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ISOLATION, IDENTIFICATION AND CROSS-INFECTION OF PATHOGENS RESPONSIBLE FOR POSTHARVEST SPOILAGE IN YAM AND COCOYAM ACROSS MARKETS IN AWKA, ANAMBRA STATE NIGERIA

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ABSTRACT: *Postharvest loss of food is highest during storage, which is caused by a number of fungi pathogens. This study aims to isolate and identify the disease-causing pathogens associated with yam and cocoyam in markets across Awka. Diseased yam (Dioscorea rotundata) and cocoyam (Xanthosoma sagittifolium) samples was collected from four different markets: Eke-Awka, Nkwo-Amaenyi, Amawbia, and Amansea all in Awka, Anambra State, Nigeria. Samples were taken to the Department of Botany laboratory, Nnamdi Azikiwe University, Awka for culturing, isolation, identification and cross-inoculation. Proximate analysis was also done on the fresh and diseased yam after cross-infection to determine the difference and effect of spoilage in the nutritional composition. Based on physical observation of the growth of the fungi on the yam and cocoyam specimens, the several fungal species isolated include: Aspergillus niger, Aspergillus flavus and Rhizopus stolonifer for both yam and cocoyam samples and Fusarium oxysporum and Aspergillus terreus for yam only, while cocoyam also had Phytophthora parasitica and Fusarium solani. The percentage occurrence of fungi pathogens on the diseased yam and cocoyam samples collected from different markets across Awka showed that most of the fungi had 100% occurrence. The pathogenicity test result for the yam sample showed that A. flavus had a higher pathogenicity on the fresh yam samples when compared with R. stolonifer. Aspergillus niger, Aspergillus terreus and Fusarium oxysporum showed no significant difference in their pathogenicity on the fresh yam sample. For cocoyam, Phytophthora parasitica and Fusarium solani had higher pathogenicity at later days. The Aspergillus species showed a slow but steady increase in their pathogenicity. The result of the pathogenicity of cross-infection of fungi pathogens isolated from the diseased yam samples on the fresh cocoyam samples showed that the pathogenicity of the fungi pathogens and the number of days were significantly different ($P<0.05$), while that of the pathogenicity of cross-infection of fungi pathogens isolated from the diseased cocoyam samples on the fresh yam samples also showed that the pathogenicity of the fungi pathogens and the number of days were significantly different ($P<0.05$). Results of the percentage proximate composition assay showed that diseased yam and cocoyam have lower ash content compared to healthy counterparts. Healthy yam has the highest carbohydrate content while diseased yam and cocoyam had lower carbohydrate levels respectively. Results obtained in this study obviously showed that cross-infection of fungi pathogens isolated from diseased yam and cocoyam samples could induce rot on healthy yam samples or cocoyam samples. Thus, cross-infection was ascertained to be possible in this study amongst the crops.*

KEYWORDS: *Aspergillus*, Cocoyam, Cross-infection, Fungi, *Fusarium*, Isolation, Pathogens, Post harvest, *Rhizopus*, Yam.

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INTRODUCTION

Reports by Magan *et al.* (2003) stated that the postharvest loss of foods is highest during storage after harvest. Magan *et al*. (2011) demonstrated that postharvest losses are caused by a wide variety of biotic and abiotic factors. These include mould, insects, mites and the key environmental factors of water and temperature. The interactions between these factors affect the dominance of fungi, especially mycotoxigenic species such as *Fusarium culmorum*, *Aspergillus ochraceus* and *Penicillium verrucosum*. Therefore, minimizing postharvest losses caused by these pathogens is an effective way to improve agricultural income (Tefera *et al*., 2011).

