NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 50 Number 1, April 2019. Pp.101-109
Available online at: http://www.ajol.info/index.php/naj

OCCURRENCE OF POST-HARVEST FUNGI AFFECTING SWEETPOTATO *(Ipomoae batatas (L) LAM)* IN EBONYI STATE, SOUTH EASTERN NIGERIA

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ABSTRACT
Postharvest fungal spoilage is one of the major constraints to sweetpotato production. The study investigated the occurrence of the spoilage fungi affecting sweetpotato in Ebonyi State. Rotten sweetpotato roots (100 each in dry and rainy seasons) and healthy roots were collected from Ebonyi State for laboratory analysis. From the rotten sweetpotato specimens, fungi were isolated and characterized using morphological properties and confirmation via Partial ITS rDNA Sequencing Analysis and a BLAST Search with the GenBank Sequence. Isolates' pathogenicity was tested on healthy sweetpotato roots. Data was subjected to frequencies and percentages. Three hundred and fifty-two isolates were obtained from 200 sweetpotato samples. Five distinct genera and 7species of fungi were identified and the later included *Rhizopus oryzae, Aspergillus flavus Fusarium solani Aspergillus awamori*, *Aspergillus niger*, *Penicillium expansum* and *Botrydiplodiae theobromae*. The frequency of occurrence of the fungi species varied with respect to pathogen and season of isolation. *Botryodplodia theobromae* was the most frequently isolated fungi, followed by *R. oryzae*, *A. niger*, *A. flavus*, *F. solani*. *P. expansum* and *A. awamori*. The percentage occurrence of fungi isolation was both qualitatively and quantitatively higher in the dry season than in the rainy season. All the isolated fungal species were confirmed positive to pathogenicity test and two major categories of rot were predominantly incited by the fungi. The need to initiate fungi rot control is emphasized. There is therefore need to encourage the farmers to mitigate root fungi infection and give the issue of storage equal attention as yield improvement if the quality of their harvested produce is to be maintained and postharvest losses reduced.

Keywords: Ebonyi State, Occurrence, Postharvest Fungi, Rot, and Sweetpotato

Introduction

The developing nations of the world have always been in short supply of food. According to existing literature, around 1 billion people are being faced by severe hunger in these nations of which 10% actually die from hunger-related complications. This problem is further compounded by the accelerated increase in human population, which creates pressure on every form of food supply (Urom, 2014). Today, one of the main global challenges is how to ensure food security for a world growing population whilst ensuring long-term sustainable development. According to the Food and Agricultural Organization, food production will need to grow by 70% to feed world population which will reach 9 billion by 2050. Worse still, in the meantime, while the number of food insecure population remains unacceptably high (FAO, 2010), each year and worldwide, massive quantities of food are lost due to spoilage and infestations from farm to fork. This problem arises due to inadequate agricultural storage and produce preservation from microbes-induced spoilages (Kana *et al.*, 2012). Nigeria population relies on sweetpotato as a food security crop. However, yield loss to postharvest fungal spoilage is one of the major constraints to sweetpotato production. Obviously, one of the major ways of strengthening food security is by reducing these losses. Understanding disease-causing microorganisms and their occurrence are critical to prevention of microbial spoilage and reduction of food loses to microbial attack. Ebonyi State, South Eastern Nigeria is notable for Sweetpotato production. The phytopathogens associated with sweetpotato spoilage in Ebonyi State seemed not to have received attention; yet there was apparently a steady increase in postharvest microbial spoilage of sweetpotato observed and reported by some farmers in the area. Therefore the objective of this study was to determine the occurrence of the postharvest fungi causing rot of sweetpotato in Ebonyi State.

Materials and Methods

Study area: Four Local Government Areas in Ebonyi State with high sweetpotato production were identified and used as sampling location and include; Ikwo, Ezza North,Izii, and Ezza South LGAs. Ebonyi State is bounded to the north by Benue State, the west by Enugu State, to the south by Imo and Abia States and to the east by Cross River State. There are two distinct seasons, the wet and the dry season. The former takes place between April and October, while the latter occurs from November to March. The state has pseudo-bimodal rainfall pattern spread from April to November with annual rainfall rang between 1700mm-2060mm. The maximum mean annual temperature is 27-36ºC all through the year. Humidity is high with lowest levels during the dry season in April before the rainy season begins (Longinus, 2015)

Collection of Sweetpotato Samples: Hundred (100) rotted sweetpotato roots (25 samples from each LGA) were collected from Ebonyi sweetpotato farmers in the dry season of 2015/2016. This was repeated in the rainy season of 2016, giving a total sample size of 200 rotted roots. In each case, collected samples were packaged in polyethylene bags that were well labeled with date, collection site and sample number and taken to the Laboratory for analysis.

