%). The Aspergilli were still the dominant isolates while the yeast *Rhodoturula glutinis* exited the stored root tubers. Ex-Igbariam and Tanzania had 11 isolates each higher than the other cultivars

Table 2: Fungal Isolates from the Peels of the Root Tubers after 2 Weeks of Storage

	Sweet		Potato		Cultivars	
Fungal Isolates	**CIP**	**EX**	**TAN**	**TIS 81**	**TIS 87**	**TOTAL**
Ascomycetes						
*Emericella nidulans (*Eidam) Vuill	-	-	-	+	-	**1**
*Eurotium herbariorum (*Wiggers) Link	-	+	-	-	-	**1**
Hyphomycetes						
Aspergillus candidus Link	-	+	-	-	-	**1**
A. clavatus Desm	+	-	-	-	+	**2**
A. flavus Link ev. Gray	-	+	+	-	+	**3**
A. fumigatus fres	-	+	+	-	+	**3**
A. niger van Tieghem	+	-	+	-	+	**3**
*A. oryzae (*Ahlburg) cohn.	-	+	-	+	-	**2**
A. terreus Ihom	-	+	+	-	-	**2**
Fusarium oxysporum Schlecht	-	-	+	-	-	**1**
Penicillum paraherguei Abe ex. G.	+	-	+	+	-	**3**
Volutella ciliata Alb.& Schw.	-	+	-	+	+	**3**
Phycomycetes						
Mucor pusillus Lindt	-	+	-	+	+	**3**
Rhizopus stolonifer Ehrenh Link	-	+	+	+	-	**3**
Yeasts						
Saccharomhyces cerevisiae Hansen	+	+	-	-	-	**2**
Hanselula sp.	-	+	+	+	-	**3**
Rhodoturula sp.	-	-	+	-	-	**1**
Debaryomyces hansenii Zopf	-	-	+	-	-	**1**
Schizosaccharomycespombe Lindner	-	-	+	+	-	**2**
Total	**4**	**11**	**11**	**8**	**6**	**40**

Key: + Present, - Absent. CIP = CIP 4400168, EX = Ex-Igbariam, TAN =Tanzania TIS81 = TIS 8164, TIS87 = TIS 87/0087

The root tubers had the same number of the isolates and percentage of occurrence after 4 weeks of storage in the barn. However the emergence of the Ascomycete *Chaetomium funicola* and the exit of the Hyphomycete: *Volutella ciliata* were observed in the stored root tubers (Table 3). The Aspergilli still maintained their dominant position in the stored root tubers after 4 weeks of storage. The Ex-Igbariam and Tanzania varieties still maintain the lead in association with more of the isolates (14) each even though there is a build-up of the isolates in the other cultivars.

Sixteen (16) species of fungi and 6 yeast species were isolated from the stored root tubers after 6 weeks of storage; 45% of the isolates belong to the Class Hyphomycetes while 23% and 27% was found to belong to the Class Phycomycetes and the Yeast respectively. The least percentage of occurrences (5%) was found to belong to the Class Phycomycetes. The emergence of the Hyphomycetes: *A. parasiticus*, *Moniliella acetoabutens* and *M. suaveolens*; Phycomycetes: *Mucor plumbens*, *Rhizopus oligosporus* and *Rhizopus stolonifer* and the Yeast: *Saccharomyces fibuligera* in the stored root tubers increased the number of the isolates to 22 species after the exit of the Ascomycetes: *Chaetomium funicola and Eurotium herbariorum.* Ex-Igbariam and Tanzania varieties had more of the isolates (18) and (17) respectively even though these had increased in number in the peels of the other cultivars.

