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AETIOLOGY OF FUNGAL DISEASES OF SWEET POTATO (*Ipomoea batatas* (L)) DURING POST HARVEST STORAGE IN OFFA KWARA STATE, NIGERIA

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ABSTRACT
Sweet potato (Ipomoea batatas (L)) is the traditional food of Offa people of Kwara State but is recently observed to be in short supply with attendant high cost for some years now. Post-harvest spoilage is found to be a major problem of the tuber. Disease survey on the incidence and severity of the fungal rot on the tuber crops within the locality was carried out. This study was carried out to isolate, identify and confirmed the microorganisms responsible for the post-harvest disease of the crop. Conventional isolation and culture techniques of diseased tissues in nutrient Agar (NA), Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) plates were adopted. Five replicate samples of healthy sweet potatoes were obtained randomly at different time intervals from farms and markets in Offa. They were homogenized and serially diluted to "thin-out" the microbial population. The fungal populations were observed after five days of incubation. The organisms were identified using morphological and biochemical tests. They were confirmed using pathogenicity tests. The isolated fungi are; Aspergillus, Mucor, Fusarivm and Penicillium. Efforts are currently going on to treat the diseases with leaves extracts of some medicinal plants. Data were obtained from disease survey was analysed with ANOVA and means were separated with FLSD.
Keywords: Post harvest spoilage, incidence and severity, Offa, Fungi, Leaves extracts.

INTRODUCTION

Offa people are known as the great consumers of Sweet Potata (*Ipomoea batatas)* in Nigeria. One of the most pressing problems facing the developing nations is food security, Salamo and Popola,

2007; Kana *et al.,* 2012). Sweet Potatoes possess many posiure health benefits including sources of anthocyanins, Phenolic compounds and other bioactive compounds (Giusti and Wrolstad, 2003; Suda, *et al.,* 2003; Kano *et al.,* 2005; TRUONG *et al.,* 2011). The vegetable crop also possess antioxidant activities (Givsti and Wrolstad, 2003; Suda *et al.,* 2003, Kana *et al.,* 2005). Salami and Popoola, (2007); Kana *et al.,* (2012), resported that nearly one billion people are challenged by severe hunger in the developing nations of which 10% are reported dead from hunger – related complications. In Nigeria, the root crops that are majorly important include cassava, yam, sweat potatoes and Irish potatoes. After harvest, these tubers suffer losses that result from physical, physiological or pathological factors or the combination of the three factors (Opara and Agugo, 2014).

Food spoilage is a metabolic process which may be brought about by microbial action and causes foods to be undesirable or unacceptable for human consumption due to deterioration in quality characteristics (Doyle, 2007). Different species of sweet potatoes have gained popularity in many countries as a result of their health benefits (Leksrisompong *et al.,* 2012). Therefore, early intervention measures, during crop development and harvesting through the use of good agricultural practices will provide dramatic reductions in yield loss due to spoilage at all subsequent steps (Barth *et al.,* 2009). Food security and sustainability is one of the crops of addressing this problem of scarcity and shortage due to activities of microorganisms after harvesting. In a FAO/WHO report of (2012), food security was defined as a situation in which all people at all times have both physical and economic access to adequate and nutritious food for an active and healthy life; the manner in which the food is produced, preserved and distributed are in a consideration of the

natural processes of the earth and thus sustainable thus reducing spoilage, scarcity, malnutrition and poverty.

According to the survey carried out in Iran, 10% pre-harvest and 20% post-harvest rots occurred in sweet potato (Bidarigh *et al.,* 2012). These rots constitute major impediments to the drives for food security in Nigeria. It has been noted that the largest post-harvest losses in vegetable crop tubers result from microbial attacks (Ray *et al.,* 2000). Reports are available on the fungi associated with spoilage of sweet potatoes during harvest storage (Ray *et al.*, 2000; Doyle, 2007).

Sweet potatoes have been described as having thin, delectate skin that is easily damaged by cuts and abrasion during harvesting, transportation or distribution. Striking roots with harvesting equipment of dropping them into containers injures their skin. The sweet potato may be cut or bruised if they are placed in containers having sharp edges or roughly hauled or handled, and this may give rise to microbial infestation (Rupsa *et al.*, 2017). The objectives of this work were to isolate, identify, confirm and characterize the fungi associated with the spoilage of sweet potato.