Isolation and Identification of Fungi Associated With Rotted Sweetpotatoes

Preparation of Growth Medium

The Potato Dextrose Agar (PDA) was prepared according to the Manufacturer's specifications. Thirty-nine grams (39g) of PDA) (Oxoid CM0139, Hamphire, England) was weighed and suspended in 1litre of distilled water, stirred to obtain a uniform mixture and autoclaved at 121^0C for 15 minutes. When the medium cooled to about 45^0C, it was amended using a broad spectrum antibiotic, Ampicillin (250mg); two capsules of which were dissolved in 2ml of autoclaved distilled water and added to 500ml molten PDA. This was gently shaken to obtain a fine mixture before pouring was done. The resulting mixture was then dispensed into 9mm diameter Petri dishes (autoclaved) when cooled and the poured medium stored at 4ºC and used when needed.

Fungal Isolation

The fungi associated with the rotted Sweetpotato roots were isolated using the methods of Anukworji *et al.* (2012). Sweetpotato samples were washed with clean water and the root tissues cut into sections of approximately 2mm cubes from the tissue at the junction between healthy and infested portion root with knife, surface sterilized in 70% ethanol and then rinsed twice in sterile distilled water. The root piece was placed on sterile paper towels in a Laminar Flow Hood chamber for 10 minutes to dry. With sterile forceps, four to five-point plating was done where the sweetpotato sections were inoculated onto PDA plates and the plates incubated at room temperature (28^0C) for five days and then examined daily for the development of fungi growth. When growth established, different fungi colonies shown on a three to five day old cultures were sub-cultured via point inoculation in which agar plugs were taken from the growing tips of different fungi colonies observed, with sterile needle and then transferred unto new plates. Plugs were placed downwards with the growing surface touching the PDA and the plates incubated at 27^0C. The Petri dishes of pure cultures of the fungi were then sealed with a masking tape to prevent contamination and the resulting pure cultures used for further investigations.

Identification of fungal isolates

Preliminary Identification of rot-associated isolates to species level was based on descriptions of Holliday (1995) and Mathur and Kongsdal (2003). Macroscopic features such as texture of mycelia, spore or conidia-producing structures, type of pigmentation were observed from fungal tissues grown on PDA. Microscopic characteristics (spore and mycelium shape and colour) of the isolates were examined by Lactophenol Cotton Blue (LPCB) wet mount preparation. Secondary identification involved subjecting some representative pathogens to Molecular Characterization through the partial ITS rDNA sequencing analysis which was done at Inqaba Biotechnology Pty South Africa and a BLAST Search using the GenBank Sequence Database.

Fungi Occurrence

The occurrence of the fungi isolated from rotted sweetpotato roots is computed as Percentage Frequency of isolation via the method of Ilondu (2013)

$$\%\ \boldsymbol{Occurrence} = \boldsymbol{PFI} = \frac{\text{Number of times a fungus is encounted}}{\text{Total number of times all fungi were encounted}} \times 100$$

Fungi Pathogenicity

Fungi pathogenicity was evaluated via the methods of Amienyo and Ataga (2007) with modification. Healthy roots of TIS 87/0087 sweetpotato cultivar obtained from the Sweetpotato Programme of National Root Crops Research Institute, Umudike were used. The Fresh sweetpotato roots were washed with tap water, rinsed with distilled water, weighed and surface sterilized with 70% ethanol. Cylindrical discs were removed from the tuber with a sterile 4mm cork borer. A disc of a five-day old pure culture of each test isolate (*Botryodiplodia theobromae, Fusarium solani, Aspergillus flavus, Aspergillus niger, Penicillium expansum, Rhizopus oryzae* or *Aspergillus awamori*) was inoculated into the hole created in the roots with the aid of another cork borer (4mm diameter). After the inoculation, the parts of the tissue bore out were carefully replaced, sealed with sterile Vaseline to prevent contamination and labeled accordingly. The inoculated roots were placed in clean polyethylene bag (one root per bag) each moistened with wet balls of

absorbent cotton wool to create a humid environment and incubated for 7 days at room temperature (28 ± 2°C). The same procedure was used for the control except that discs of un-inoculated PDA were placed in the holes created in the roots. After the incubation period, the sweetpotato roots were weighed, incised horizontally with sterile knife and examined for infection and disease development. The causal agents were re-isolated from the infected Sweetpotato roots and compared with the original isolates. This experiment was replicated three times.