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Table 3: Fugal Isolates from the Peels of the Root Tubers after 4 Weeks of Storage

		Sweet	Potato	Cultivars		
Fungal Isolates	**CIP 44**	**EX**	**TAN**	**TIS 81**	**TIS 87**	**TOTAL**
Ascomycetes						
Chaetomium funicola Cooke	+	+	-	+	+	**4**
*Emericella nidulans (*Eidam) Vuill	+	-	+	+	-	**2**
*Eurotium herbariorum (*Wiggers) Link	-	+	+	-	-	**2**
Hyphomycetes						
Aspergillus candidus Link	-	+	-	+	-	**2**
A. clavatus Desm	+	-	+	-	+	**3**
A. flavus Link ev. Gray	-	+	+	-	+	**3**
A. fumigatus fres	+	+	+	-	+	**4**
A. niger van Tieghem	+	+	-	+	+	**4**
*A. oryzae (*Ahlburg) cohn.	-	+	-	+	+	**3**
A. terreus Ihom	-	+	+	+	-	**3**
Fusarium oxysporum Schlecht	+	-	+	-	-	**2**
Penicillum paraherguei Abe ex. G.	+	-	+	+	+	**4**
Phycomycetes						
Mucor pusillus Lindt	-	+	+	+	+	**4**
*Rhizopus stolonifer (*Ehrenh) Link	-	+	+	+	+	**4**
Yeast						
Debaryomyces hansenii Zopf	+	+	+	+	-	**4**
Hanselula sp.	+	+	+	+	-	**4**
Rhodoturula sp.	-	-	+	-	+	**2**
Saccharomyces cerevisiae Hansen	+	+	-	+	-	**3**
Schizosaccharomyces pombe Lindner	-	+	+	+	+	**4**
Total	**10**	**14**	**14**	**13**	**11**	**62**

Key: + Preset, - Absent; CIP = CIP 4400168, EX = Ex-Igbariam, TAN =Tanzania TIS81 = TIS 8164, TIS87 = TIS 87/0087

Table 4: Fungal Isolates from the Peels of the Root Tubers after 6 Weeks of Storage

		Sweet	Potato	Cultivars		
Fungal Isolates	**CIP**	**EX**	**TAN**	**TIS 81**	**TIS 87**	**TOTAL**
Ascomycetes						
Emericella nidulans (Eidam) Vuill	+	-	+	+	-	**3**
Hyphomycetes						
Aspergillus candidus Link	-	+	+	+	-	**3**
A.clavatus Desm	+	-	+	-	+	**3**
A flavus Link ex. Gray	-	+	+	+	+	**4**
A.fumigatus fres	+	+	+	-	+	**4**
A parasiticus Speare	-	+	+	-	+	**3**
A.terreus Ihom	-	+	+	+	-	**3**
Fusarium oxysporum Schlecht	+	+	+	-	-	**3**
Moniliella acetoabutens Stock Dakin	+	+	-	-	-	**2**
M. suaveolens (Lindner) v. Arx	+	+	-	-	+	**3**
Penicillum paraherquei Abe ex G. Smith	+	-	+	+	+	**4**
Phycomycetes						
Mucor plumbens Bon	+	+	+	+	-	**4**
M. pusillus Lindt	-	+	+	+	+	**4**
Rhizopus oligosporus Saito	-	+	-	+	+	**3**
R. oryzae Wentr Prinsen Geerlings	+	+	+	+	-	**4**
*R. stolonifer (*Ehrenb) Link	-	+	+	+	+	**4**
Yeast						
Debaryomyces hansenii Zopf	+	+	+	+	-	**4**
Hanselula sp.	+	+	+	+	-	**4**
Rhodoturula sp.	-	-	+	+	+	**3**
Saccharomhyces cerevisiae Hansen	+	+	-	+	+	**4**
Saccharomycopsis fibuligera Lindner	-	+	-	-	+	**2**
Schizosaccharomyces pombe Lindner	-	+	+	+	+	**4**
Total	**12**	**18**	**17**	**15**	**13**	**75**

Key: + Present, - Absent; CIP = CIP 4400168, EX = Ex-Igbariam, TAN =Tanzania TIS81 = TIS 8164, TIS87 = TIS 87/008

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Eighteen (18) species of fungi and 6 species of yeast totalling 24 isolates were associated with the stored root tubers after 8 weeks of storage (Table 5). Fifty percent (50%) frequency of occurrence of the fungi belonged to the Hyphomycetes, 21% to the Phycomycetes the least 4% to the Ascomycetes. The Yeast had 25% frequency of occurrence of the isolates. The Hyphomycetes: *Aspergillus candidus, A. clavatus, A. flavus, A. fumigatus, Fusarium oxysporum,* and *P. paraherguei;* Phycomycetes: *M. pusillus and R. stolonifer* and the Yeasts: *Debaryomyces hansenii* and *Rhodoturula sp* which were identified on the root tubers prior to storage persisted on their peels throughout the storage period of 8 weeks.