In terms of microorganisms, fungi and their associated secondary metabolites known as mycotoxins are of high concern in farm produce or storage facilities due to the production of mould, odours, the presence of microbial 'hot-spots', and the production of secondary metabolites which can lead to subsequent poisoning of food and animal feed, thus negatively impacting food safety (Tefera *et al*., 2011). There are a number of postharvest fungi that can attack and cause damage to foods, such as yam and cocoyam, and they can be divided into two groups: field fungi and storage fungi (Miller, 1995). Field fungi may modify the structure and quality of produce (Chelladurai *et al*., 2010); these cause damage to the tubers and corms before harvest and can generally be detected by routine assessment. Storage fungi are those that cause damage to grain during storage and usually do not occur at a serious level prior to harvest (Muir & White, 2000). The mycoflora of stored grains predominantly consist of the ubiquitous mould genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Rhizopus* and *Penicillium* (Mathew *et al*., 2010). They are usually introduced into the stored produce as spores in minute quantities during handling and storage. Other microorganisms such as certain bacteria can also colonise the stored food materials. These bacteria mainly belong to the families *Pseudomonadaceae*, *Micrococcaceae*, *Lactobacillaceae* and *Bacillaceae* (Laca *et al*., 2006). In Australia, Europe, and the US, *Salmonella* spp., *Escherichia coli*, and *Bacillus cereus* are also present in wheat and flour at low levels but are prevalent in Africa and Asia Minor (Cicognani *et al*., 1975; Ottogalli & Galli, 1979; Spicher, 1986; Eyles *et al*., 1989; Richter *et al*. 1993; Aydin *et al*. 2009).

Yams are monocotyledonous plants belonging to the genus Dioscorea of the family Dioscoreaceae which constitute a multi-species of crops that are important for food, socio-cultural activities and income. Dioscorea species are important food crops in West Africa, and other tropical countries including East Africa, Central Africa, The Caribbean, South America, South East Asia and India (Coursey, 1967). The most important areas for the cultivation and usage of yam stretches from Ivory Coast through Ghana, Nigeria, Togo, Cameroon, Garbon, Central African Republic and the Western part of the Democratic Republic of Congo. According to F.A.O. (2000), these regions produce about 93% of the World's annual yam production, estimated at 38.5 million metric tons and Nigeria alone accounts for about 26.4 million tons (70%) in the year 2000.

Cocoyam is a monocotyledonous plant of a genus of flowering plants in the family Araceae; it is a popular tuber crop in Southeastern Nigeria. *Colocasia esculenta* and *Xanthosoma sagittifolium* serve as staple foods in Southeastern Nigeria, and are commonly known as cocoyam. Various ethnic groups in Nigeria have different names which attests to its nationwide distribution and use. It is known as ede/akaso/uli in Ibo, guaza in Hausa, koko in Yoruba, mkpon in Efik and ikereburu in Ijo. *Colocasia esculenta* (Taro) is a member

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of the Araceae family; it is an ancient crop grown throughout the humid tropics for its edible corms and leaves, as well as for its traditional uses (Wang 1983). The Araceae family is a large one, comprising about a hundred genera and more than fifteen hundred species, mostly tropical or subtropical plants. The aim of this study is to isolate, identify and cross-infect the disease-causing fungi pathogens associated with yam and cocoyam in markets across Awka.

MATERIALS AND METHODS

Study Area

This research was carried out in Awka metropolis; Awka lies between latitude ($7^{0}00$ and $7^{0}10^{1}$) E and ($6^{0}05$ and $6^{0}15^{1}$) N in Anambra State.

Source of Materials

Diseased yam *(Dioscorea rotundata*) and cocoyam (*Xanthosoma sagittifolium*) samples were collected from four different markets: Eke-Awka, Nkwo-Amaenyi, Amawbia, and Amansea all in Awka, Anambra State, Nigeria. They were placed in a sterile polythene bag and brought to the Department of Botany laboratory, Nnamdi Azikiwe University, Awka for culturing, isolation, identification and cross-inoculation.

Media Preparation

The medium used for the fungal isolation was Sabouraud Dextrose Agar (SDA). Ten grams of the powder was dispensed into 100 ml of distilled water in a conical flask and then stopped tightly with cotton wool and foil; it was heated in a water bath for about 2 hours until the agar was melted. The prepared medium was then sterilized using an autoclave at 120^{o}C and 30 psi (Cheesbrough, 2000; Jawetz *et al*., 2004) for 15 minutes. Thereafter, it was allowed to cool and then dispensed into the petri dishes.

Preparation of Sample Inocula

Inocula were prepared from four (4) unhealthy yam and cocoyam tubers. The unhealthy yam tubers were first washed in sterile water and then surface sterilized using 70% ethanol. A sterile kitchen knife was used to cut each of the tubers so as to reveal the boundary zone between the rotten and healthy parts of the yam tuber. Small bits were cut from the boundary zone of each tuber and transferred to sterile petri dishes and later used for isolation of fungi pathogens. The same process was repeated for cocoyam tubers.