Symptoms of Sweetpotato rot

Rots were categorized using the descriptions of Amusa *et.al* (2003) and Brook *et.al* (2003).

Results and Discussion

Isolation and Identification of Fungi Associated With Rotted Sweetpotatoes

Hundred percent (100%) of the 200 rotted Sweetpotato samples yielded viable postharvest fungal pathogen. Colonies of isolated fungi are presented in Figures 1 (A-F). The BLASTn analysis against the NCBI database of the nucleotide sequences resulted in 100% homology with *Rhizopus oryzae, Fusarium solani, Aspergillus awamori and Aspergillus flavus* with respective GenBank database Accession Number KJ439050, KJ863521, FJ441004 and KF908788. Five fungi genera (Aspergillus, fusarium, Rhizopus, Penicillium and Botrydiplodiae) comprising 7 fungi species (*Aspergillus flavus, Aspergillus niger, Fusarium solani, Rhizopus oryzae, Penicillium spp, Botrydiplodiae theobromae* and *Aspergillus awamori)* were identified.

Occurrence of Fungal Isolates obtained from rotted Sweetpotato

The frequency of occurrence of these seven species of fungi varied with respect to pathogen and season of isolation (Figure 2). In the dry season of 2015/2016, out of the 100 sweetpotato samples, 194 fungal isolates (55.11%) were obtained. Conversely in the rainy season of 2016, out of the 100 rotted sweetpotato roots sampled, 158 fungi (44.89%) were isolated. Thus, in both seasons, 352 fungi were isolated from 200 rotted Sweetpotato root samples, with a mean percentage occurrence of 176.

In the dry season of 2015/2016, seven species (194 isolates) were isolated. These included 26 *Aspergillus flavus* (13.4%), 42 *Aspergillus niger* (21.65%), 26 *Fusarium solani* (11.86), 36 *Rhizopus oryzae* (18.55%), 5 *Peniccilium expansum* (2.58%), 56 *Botryodiplodiae theobromae* (28.87%) and 6 *Aspergillus awamori* isolated (3.09%). Conversely, in the rainy season of 2016, six fungal species (158 isolates) were obtained (Figure 2). These included 20 *Aspergillus flavus* (12.66%), 29 *Aspergillus niger* (17.72%), 16 *Fusarium solani* (10.13%), 42 *Rhizopus oryzae* (26.56%), 9 *Penicillium expansum* (5.69%) and 43 *Botrydiplodiae theobromae* (27.22%).

Figure 3 represents the mean percentage occurrence of fungi isolated obtained in both seasons. In both seasons, out of the 200 samples, 352 fungal isolates were isolated and consisted *Aspergillus flavus* (13.03%), *Aspergillus niger* (19.68%), *Fusarium solani* (10.99), *Rhizopus oryzae* (22.56%), *Penicillium expansum* (4.08%), *Botrydiplodiae theobromae* (28.45%) *and Aspergillus awamori* (1.50%).

Table 1 is a presentation of the qualitative distribution of fungal pathogens associated with root rot of Sweetpotato across the LGAs. The results showed that *A. niger* and *B. theobromae* were isolated from all the studied LGAs and in both seasons; *A. flavus* and *Rhizopus oryzae* were isolated in both seasons and in three LGAs and none from Izii LGA. *F. solani* was isolated in both seasons and from two LGAs, namely Ikwo and Ezza North and Penicillium also in both seasons and from two LGAs – Ezza North and Izii while *A. awamori* was isolated only in the dry season and from only one LGA.