The peels mycoflora contained more of Aspergilli than any of the species of `fungi but *A. flavus, F. sporotrichoides, M. pusillus, R. stolonifer* and the Yeast: *Debaryomyces hansenii* were isolated in all the samples. The emergence of *Botrytis aclada, F. sporotrichoides, Penicillium lanusum* and *P. roqueforti* in the stored root tuber brought about the increased of the isolates. However the isolates *Emericella nidulans A.terreus* and *M. suaveolens* had exited the root tubers during this storage period. The isolates were now almost evenly distributed within the stored cultivars though still higher in Ex-Igbariam and Tanzania with 21 isolates each.

Table 5: Occurrence of the Isolates from the Cultivars after 8 Weeks of Storage

	Sweet Potato Cultivars					
Fungal Isolates	**CIP**	**EX**	**TAN**	**TIS 81**	**TIS 87**	**TOTAL**
Ascomycetes						
Chaetomium nozdrenkoae Serg**.**	-	+	-	+	+	**3**
Hypomycetes						
Aspergillus candidus Link	-	+	+	+	-	**3**
A. clavatus Desm	+	+	+	-	+	**4**
A. flavus Link ex Gray	-	+	+	+	+	**4**
A. fumigatus fres	+	+	+	+	+	**5**
A. parasiticus Speare	-	+	+	-	+	**3**
Botrytis aclada Fres	-	+	-	+	-	**2**
Fusarium oxysporum Schlecht	+	+	+	+	-	**4**
F. sporotrichoides Sherb	+	+	+	+	+	**5**
*Moniliela suaveolens (*Lindner) v. Arx	+	+	-	-	+	**3**
Penicillium lanusum Westling	+	-	+	+	+	**4**
P. paraherguei Abe ex. G Smith	+	-	+	+	+	**4**
P. roqueforti Ihom	+	+	+	-	+	**4**
Phycomycetes						
Mucor plumbens Bon	+	+	+	+	-	**4**
M. pusillus Lindt	+	+	+	+	+	**5**
Rhizopus Oligosporus Saito	-	+	+	+	+	**4**
R.oryzae Went & Prinsen Geerlings	-	+	+	+	-	**3**
*R. stolonifer (*Ehrenb) Link	+	+	+	+	+	**5**
Yeasts						
Debaryomyces hansenii Zopf	+	+	+	+	+	**5**
Hanselula sp.	+	+	+	+	-	**4**
Rhodoturula sp.	+	-	+	+	+	**4**
Saccharomyces cerevisiae Hansen	+	+	+	+	-	**4**
Saccharomycopsis fibuligera Lindner	-	+	+	-	+	**3**
Schizosaccharomyces pombe Lindner	-	+	+	+	+	**4**
Total	**15**	**21**	**21**	**19**	**17**	**93**

Key: + Present , - Absent; CIP = CIP 4400168, EX = Ex-Igbariam, TAN =Tanzania TIS81 = TIS 8164, TIS87 = TIS 87/0087.

The cultures of the yeasts were cream, white and occasionally pink and red colored. Their colony average diameter ranged between 10 to 30mm and creamy colonies grew faster and best at 37^0C. The colonies became much larger after 48 hours of incubation. Color change from white in some species to cream, yellow and tan while others remained mucoid and fluidy.

The biochemical reactions of the Yeast isolates revealed a varied carbohydrate fermentation and assimilation pattern (Table 6). The Yeasts assimilated more sugars than fermented them. *Rhodotorula* species did not ferment any of the sugars but assimilated only glucose while other Yeasts were weak and varied in their reactions to the entire test carried out.