Isolation of Test Fungi from Rotten Yam

Isolation of fungi was done by agar dilution plate method. The method was used by Humaidi *et al*. (1999). The inoculum prepared from the diseased yam was used for isolation of the fungi. Three pieces each of the four different samples of the yam were placed in each petri dish containing SDA media (making three plates of sample, giving a total of 12 plates). All plates were wrapped externally with masking tape and incubated at +/- 27^{o}C for 72 hours and observed daily for growth of fungi.

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Isolation of Test Fungi from Rotten Cocoyam

The inoculum prepared for the fungi isolation was inoculated into the petri dishes containing SDA media. After inoculation, the plates were placed in the incubator at +/- 27^{o}C and the growths were monitored and recorded daily.

Subculturing and Identification of Test Fungal Pathogens

Subcultures were prepared using inocula from different organisms in the mixed cultures to obtain a pure culture; this was done by transferring from the colony edge of the mixed cultures to fresh sterile SDA plates with the aid of a scalpel. The plates were wrapped externally with masking tape and incubated for 72 hours. The resulting pure cultures were used for the subsequent identification of fungi isolates. The identification was on the basis of their micro- and macro-morphological characteristics using standard taxonomic keys used previously by Samson *et al*. (2010).

Pathogenicity Test for Yam

Fresh yam was brought and the fungi colonies were inoculated in the fresh healthy tuber crops. Ten grams of yam was weighed into four places and placed in sterile petri-dishes. A sterile knife was used to create wounds in the sliced tuber samples and the isolated fungi were incubated into the wounds separately, labeled and incubated for 7 days. At the end of the 7th day, the extent of the rot caused by the fungi was determined using the method as described by Kassim (1986).

$$\text{Rot (\%)} = \frac{A-a}{A} \times 100$$

where:

A= Initial weight of tubers

a = Final weight of tubers after the removal of the rotten portion.

Pathogenicity Test for Cocoyam

The method of Okigbo and Nmeka (2005) was used in the pathogenicity test. Healthy cocoyam corms were washed with distilled water and thereafter disinfected with 70% ethanol. A flamed 4 mm cork was used to bore a hole into the healthy cocoyam corm; a 4 mm disc from the pure culture was inoculated into the hole made with the aid of another cork borer of 4 mm diameter. After inoculation, the part of the cocoyam corm bored out was carefully replaced and sealed with sterile vaseline to prevent contamination.

Cross-infection of Fungi Pathogen of Cocoyam on Fresh Yam Tuber

This was carried out using fresh yam. Fungi isolates from the cocoyam samples were inoculated into the healthy yam tubers. Holes were bored in the healthy tubers and fungi isolates from cocoyam pure culture were inoculated into the healthy yam tubers and covered. The edge was sealed with sterile vaseline and labeled accordingly.

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Cross-infection of Fungi Pathogen of Yam on Fresh Cocoyam Corm

The same process above was repeated in the cross-infection of fungi pathogens of yam on fresh cocoyam corm. A pure culture from the yam samples was incubated into the healthy cocoyam corms and covered. This was then monitored daily and observed for any possible infection.

Proximate Analysis

This was carried out mainly by using the method described by the Association of Official Analytical Chemists (A.O.A.C). It involved the determination of crude protein, ash, crude fiber, ether extract (fat), moisture content and carbohydrate content.

Data Analysis

Completely Randomized Design (CDR) was used for this study. The data obtained was analysed using the statistical package SPSS version 2023. Data obtained from the study were subjected to Analysis of Variance (ANOVA) at 5% significance level.

RESULTS

Table 1 shows the percentage occurrence of fungi pathogens on the diseased yam samples collected from different markets across Awka. *Rhizopus stolonifer* and *Fusarium oxysporum* had 100% occurrence in all the markets (Eke Awka, First market, Amaenyi market and Amansea market); *Aspergillus niger* and *Aspergillus flavus* had 75% occurrence across board while *Aspergillus terreus* had only 50% occurrence on yam samples collected from the four markets. A pie chart representation of the percentage occurrence of the fungi pathogens isolated from the yam samples was shown.