Fungi Pathogenicity and Categories of Induced Rot

The result of the pathogenicity test is shown in Table 2. All the seven fungal isolates induced rot in sweetpotatoes after 7 days of inoculation. Roots artificially inoculated with test fungi showed typical rot symptoms with the root tissue rotting around the inoculation point within seven days and when cut longitudinally into two halves through the inoculation point, showed variation in tissue degradation while the control root showed no tissue degradation. All the fungi were successfully re-isolated and on re-isolation, exhibited morphological characteristics and growth patterns similar to those earlier observed on axenic cultures, confirming their pathogenicity. The type of rot incited by isolates on mechanically wounded and artificially pathogen inoculated sweetpotato roots after 7 days of storage are also presented in Table 2 and Figure 4. They include soft rot and dry rot.

During this study, 352 fungal isolates were obtained from 200 rotted Sweetpotato roots, suggesting that postharvest rots of sweetpotatoes in Ebonyi state occur together as a complex rot involving many fungi which were more prevalent in the dry season than in the rainy season and would demand a broad spectrum control strategy. Qualitatively, 5 fungi genera (Aspergillus, Fusarium, Botryodipladea, Rhizopus and Penicillium) and 7 species (*A.niger, A. flavus, A. awamori, F. solani, B. theobromae, R. oryzae* and *Penicillium expansum*) were the spoilage fungi of Sweetpotato in Ebonyi state. In a related study in Anambra state, Agu *et al* (2015) examined fungi associated with the post-harvest loss of sweetpotato using a total of ten tubers obtained from Eke-Awka market, Awka South Local Government Area, Anambra State. The spoilage molds they

identified were three species: *Aspergillus fumigatus*, *Aspergillus niger* and *Rhizopus stolonifer*. The reason for the variation in occurrence of up to 7 different species of fungi in the present study and those (3 species) of Agu *et al* (2015) may be due to several factors, some of which are the fact that whereas this study investigated multiple samples (200) and these samples were collected from four LGAs, in the study by Agu *et al* (2015) only ten samples which collection was limited to one LGA were screened. A different report buttressing this point can be seen with the findings of Amienyo and Ataga (2007) who analyzed Sweetpotato samples collected from different markets in Port Harcourt and identified six fungi comprising four each of the fungi genera (*Fusarium*, *Rhizopus*, *Aspergillus* and *Botryodiplodea*) and species (*Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae*, *Fusarium solani*) recorded in the present study as agents of postharvest rot of sweetpotatoes. In a similar vein, four of the genera of fungi (*Fusarium, Rhizopus, Aspergillus and Penicillium*) reported in this work were also implicated with causation of Sweetpotato rot by Salami (2007) but with different species composition with the exception of *A. niger (Fusarium roselens, Rhizopus stolonifer, Aspergillus fumigatus*, penicillium, *Aspergillus niger*).

Botryodplodia theobromae was the most isolated fungi (28.45%) from the decaying sweetpotato roots. This finding is in accord with results from related studies by Amienyo and Ataga (2007) and Clark and Hoy (1994). *Rhizopus oryzae* was the 2nd most frequently isolated fungi (22.56 %) from sweetpotato rot in both seasons. Agu *et al* (2015) reported *Rhizopus spp (R. stolonifer)* as one of the most frequently isolated fungus from spoilt sweetpotato tubers in Anambra State. Similar scenario was found between two studies done in south west by Amienyo (2007) and Salami (2007) where Amionye reported that Rhizopus spp (*R stolonifer*) was the most frequently isolated fungus from spoilt sweetpotato tubers in South western, Nigeria. *Aspergillus niger* was the 3rd most isolated fungi (19.68%) from the rotted sweetpotato roots followed by *A. Flavus* (13.03%). Oyewole (2006) also eported *A. flavus* as one of the fungi associated with postharvest fungal rots. *F. solani* was the 5th most isolated fungi (10.99%) from the rotted sweetpotato roots. Amienyo and Ataga (2007) also isolated *F. solani* from rotting sweetpotatoes. Besides damaging sweetpotato roots in storage, *F. solani* was identified as a pathogen of a number of tropical agricultural crops such as tomatoes, potato, soybeans, peas and peppers (Amadioha and Uchendu 2003). *Penicillium expansum* was the 6th most isolated fungi in this study. Oladoye *et al.* (2016) also implicated *P. expansum* with causation of sweetpotato rot. *Aspergillus awamori* was the 7th most isolated fungi in this study. This to the best of our knowledge represents the first report on the involvement of *A. awamori* in post-harvest rot of root crops in South-eastern Nigeria.