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Table 6: The Biochemical Features of the Yeast Isolated from the Sweet Potato Peels

			Fermentation Test					Sugar Assimilation Test									
Gram Staining	**GermTube Test**	**India Ink Test**	**Galactose**	**Glucose**	**Lactose**	**Maltose**	**Sucrose**	**Galactose**	**Glucose**	**Lactose**	**Matt**	**Sucrose**	**Urease Test**	**Growth at 50^{0}C**	**Growth at 70^{0}C**	**Growth at 45^{0}C**	**Growth at 50^{0}C**	**Probable Yeast Identity**
+	+	-	+	+	-	+	+	+	+	-	+	+	-	+	+	-	-	*Candida aIbicans* (Berkhout)
+	-		-	+	-	-	+w	+	+	-v	+	+	-	+	+	-	-	*Debaryomyces hansenii* (Zopt)
+	-	-	-	+v	-	-	+	-v	+	-	+	+	-	+	+	-	-	*Hansenula* sp
+	-	-	-	-	-	-	-	+w	+	-	+w	-	-	+	+	-	-	*Rhodotorula glutinis* (Fres) Harrison
+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	*Rhodotorula* sp.
+	-	-	+v	+	-	+v	+v	-v	+	-	+v	+v	-	+	+	-	-	*Saccharomyces cerevisiae* (Hansen)
+	-	-	-	+w	-	+w	+w	-	+	-	+	+	-	+	+	-	-	*Saccharomycopsis fibuligera* Lindner
+	-	-	-	+	-	+	+	-	+	-	+	+	-	+	+	-	-	*Schizosaccharomyces pombe* Lindner

Key: + = positive; - = negative; w= weak reaction v = variable reaction.

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The different Classes and their isolates identified during the storage of the freshly harvested stored root tubers in the barn for a period of 8 weeks is presented in Figure 1. Seventeen percent (17%) of the fungal species were isolated from the root tubers prior to storage in the barn, 19% of the species after 2 and 4 weeks, 22% of the species after 6 weeks and 24% of the species after 8 weeks. Fifty percent (50%) of the isolates belong to the Class Hyphomycetes while the Class Ascomycetes had the least of 9% of the isolates. The Yeast and the Class Phycomycetes had 26 and 16% of them respectively. Succession of the fungal species was observed in the stored root tubers with the emergence of some of them and the exit of others during the storage period.

The aerial mycoflora of the storage environment where the root tubers were stored included: *Alternaria alternater, Aspergillus flavus, A. niger, Botryodiplodia theobromae, Cladosporium herbarum, Curvularia lunata, Fusarium oxysporum, Monilia species, Mortierella ramaniana, Mucor pusillus, Rhizopus stolonife, Rhodotorula* sp and *Saccharomyces cerevisiae*. These isolates were similar with those of the stored root tubers. However *Alternaria alternata, Botryodiplodia theobromae, Cladosporium herbarum, Curvalaria lunata, Monilia* and *Mortierella ramaniana* species were not isolated from the freshly harvested stored root tubers. It appears that these species of fungi might have originated from the storage aerial environment.