TABLE 1: Percentage Occurrence of Fungi Pathogens on Diseased Yam across Markets in Awka

PATHOGEN	EA	FM	AEM	AMN	PERCENTAGE PREVALENCE
Rhizopus stolonifer	+	+	+	+	100%
Fusarium oxysporum	+	+	+	+	100%
Aspergillus niger	+	+	+	-	75%
Aspergillus flavus	+	-	+	+	75%
Aspergillus terreus	-	+	-	+	50%

EA = Eke Awka, FM = First Market, AEM = Amaenyi Market, AMN = Amansea Market.

Presence = +, Absence = - .

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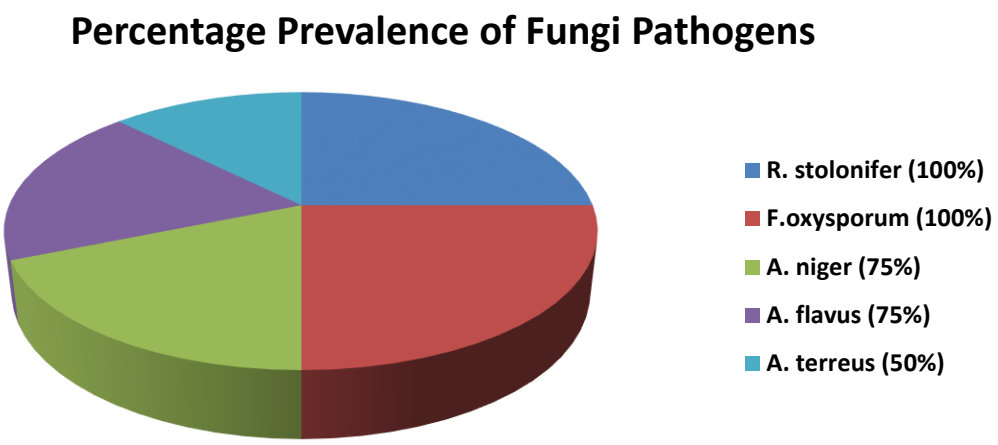

Figure 1: Percentage Prevalence of Fungi Pathogens in Diseased Yam Samples across the Study Area

Table 2 shows the percentage occurrence of fungi pathogens on the diseased cocoyam samples collected from different markets across Awka. *Phytophthora parasitica* and *Fusarium solani* had 100% occurrence in all the markets (Eke Awka, First market, Amaenyi market and Amansea market); *Aspergillus niger* and *Aspergillus terreus* had 75% occurrence across board while *Aspergillus flavus* had only 50% occurrence on cocoyam samples collected from the four markets. A pie chart representation of the percentage occurrence of the fungi pathogens isolated from the cocoyam samples was shown.

TABLE 2: Occurrence of Fungi Pathogens on Diseased Cocoyam across Markets in Awka

PATHOGEN	EA	FM	AEM	AMN	PERCENTAGE PREVALENCE
P. parasitica	+	+	+	+	100%
F. solani	+	+	+	+	100%
A. niger	+	+	+	-	75%
A. terreus	+	-	+	+	75%
A. flavus	-	+	-	+	50%

EA = Eke Awka, FM = First Market, AEM = Amaeyi Market, AMN = Amansea Market.
Presence = +, Absence = - .

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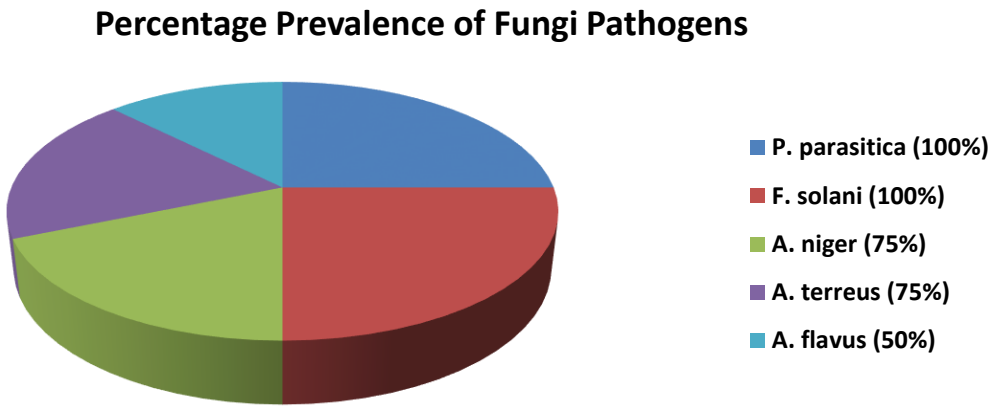