A test of pathogenicity confirmed that all the isolated fungi *(Aspergillus flavus, Aspergillus niger, Fusarium solani, Rhizopus oryzae, Penicillium spp, Botrydiplodiae theobromae* and *Aspergillus awamori)* were responsible for rot induction in sweetpotatoes evidenced by typical rot symptoms shown by roots artificially inoculated with the test fungi within 7days of incubation and the fact that the isolates on re-isolation, exhibited morphological characteristics and growth patterns similar to those earlier observed on axenic cultures. The isolated fungi were found to incite different categories of rot, with dry rot being the most. The finding lends credence to the report of Snowdor (1991) that postharvest fungi create local discoloration of the surrounding tissues of infected tubers resulting in changes in appearance, deterioration of texture and possibly flavour or taste; thus causing postharvest losses, reduction in the market value and misfortune to farmers. According to Ray and Misra (1995), the fungus could infect sweetpotato roots through injuries to the tubers due to deficiencies in the handling or packaging processes. Though the mechanisms of fungi action was not experimentally elucidated in the present study, several reports show that the Phytopathogenic microorganisms are assisted by the enzymes they secrete (Ray, 2004)

Conclusion

The fungi associated with the spoilage of sweetpotato in Ebonyi State include; *A.niger, A. flavus, B. theobromae, R. oryzae, F. solani, Penicillium expansum and A. awamori*, with percentage occurrence quantitatively and qualitatively greater in the dry season than in the rainy season and varies with pathogen. The fungi on infection of the food crop, cause root decay ranging from soft rot, dry rot to java rot. Based on the results, control measures should be embarked upon in subsequent studies. There is therefore need to encourage the farmers to mitigate root fungi infection and give the issue of storage equal attention as yield improvement if the quality of their harvested produce is to be maintained and postharvest losses reduced.

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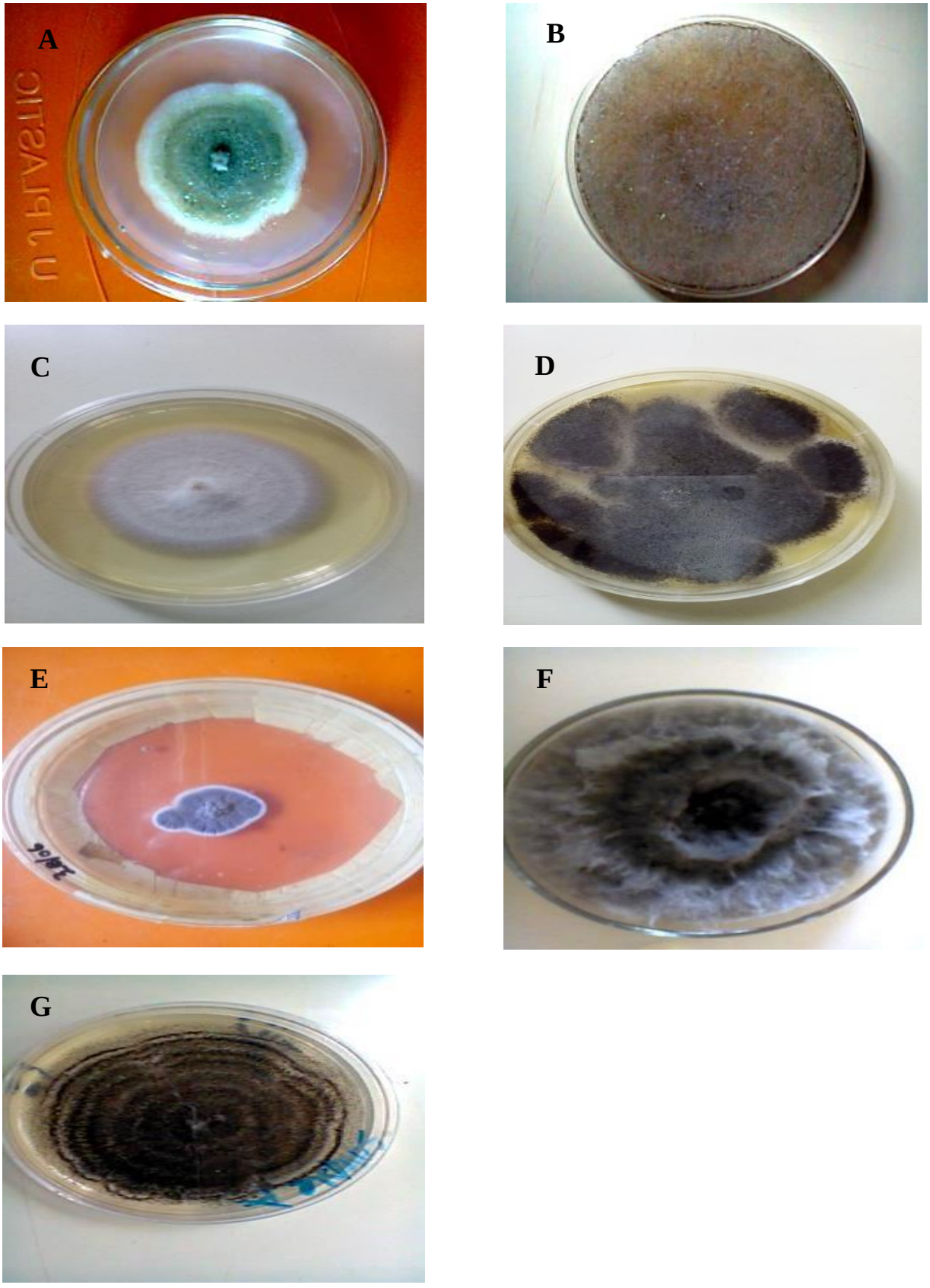