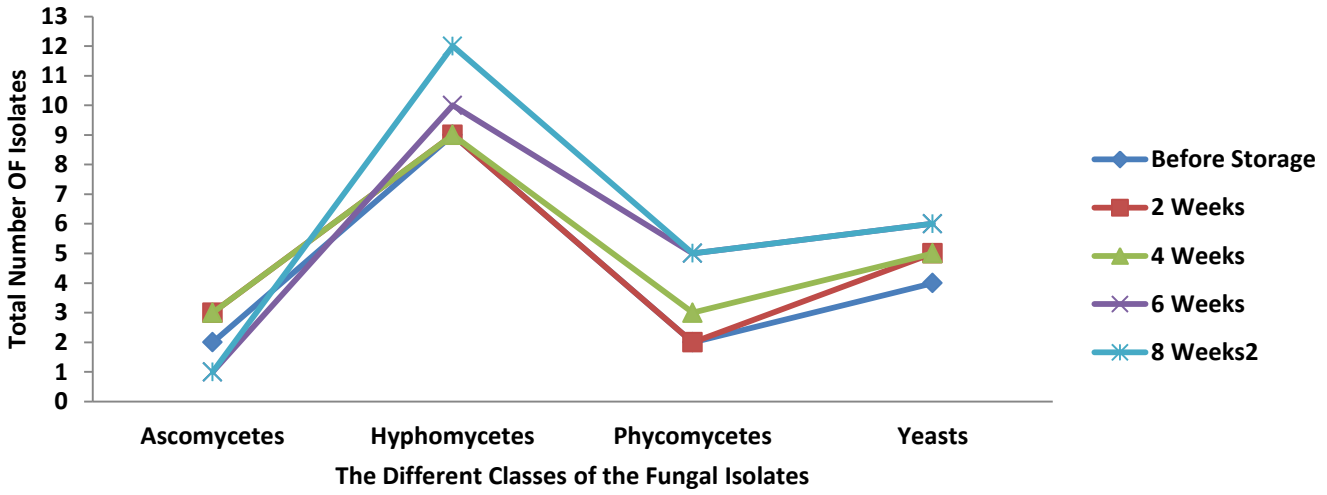

Figure 1: The Distribution of the Isolates from the Freshly Harvested Stored Root Tubers

Discussion

All the fungal species on the surface of the peels of the root tubers prior to storage must have originated from the experimental farm soil propagules. Their presence on the root tubers meant that these species of soil fungi could become post-harvest fungi of the root tubers in storage. Harvesting faults like cracks or wounds could facilitate the entry of the fungal propagules into the root tubers which would lead to their eventual decay (Rupsa *et al.*, 2017). That was why healthy harvested root tubers were selected for the root tuber storage studies. The storage aerial environment of the root tubers also played a significant role in the preservation of the cultivars.

Seventeen (17) isolates were identified in the freshly harvested root tubers prior to storage, the number of the fungal isolates was 13, with 4 Yeasts species and all the isolates originated from the experimental farm soil propagules. The Aspergilli were the dominant species.

Fungal succession was observed in the freshly harvested root tubers during their storage in the barn for 8 weeks. *Chaetomium funicola* which was not isolated from the peels of the root tubers prior to storage emerged in the peels after 2 weeks of storage in the barn. Succession of the fungal isolates continued to be observed in the stored root tubers after 6 weeks of storage with the exit of some of the fungal isolates:

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Aspergillus niger, A. oryzae, Chaetomium funicola and *Eurotium herbariorum* which were associated with the peels of the stored root tubers after 2 weeks of storage were not isolated after 4 and 6 weeks of storage in the barn. The emergence of *A flavus, A.fumigatus, Penicillum paraherquei, Mucor plumbens, M. pusillus, Rhizopus.Oryzae, R. stolonifer, Botrytis aclada, F. sporotrichoides, Penicillium lanusum* and *P. roqueforti* in the stored root tuber brought about the increased of the isolates.in the stored root tubers after 6 weeks of storage. However the isolates *Emericella nidulans A.terreus* and *M. suaveolens* had exited the root tubers during this storage period. Also the emergence of *Botrytis aclada, F. sporotrichoides, Penicillium lanusum* and *P. roqueforti* in the stored root tuber was observed after 8 weeks of storage but the isolates *Emericella nidulans A.terreus* and *M. suaveolens* had exited the root tubers during this storage period.

Succession occurred in the stored root tubers because the storage environmental conditions might not have favoured some of the fungal species that were brought from the farm with the root tubers into store while others were favoured. Those that were favoured continue to thrive in the root tubers causing their decay but the fungal species the conditions did not favour withered away (Oyewale, 2002; Oladoye *et al.* 2013).

The emergence of some of the isolates much later in the root tubers in the barn might have been due to the availability of glucose exuded by the root tubers much later during storage. In another instance the carbohydrates of the root tubers may have been hydrolyzed by enzymes of other fungal species thereby increasing the glucose concentration of the peels of the stored tubers which was available for decomposition by other fungal isolates. The availability of abundant sugars and other factors must have encouraged sporulation and the germinability of fungal propagules much later during storage in the barn.