Figure 2: Percentage Prevalence of Fungi Pathogens in Diseased Cocoyam Samples across the Study Area

Table 3 shows the result of the pathogenicity test of fungi pathogens isolated from the diseased yam samples on the fresh yam samples. The result shows that *Rhizopus stolonifer* and *Aspergillus flavus* had a progressive but slow increase in pathogenicity from Day 3 to the 6th day, although *A. flavus* had a higher pathogenicity on the fresh yam samples when compared with *R. stolonifer*. *Aspergillus niger, Aspergillus terreus and Fusarium oxysporum* showed no significant difference in their pathogenicity on the fresh yam sample. *A. terreus* had the least pathogenicity in all the days. The pathogenicity of the fungi isolates and the number of days were significantly different (P<0.05)**.**

Table 3: Pathogenicity of Fungi Pathogens Isolated from Yam Samples (cm)

Fungi Isolates	DAY 3	DAY 4	DAY 5	DAY 6
Rhizopus stolonifer	3.33 ± 0.08^{b}	3.41 ± 0.10^{b}	5.01 ± 0.02^{a}	5.23 ± 0.04^{a}
Aspergillus flavus	3.38 ± 0.04^{c}	4.00 ± 0.08^{bc}	5.22 ± 0.06^{b}	6.20 ± 0.10^{a}
Aspergillus niger	3.53 ± 0.04^{b}	3.60 ± 0.04^{b}	3.64 ± 0.01^{b}	3.70 ± 0.00^{a}
Aspergillus terreus	2.74 ± 0.06^{b}	2.76 ± 0.05^{b}	2.80 ± 0.08^{a}	2.89 ± 0.04^{a}
Fusarium oxysporum	5.41 ± 0.08^{a}	5.41 ± 0.01^{a}	5.44 ± 0.02^{a}	5.50 ± 0.04^{a}
LSD	0.242	0.721	0.487	0.559

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column/row are significantly different (P<0.05).

Table 4 shows the result of the pathogenicity test of fungi pathogens isolated from the diseased cocoyam samples on the fresh cocoyam. The result shows that all the fungi tested had a progressive increase of the fresh cocoyam. Although, *Phytophthora parasitica* had higher pathogenicity at Days 5 and 6; *Fusarium solani* had a higher pathogenicity on Day 6 only. The *Aspergillus* species showed a slow but steady increase in their pathogenicity. The pathogenicity of the fungi isolates and the number of days were significantly different (P<0.05).

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Table 4: Pathogenicity of Fungi Pathogens Isolated from Cocoyam Samples

Fungi Isolates	DAY 3	DAY 4	DAY 5	DAY 6
P. parasitica	2.23±0.08^{c}	3.43±0.10^{b}	4.03±0.02^{a}	4.51±0.04^{a}
Aspergillus flavus	2.30±0.04^{d}	2.62±0.08^{c}	3.06±0.06^{b}	3.22±0.10^{a}
Aspergillus niger	3.30±0.06^{bc}	3.34±0.05^{bc}	3.64±0.08^{b}	3.98±0.04^{a}
Aspergillus terreus	2.54±0.04^{b}	2.55±0.04^{b}	2.55±0.01^{b}	2.64±0.00^{a}
Fusarium solani	2.25±0.08^{c}	2.45±0.01^{b}	2.55±0.02^{b}	4.51±0.04^{a}
LSD	0.441	0.394	0.418	0.388

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column/row are significantly different (P<0.05).

Table 5 shows the result of the pathogenicity of cross-infection of fungi pathogens isolated from the diseased yam samples on the fresh cocoyam samples. The result shows that *Rhizopus stolonifer* and *Aspergillus flavus* had almost similar pathogenicity from Day 3 to the 6th day, although *R. stolonifer* had a higher pathogenicity on the fresh cocoyam sample on Day 6. *Aspergillus niger* and *Aspergillus terreus* also showed similar pathogenicity on Day 3 (2.68±0.03^{b} and 2.04±0.01^{c} respectively) and Day 4 (2.82±0.06^{b} and, respectively), although they went up drastically on Day 5; *A. terreus* (4.11±0.06^{b}) showed more pathogenicity than *A. niger* (3.94±0.07^{a}). *Fusarium oxysporum* showed no significant difference in their pathogenicity on the fresh cocoyam sample after Day 3. The pathogenicity of the fungi pathogens and the number of days were significantly different (P<0.05).