Figure 1: Colonies of Fungi Isolated from rotted Sweetpotato
Colonies of *A. flavus (*B) Colonies of *Rhizopus oryzae (*C) Colonies of *Fusarium solani* (D) Colonies of *Aspergillus awamori* (E) Colonies of *Penicillium expansum* (F) Colonies of *B. theobromae* (G) Colonies of *Aspergillus niger*

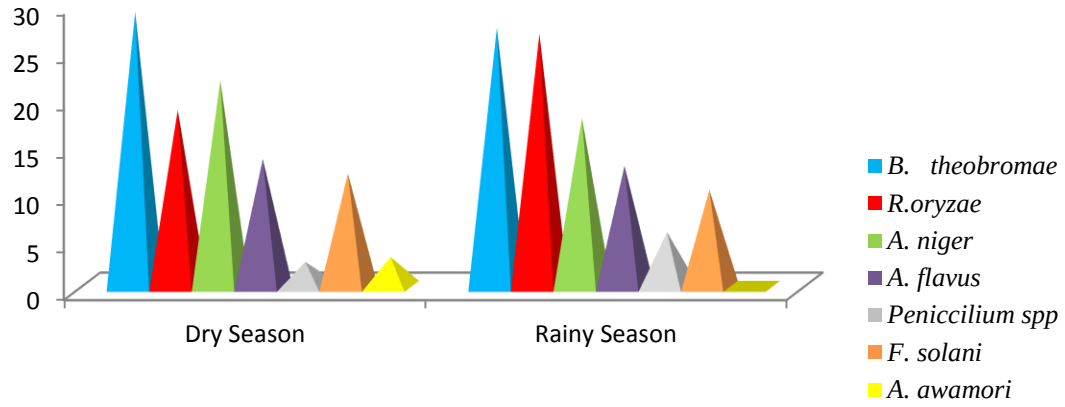

Figure 2: Seasonal percentage occurrence of fungi isolated from rotted sweetpotato from Ebonyi State