Therefore succession was inevitable during the storage of the root tubers due to these and other factors. Oyewale (2002) illustrated the concept of succession in microorganisms by the transformations that take place at the root of cassava during fermentation. The *Bacillus* strains produce amylase enzymes which are involved in the initial breakdown of the cassava starch into simple sugars which are required by other organisms but the organism became extinct towards the end of the process.

The yeasts took over the fermentation process and brought it to a perfect conclusion.

Some of the genera of fungi identified in this study are similar with those reported by Garret (1963); Hattori (1973); Rangaswami (1988) and Arora and Arora (2007). These authors identified the genera: *Aspergillus, Mucor, Penicillium, Trichoderma, Cladosporium, Alternaria, Rhizopus, Fusarium, Verticillium Cephalosporium, Botrytis, Scopulariopsis, Acrostalagmus, Zygorhynchus, Pullularia, Gliocladium, Monilia, Chaetomuim* and *Pythium* to be among the most commonly encountered on the farm and within the storage environment of the sweet potato root tubers.

The sweet potato tuber is capable of supporting the growth of fungi due to its high water activity a_w and nutrients. The Periderm (skin) serves as a physical barrier against potentially pathogenic fungi which can degrade the complex carbohydrate into forms more usable by the organisms. Bovell-Benjamin (2002) examined the microbiological quality of field sweet potato and hydroponically grown sweet potatoes stored at 13^0 and 21^0C and 85% RH for five weeks. The genera of fungi found on HSP and FSP at both temperatures included*: Absidia, Crytococcus, Fusarium* and *Alternaria.* The fungal isolates were fewer in number compared with the number of isolates identified in this study. The difference in the number of the isolates could be due to the difference in storage time of the root tubers.

Fungal deterioration of sweet potato root tubers under different storage methods and time have been studied and reported by Charles *et al*. (2010). They found *Aspergillus flavus* as the most dominant fungus occurring in all of the three different storage structures followed by *A. niger, Rhizopus stolonifer, Trichoderma viride, Fusarium oxysporum, Penicillium digitatum, Cladosporium herbarum* and *Aspergillus ochraceus* in that order. These isolates are similar with the isolates identified in the stored root tubers after 2 weeks of storage in the barn.

Jonathan *et al*. (2012) analyzed samples of fresh and stored sweet potato chips for proximate composition, presence of bio-deteriorating fungi and mycotoxins (Aflatoxin B_1, B_2 G_1 and G_2) contamination. The result revealed that the nutrient contents decreased with increasing period of storage in all the samples because of the presence of *Aspergillus tamari, A.niger,*

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Fusarium oxysporum, A. flavus, Penicillium chrysogenum, Fusarium compactum and *Saccharomyces* species. The fungal isolates are similar with those identified in the stored root tubers of this research. Oyeyipo (2012) studied the bio-deterioration of sweet potato root tubers during storage at Port Harcourt, South Southern Nigeria. *Fusarium oxysporum, Rhizopus stolonifer, Botryodiploda theobroma and Penicillium* species were found to be associated with deterioration of the stored sweet potato root tubers. Some of the fungi in this present study are also similar with those identified in this research.

Most of the stored root tubers had become discoloured after 8 weeks of storage in the barn. Discolouration of the stored root tubers is due to heat produced by the respiring stored root tubers and is caused by pigments imparted on them by mycelia and spores of the storage fungi. The Penicillia have been referred to as the predominant green and blue stain fungi while the *Aspergillus niger* group has been referred to as the black stain fungi (Onwuka, 1982).

A number of factors have been attributed to the cause of spoilage of sweet potato root tubers during storage. These include high level of fungal metabolic activity within or on the stored root tubers by one or more species of fungi or a combination of species. It involves also the action of the fungus metabolites on the pigments within the product or by the pigments synthesized by the fungi themselves, resulting from the diffusibility of these pigments into the stored root tubers.