Table 5: Pathogenicity of Cross-infection of Fungi Pathogens Isolated from Diseased Yam Samples inoculated on Fresh Cocoyam Samples

Fungi Isolates	DAY 3	DAY 4	DAY 5	DAY 6
Rhizopus stolonifer	3.18±0.07^{b}	4.23±0.06^{a}	4.31±0.08^{a}	4.88±0.00^{a}
Aspergillus flavus	3.99±0.07^{c}	4.32±0.07^{a}	4.38±0.09^{a}	4.49±0.05^{a}
Aspergillus niger	2.68±0.03^{b}	2.82±0.06^{b}	3.94±0.07^{a}	3.98±0.00^{a}
Aspergillus terreus	2.04±0.01^{c}	2.23±0.04^{c}	4.11±0.06^{b}	4.89±0.01^{a}
Fusarium oxysporum	3.48±0.03^{b}	4.17±0.09^{a}	4.21±0.05^{a}	4.26±0.05^{a}
LSD	0.354	0.367	0.294	0.358

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column/row are significantly different (P<0.05).

Table 6 shows the result of the pathogenicity of cross-infection of fungi pathogens isolated from the diseased cocoyam samples on the fresh yam samples. The result shows that *P. parasitica* had a high and consistent increase in pathogenicity on the fresh yam, with the highest being (5.40±0.00^{a}) on Day 6. *Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus* had almost similar pathogenicity from Day 3 to the 6th day, although *A. niger* had a lower pathogenicity on the fresh yam sample on Day 3 (1.89±0.08^{c}). *Fusarium oxysporum* showed similar pathogenicity on Days 3 and 4 (3.00±0.00^{c} and 3.00±0.08^{c}) respectively and had its highest pathogenicity on Day 6 (3.67±0.07^{a}). No significant difference in their pathogenicity on the fresh cocoyam sample after Day 3. The pathogenicity of the fungi pathogens and the number of days were significantly different (P<0.05).

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Table 6: Pathogenicity of Cross-infection of Fungi Pathogens Isolated from Diseased Cocoyam Samples inoculated on Fresh Yam Samples

Fungi Isolates	DAY 3	DAY 4	DAY 5	DAY 6
P. parasitica	4.00±0.01^{c}	4.67±0.03^{c}	5.04±0.05^{b}	5.40±0.00^{a}
Aspergillus flavus	2.00±0.03^{b}	2.00±0.00^{b}	2.00±0.02^{b}	2.33±0.05^{a}
Aspergillus niger	1.89±0.08^{c}	2.00±0.06^{bc}	2.45±0.02^{b}	2.67±0.05^{a}
Aspergillus terreus	2.23±0.07^{bc}	2.27±0.07^{b}	2.30±0.04^{b}	2.58±0.01^{a}
Fusarium solani	3.00±0.00^{c}	3.00±0.08^{c}	3.33±0.00^{b}	3.67±0.07^{a}
LSD	0.448	0.437	0.395	0.452

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column/row are significantly different (P<0.05).

Results of the percentage proximate composition assay in Table 7 below show that the fresh yam and cocoyam tested had more carbohydrate content (78.50±0.30^{a} and 69.21±0.26^{ab} respectively) as against the very low carbohydrate content in the decaying yam and cocoyam samples (13.80±0.22^{b} and 10.62±0.02^{b} respectively). There was variance in the figures for moisture content in the samples tested with healthy yam and cocoyam having (62.68±0.35^{a} and 58.27±0.23^{a} respectively) and the diseased yam and cocoyam having (50.23±1.24^{a} and 64.21±0.76^{a} respectively), although there was no significant difference in the moisture contents of the samples (P<0.05). Healthy cocoyam had the highest protein content (1.62±0.04^{d}) while diseased yam and healthy yam had similar protein levels (0.28±0.22^{d} and 0.77±0.08^{c} respectively). Also, healthy cocoyam contained more fat (1.89±0.06^{d}) while diseased yam had the lowest fat content (0.11±0.06^{d}). The fiber content in diseased yam and cocoyam were both low (0.98±0.06^{c} and 1.21±0.07^{c} respectively) compared to the healthy yam and cocoyam had higher fiber levels (3.97±0.11^{d} and 6.34±0.07^{c} respectively). More so, ash content represents mineral content. Diseased yam and cocoyam have lower ash content compared to healthy counterparts. Healthy yam has the highest carbohydrate content (78.50±0.30^{a}) while diseased yam and cocoyam have lower carbohydrate levels (13.02±0.22^{b} and 10.62±0.02^{b}, respectively).