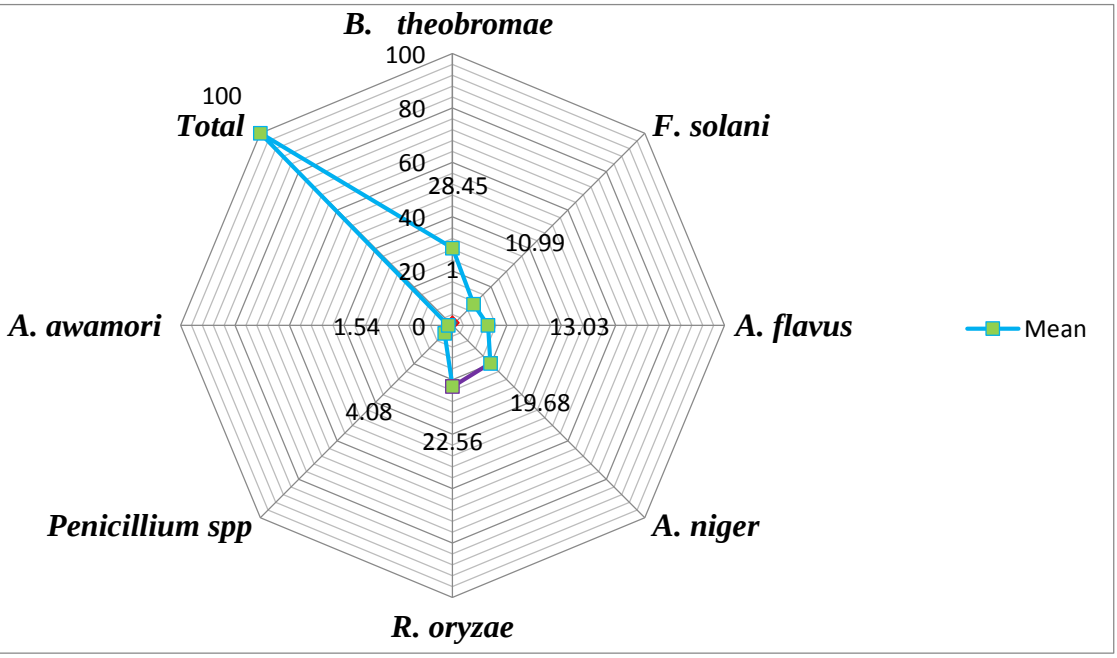

Figure 3: Mean percentage occurrence of fungi isolated in both seasons

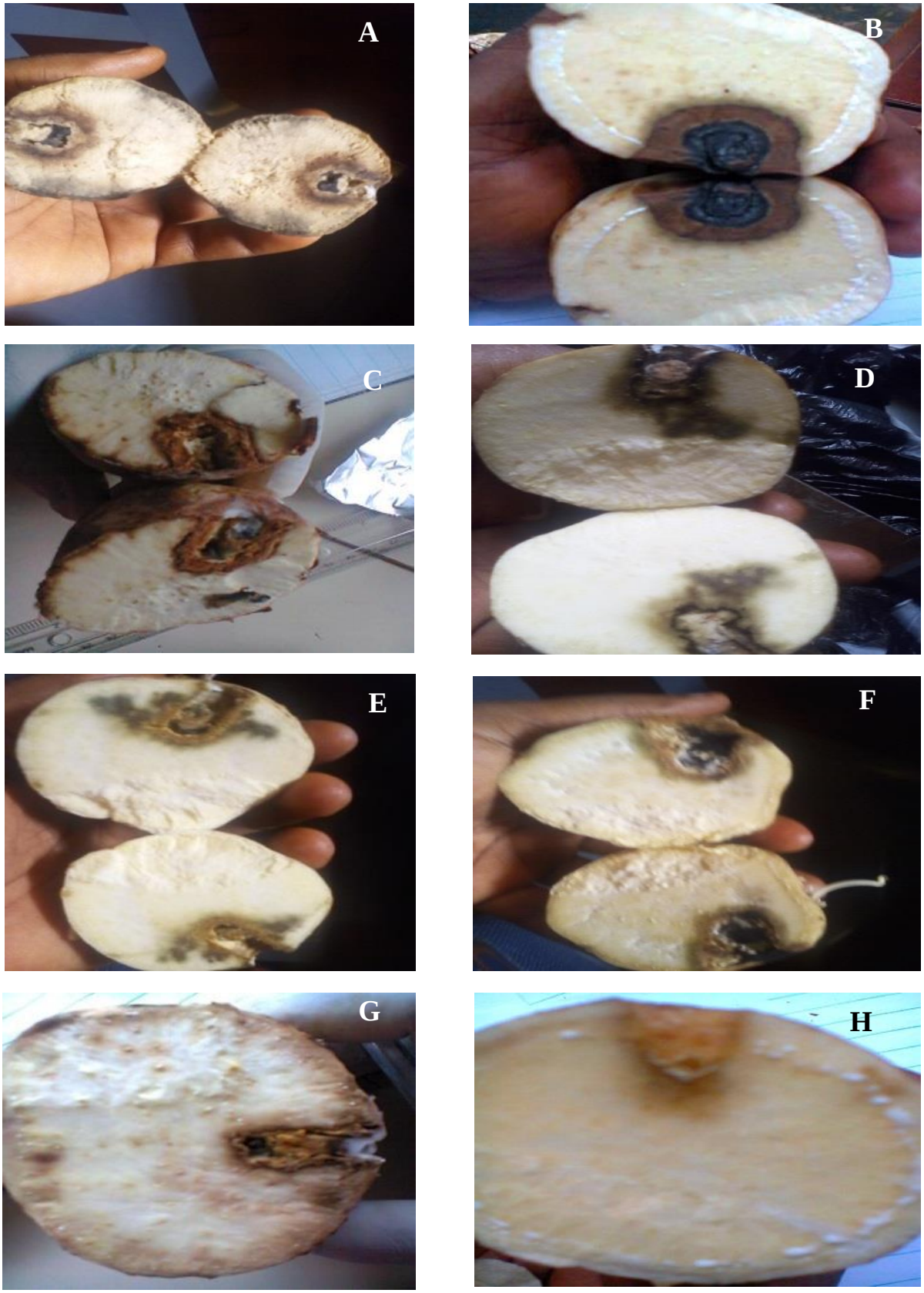

Figure 4: Sweetpotato roots showing lesions of postharvest rot at the point of inoculation with fungi
(a) Sweetpotato root showing lesions of soft rot at the point of inoculation with *R. oryzae* (b) Sweetpotato root showing lesions of java rot at the point of inoculation with *B. theobromae* (c) Sweetpotato root showing lesions of dry rot at the point of inoculation with *A. niger* (d) Sweetpotato root showing lesions of dry rot at the point of inoculation with *Penicilium expansum* (e) Sweetpotato root showing lesions of dry rot at the point of inoculation with *A. flavus* (f) Sweetpotato root showing lesions of dry rot (blue mold) at the point of inoculation with *fusariun solani* (g) Sweetpotato root showing lesions of dry rot at the point of inoculation with *A. awamori* (h) Control showing absence of rot

Table 1: Distribution of fungal pathogens associated with root rot of Sweetpotato across the LGAs

Locality	Fungal pathogens isolated
Ikwo	
RS	*A. niger, B. theobromae, A. flavus,Rhizopusoryzae, F. solani,*
DS	*A. niger, B. theobromae, A. flavus,Rhizopusoryzae, F. solani,*
Ezza South	
RS	*A. niger, B. theobromae, A. flavus,Rhizopusoryzae*