The genus *Aspergillus* had more species isolated from the stored root tubers than all species of the other genera isolated in this research. The genus has close to 200 species and varieties and it is widely distributed in nature, from the arctic to the tropics (Bessey, 1950). *Aspergillus* species are frequently found in the air and soil. As concerns indoor air quality the most important species are: *Aspergillus fumigatus, A. flavus, A. clavatus, A. niger and A. versicolor* which were all isolated from the stored root tubers and the environment in which plates of MEA were suspeneded. *Aspergillus* species are capable of utilizing an enormous variety of organic material for food because of their ability to produce a large number of enzymes. Growth of commonly occurring fungi like those isolated from the stored root tubers may result in the production of toxic complex secondary metabolic by-products referred to as mycotoxins.

Fungi that produce mycotoxins are said to be toxigenic. According to experts in the field, five kinds of mycotoxins: aflatoxins, ochratoxin A, fumonicins, certain trichothecenes and zearalenone are important in human and animal health. It is believed that the effect of mycotoxins as a cause of human mortality is underestimated.

Succession also occurred in the yeasts that were associated with the freshly harvested root tubers. Four yeast species: *Debaromyces hansenii, Rhodotorula glutinis, Rhodotorula* sp and *Saccharomyces cerevisiae* were isolated from the peels of some of the root tubers prior to storage in the barn. These yeasts might have originated from the farm soil propagules as it was with the fungi but the storage conditions did not favour the early growth of *Rhodotorula glutinis* and so it exited in the stored root tubers.

However, the yeasts might have also originated from the storage environment of the root tubers. The yeasts *Hansenula* sp and *Schizosaccharomyces pombe* which were absent in the root tubers prior to storage emerged on the root tuber peels after 2 weeks of storage in the barn. It was observed that after 6 and 8 weeks of storage of the root tubers the yeasts had increased in number to 6 species: *Debaryomyces hansenii, Hanselula* sp., *Rhodoturula, Saccharomyces cerevisiae, Saccharomycopsis fibuligera and Schizosaccharomyces pombe*. The increase in number of the yeast isolates may be due to the availability of sugars exuded by the stored root tubers which the organisms utilized to grow and develop and cause their decay.

The yeasts are popular for enzyme production which is employed to ferment the sugars of the root tuber peels to initiate the decay process. Oladoye *et al.* (2013) studied the postharvest losses of sweet potato root tubers initiated by the activities of yeasts. Six yeasts were identified as *Rhodotorula* sp, *R, minuta, Picha guilliemondii, P. anumala, Sporobolomyces marcillae* and *Saccharomycopsis fibuligera.* Some of these yeasts were isolated in this study and might have infected the sweet potato root tubers by secreting varying amounts of extracellular enzymes: cellulase, amylase, polygalacturonase, glucanase, xylanase, xylosidaze arabinofuranosidase and ferulic acid esterase as the yeast are known for. These enzymes had the capacity to degrade plant cell walls and possibly enhanced the pathogenicity of the yeasts.

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Prior to storage Ex-Igbariam peels had 10 isolates followed by Tanzania with 9 isolates but after 8 weeks of storage they both had 21 isolates each. This means that these root tubers have the potential of being decayed faster in storage if there was to be mismanagement in terms of storage facilities and techniques and if such surface fungal propagules are equipped with the necessary enzymes needed for the hydrolysis of their carbohydrate and other nutritional components.

In conclusion, the findings of this study has clearly shown that freshly harvested sweet potato root tuber is in association with farm soil propagules that constitute fungi and yeasts which might have commenced the ravaging of the produce in the field without any symptom of detection. Therefore the retention of the produce over 2 weeks of storage would encourage fungal and yeast succession which encouraged the retention of the farm colonisers, the exit of some of them and the emergence of newer forms an inter-play that ravaged, damaged and finally decayed the commodity holistically by reducing its shelf life drastically. To avoid fungal and yeasts succession during storage and possible public health challenges, the produce should be processed into secondary products with extensive shelf-life using most appropriate technology.

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