Table 7: Proximate Composition of Healthy and Diseased Yam and Cocoyam (g/ml)

Proximate	Yam		Cocoyam	
content	Healthy	Diseased	Healthy	Diseased
Moisture	62.68±0.35^{a}	50.23±1.24^{a}	58.27±0.23^{a}	64.21±0.76^{a}
Protein	0.77±0.08^{c}	0.28±0.22^{d}	1.62±0.04^{d}	0.58±0.03^{d}
Fat	0.25±0.03^{d}	0.11±0.06^{d}	1.89±0.06^{d}	0.39±0.04^{d}
Fibre	3.09±0.11^{d}	0.98±0.06^{c}	6.30±0.07^{c}	1.21±0.07^{c}
Ash	1.70±0.04^{d}	0.42±0.04^{c}	1.55±0.04^{d}	0.93±0.08^{c}
CHO	78.50±0.30^{a}	13.80±0.22^{b}	69.21±0.26^{ab}	10.62±0.02^{b}
LSD	5.824	3.459	4.636	2.465

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column are significantly different (P<0.05).

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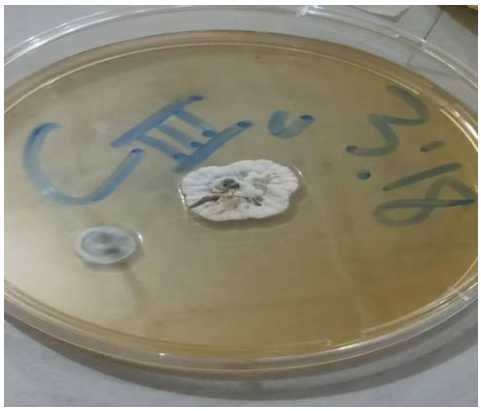