DS	*A. niger, B. theobromae, A. flavus, Rhizopusoryzae*
Ezza North	
RS	*A. niger, B. theobromae, A. flavus,Rhizopusoryzae, F. solani, Penicilliumsp,*
DS	*A. niger, B. theobromae, A. flavus, Rhizopusoryzae, F. solani, Penicilliumsp,*
Izii	
RS	*A. niger, B. theobromae, Penicillium spp*
DS	*A. niger, B. theobromae, Penicillium spp, A. awamori*

DS=Dry season RS=Rainy season

Table 2: Fungi Pathogenicity and Type of Rots Induced

S/N	Control/ Inoculated Fungi	Pathog-enicity status	Charateristic of rot	Rot Type
	Control	×	nil	Assymptomatic
1	*Aspergillus niger*	√	Infected tissues became light brown, hard and dry	Dry rot
2	*Aspergillus flavus*	√	Infected tissues became light yellow, hard and dry	Dry rot
3	*Botryodiplodiae theobromae*	√	Infected tissue was clearly demarcated, firm and dark surrounded by a brown portion	Dry (Java) rot
4	*Aspergillus awamori*	√	Infected tissues became light brown, hard and dry	Dry rot
5	*Fusarium solani*	√	Infected tissues become firm, dry and dark brown, with internal cavities filed with white fungus mycelium	Dry rot
6	*Rhizopus oryzae*	√	The infected tissues became brownish, soft and moist with stringy flesh	Soft rot
7	*Penicillium expansum*	√	Infected tissues became blue-green, hard and dry	Dry rot

× =did not produce rot symptom ;√ = Produced rot symptom with successful re-isolation