MATERIALS AND METHODS

Healthy potatoes were obtained from Owode market, Erinle market adjacent Offa town and farms at Igosun town in Offa. They were protectively taken to Science Laboratory Technology Laboratory in Delta State Polytechnic, Ozoro in sterile containers and stored at 29^0C for five weeks. Survey was carried out in the three locations within Offa town and its environs of Erivile and Igosun. Assessment began 20th February 2020 with the onset of harvet and was completed within three weeks.

Disease Incidence

For the rot incidence, experiment sites were used. Each site contained two farms (replicates). In each farm, a square measuring 4m x 4m was carried out using a measuring tape and total number 7 sweet potatoes (Tnc) within that given area counted and recorded. The number of rotten tubers dug (Tdc) per the given area was also calculated for a farm and the mean incidence was obtained from each site. Thus:

PRI = $\frac{Tdc}{Tnc}$ x 100

Where PRI = Percentage rotten tuber incidence (%)

Tdc = Total number of rotten sweet potatoes

Tnc = Total number of sweet potatoes harvested

Disease Severity Rating

In each location, three sites were assessed and each site comprised two farms. In each farm, five randomly selected tubers were assessed for disease severity and mean of the data obtained. The disease severity rating of the rotten tubers by the pathogens was assessed using a modified method of Eze and Maduewesi (1990), as cited by Chiejina (2006), using a rating scale of 1 – 5. Where 1 = No decay, 2 = slight decay 10 – 30%, 3 = moderate decay 31 – 60%, 4 = severe decay 61 – 90% and 5 = complete decay 91 – 100%.

Preparation and Sterilization of Media

Potato Dextrose Agar and Malt extract Agar (MEA) were aseptically sterilized in an autoclave at 103 KNM^{-2} pressure and 121^{0}C for 15 minutes. Preparation and sterilization of media, water and glass ware for the experiment all preceded the collection of the sample. Petri dishes, beakers, test tubes filter paper, spatula and forceps were sterilized in a hot air oven at a temperature of 160^{0}C for 1hour. The wire loops were also

sterilized by heating over a sprit lamp until red hot and allowed to cool. Seventy percent alcohol was used to wipe the work tops to prevent contamination.

Preparation of Culture Media

All culture media were prepared according to instructions by manufactures.

Isolation of Fungi from *I. Batatas* Tuber

The isolation technique of Chiejina (2008) was used. Thin sections (2mm diameter) were cut from the periphery of diseased potato tubers and sterilized in 0.1% mercuric chloride for 2 minutes. These sections were rinsed in 3 changes of sterilize distilled water and plated in PDA and MEA plates. The plates were incubated at room temperature (27 ± 2^0C) for days. Pure cultures were obtained by several transfers of the colony growth from the plates to clean plates aseptically.

Pathogenicity Test

Pathogenicity test was carried out for all the isolates from the different sweet potato tubers using the techniques of Okigbo *et al* (2009). Healthy tubers of sweet potato were washed in sterile distilled water and surface sterilized with 0.1% mercuric chloride solution. With the aid of sterile 5mm cork borer, a cylindrical core was removed from each potato tuber. A pure culture of the isolate was introduced into the hole and the core was replaced and sealed with sterile petroleum jelly. Un-inoculated tubers (control) were also set up (Okigbo and Emeka 2010). The fruits were kept at room temperature for 7 – 10 days. On establishment of diseases symptoms, inoculate from the infected fruits were taken and cultured. Pure cultures were identified according to Barnett and Hnter (1999) and Alexopoullus *et al.,* (2002). The

symptoms were compared to those of naturally infected potato tubers. Morphological characteristics of conodia and mycelia of the fungi that were re-isolated from inoculated fruits were used to prove koch's postulates.

Data Analysis

A multi-locational Analysis of Variance (ANOVA) was done separately for the disease incidence and for disease severity. Means separations, was down with Fischer's Least Significant (FLSD) at P = 0.05.

RESULTS

Characteristics of Fungal Isolates

Four fungi were isolated and identified as *Aspergillus, Mucor ramossissimus, fusarium* and *Penicillin.* Pathogenicity tests confirmed that the isolated fungi were the causal agents of the rot. The total fungi count was measured in spore-forming unit per/gram (sfu/g). Most of the reported cases have identified fungi (Ray *et al.*, 2000; Doyle, 2007). The mean value of the different samples is the Table 1, the morphological characteristics of the fungal isolates of the fungal isolates are in table 2, Identification of fungal descriptively in Table 3, Severity rating in Table 4 while Percentage occurrence is in Table 5.

Table 1, Mean of Fungal Load of Samples

Replicate Samples	Total Fungi count (sfu/g) (Mean value
First Isolation	2.11×10^7
Second isolation	$2 \times 90 \times 10^7$
Third isolation	$1 \times 95 \times 10^7$
Fourth isolation	2.50×10^7

Table 2. Morphological Characteristics of Fungal Isolates

S/N	Isolate code	Pigmentation	Colour of Spore	Reverse side of the Agar	Aerial Hyphaphae	Abundance Growth
1	KF1	No	Black	Powdery, spores embedded	Abundant	Fast
2	KF 2	No	Black	"	"	"
3	KF 3	No	Blue-green	"	"	"
4	KF 4	No	White	Fluffy, raised a	"	"
5	KF 5	No	White	Fluffy, not raised	"	"
6	KF 6	No	White	Fluffy, not raised	"	"
7	KF 7	No	White	Fluffy raised	"	"
8	KF 8	No	Brown	Powdery raised	"	"
9	KF 9	Yes	Creamy green	Embedded	"	"

Table 3: Disease severity rating of the isolates in Sweet Potato

Days	1	2	3	4	5	6	7	8	9	10
Fungal Isolates	**Severity rating**									
Aspergillus	1	2	2	3	3	3	4	4	5	5
Mucor	1	2	2	3	3	2	3	3	4	4
Fusariom	1	2	2	2	3	3	3	5	4	4
Penicillin	1	2	2	2	2	2	3	3	3	4
Control	1	1	1	1	1	1	1	1	1	1

Rating scale of 1 – 5 (Chiejina, 2006) where I = No decay, 2 = slight decay: 10 – 30%, 3 = Moderate decay: 31 – 60%, 4 = Severe decay <61 – 90%, 5 = complete decay.

Table 5 – Identification of Fungi

S/N	Isolate code	Description	Probable identity
1	KF 1	They are typically powdered black. Conidiophores arsing from long, broad thick walled, sometimes branched foot cells, it has tall conidiophores. Conidia are large with radiating heads, mostly glucose and irregularly roughed.	*Aspergillus, niger*
2	KF 2	They are typically black. Conidiophores arising from long, broad thick-walled, sometimes branched foot cells, it has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed	*Aspergillus, niger*
3	KF 3	Colonies spread thinly; blue-green with strictly columnar conidial heads. Pigmented conidiophores present with clavate resides arising from clearly differentiated thick walled foot cells. Conidia are globose.	*Aspergillus fumigates*
4	KF 4	Colonies are whitish to olivaceous-bluff, odour aromatic; in the dark differentiated into tall and short sporangiophores. Sporangia blackish with ellipodial, pyriform or subglobose, chlamydospores	*Mucor Sp*
5	KF 5	Colonies are whitish to olivaceous-bluff, odour aromatic; in the dark differentiated into tall and short sporangiophores. Sporangia blackish with ellipsoidal, pyriform or subglucose. Chlamydospores absent.	*Mucor Sp*
6	KF 6	Colonies smoke-grey in the dark, yellowish brown in the light; odour aromatic, it has wide spor angiophore and a denser larger layer of short repeatedly branched sporangiophores. Sponrangiophores thick-walled with granular contents	*Mucor mucedo*
7	KF 7	Colonies are fast growing, aerial mycelium sparse to abundant and floccose, becoming felted, white or peach, but with a violet tinge. Characteristic aromatic odour suggesting lilae.	*Fusorium*
8	KF 8	Colonies growing rapidly, appearing cinnamon to orange-brown or brown. It appears velvety in appearance. Conidiophores are long smooth walled hyaline, with hemispherical resides. Metulac are present, conidial heads strictly columnar conidia appear globose to slightly ellipsoidal and smooth-walled.	*Aspergillus terneus*
9	KF 9	Colonies fast growing conidiophores in fresh isolates typically loosely synematous, giving the colon a donate appearance. Colonies are light-green, reverse colourless yellow-brown condiophores usually smooth walled, pencil 2-3 stages branched with numerous usually oppressed mutualc, conidia sub-globose to ellipsoidal, smooth-walled, odour aromatic, fruity and suggesting apples.	

Table 5: Percentage Occurrence of Fungal Isolates

Fungi genera	Frequency	Percentage %
Aspergillus	4	45
Mucor	2	25
Fusarium	2	15
Penicillium	2	15
Total	**10**	**100**

DISCUSSION

Fungi isolated give 1.80 x 10^7 sfu/g to 2.9 x 10^7 sfu/g. this confirms fungi have been implicated in the spoilage of sweet potato which agreed with Onuegbu (2002) who reported *Penicillium* Sp., *Ceratocy* Stis *Fombriata, Aspergillus niger, Diaporthe batatalis* and *Aspergillus flavus.* It is also in agreement with Oyewole (2006) finding on fungi that were associated with post-harvest fungal rot of sweet potato to include: *Motierella ramanniana, Rhizopus stoloifer, stoloifer, Tyidhadevrnas, Mucor, Pusiluss, Botrytis, Cinerea, Erysiphe polygons* and *Aspergillus flavus.* During the investigation, *Aspergillus flavus* is the most dominant fungal species, followed by *Musor ramosissimus* and *Penicillium* been the least which is in consonance with (Chiejina and Ukeh, 2012).

The percentage of occurrence includes *Aspergillus* (45%), *Mucor* (25%), *Fusarium* (15%) and *Penicilium* (15%). This is also in tandem with (Khatoon *et al.,* 2012; 2016). Enyiukwu *et al* (2014) who isolated *Aspergillus, Fusarium* and *Geotrichum* as fungi responsible for the spoilage of potato and *Aspergillus fumigates* respectively. Washington (2013) also reported the soft rot disease of sweet potato storage and post-harvest storage rot by the fungi *Fusarium solani* and *Macrophomina Phaseolina* and *Geotrichum Phaseolina* which this work agreed with. However, few works has been carried out on the post-harvest fungal

storage loss of sweet potato root tubers throughout the world (Akhtari *et al.,* 2007, Rupsa *et al.,* 2007).

The toxins produced by the fungal isolates are dangerous in small quantities and presents extreone toxicity because of their resistance to heat. Fungal toxins contaminate food products and cause acute or chronic intoxications. This leads to a reduction in life expectancy, worsen disease conditions in humans leading to 40% loss of economic productivity (Okigbo, 2004; Shukla *et al.,* 2012).

Four pathogens: *Aspergillus niger, Mucor ramosissimus, Fusarium Sp* and *Penicillum* were isolated from sweet potato tubers in this work which is in agreement with the earlier finding Adaskave *et al.,* (2002) who reported the species of *Mucor, Aspergillus* and *Penicillium* were common post-harvest fungi. *Rhizopus* and *Mucor* have been shown to cause *Mucorosis* in the immune system of man, which could be a serious ailment if contacted via consumption of potato tubers. The toxins from these pathogens cause respiratory and ulceration diseases in human beings. This in supported by the work of Kurup (2003).

CONCLUSION

This work was able to associate the following fungi with the post-harvest diseases of sweet potato. *Aspergillus niger, Mucor ramosissimus, Fusarium* and *Penicillin Sp.* This causes a reduction in the availability of sweet potato in circulation. We are currently working on the fungitoxk effort of methanolic leaf extracts from some medicinal plants and aqueous extracts of the same leaves to control the post-harvest fungal diseases of sweet potato tubers. More researches are also suggested to eradicate such pathogens.

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