Plate 1: Photo of *Fusarium solani* observed

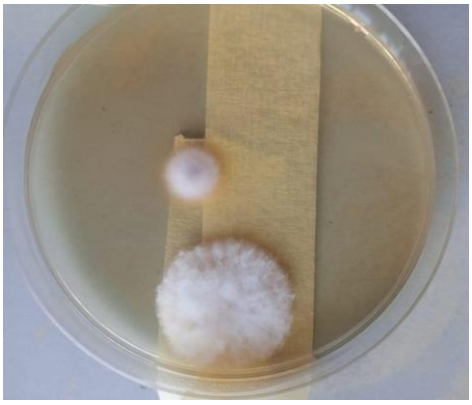

Plate 2: Photo of *Fusarium oxysporum* observed

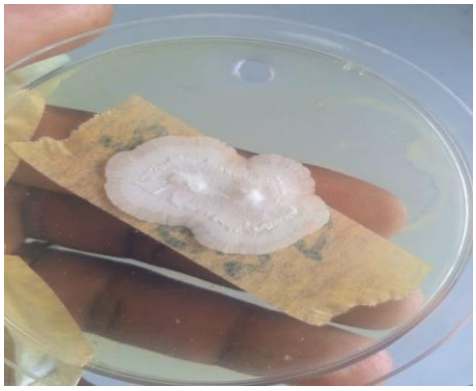

Plate 3: Photo of *Phytophthora parasitica* observed

Plate 4: Photo of *Rhizopus stolonifer* observed

Plate 5: Photo of *Aspergillus niger* observed

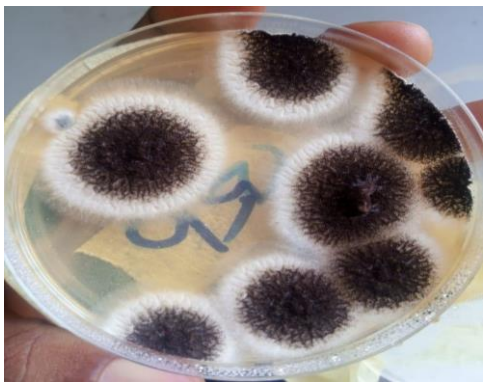

Plate 6: Photo of *Aspergillus niger* observed

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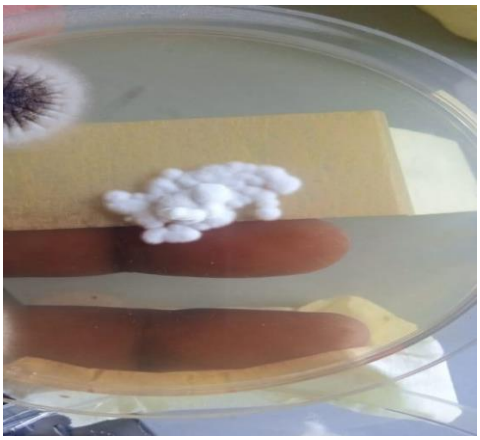

Plate 7: Photo of *Aspergillus terreus* observed

DISCUSSION

Contamination of foods was reported to be a function of many factors which may include infestation in the field (farm) prior to harvest, contamination during harvesting by handlers and materials used, method of packaging and distribution of plant produce to the market. Mode of preservation plays a role too (Okoye & Amadi, 2011).

The percentage occurrence of fungi pathogens on diseased cocoyam in markets across Awka showed that *Phytophthora parasitica* and *Fusarium solani* had 100% occurrence in all the markets (Table 2). *Aspergillus niger* and *Aspergillus tereus* had 75% percentage occurrence across board while *Aspergillus flavus* had only 50% percentage occurrence on cocoyam samples collected from the four different markets. The findings contrast with Okigbo and Nnadiri (2017)who observed that *Botryodiplodia theobromae* had the highest percentage occurrence, followed by *Aspergillus niger.*

Pathogenicity tests of the fungi pathogens isolated from the diseased yam samples revealed that all the test fungi were pathogenic with varying degrees of virulence (Table 3). *Fusarium oxysporum* was the most virulent amongst the fungi pathogens isolated from yam samples across the number of days for incubation. This contrasts with Okigbo *et al.* (2015), who observed that *Sclerotium rolfsii* was very pathogenic to the healthy white yam tubers, causing rot of 42.4% on the total tissue surface within 7 days. This was followed by *Fusarium oxysporum* isolated with 36.2% of the rotted tissue surface area of the healthy white yam tuber after 7 days of incubation.

In the pathogenicity test of the fungi pathogens isolated from the diseased cocoyam samples, on the healthy cocoyam, there was a progressive increase on all the fungi tested. *Phytophtora parasitica* had higher pathogenicity values at Days 5 and 6 while *Fusarium solani* had a higher pathogenicity on Day 6 only. The *Aspergillus* species showed a slow but steady increase in their pathogenicity. These findings differed from the observations by Okigbo and Nnadiri (2017) who reported that all the four test fungi they isolated were pathogenic with the most virulent being *Botryodiplodia theobromae*. Pathogens play a significant role in the cultivation of yam and cocoyam, two staple crops in many regions around the world. These pathogens can cause devastating diseases that result in reduced crop yield and quality, posing a threat to food security.

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More so, the pathogenicity of these fungi might be due to the report of their ability to grow faster, and high pH tolerance. Hence, this makes them important cosmopolitan fungi associated with post harvest decay and soft rot of different substrates (Perrone *et al.,* 2007). These organisms are soil saprobes with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses and this is because of their ability to produce extracellular organic acids. Relatively, Sharma and Thiyam (2013) reported that *Mucor* sp. were pathogenic on some local fruits and vegetables among which were *Psidium guajava, Citrus lomon, Mangifera indica, Musa paradisiacal, Phyllanthus emblica, Talinum triangulare* and *Carambola* sp. Bhale (2011) reported the prevalence of *Aspergillus* sp., *Fusarium* sp. and *R. solani* on the post-harvest rot of vegetables and fruits. *A. niger, R. solani*, and *P. parasitica* were established as soft rot organisms.

Cross-infection of yam (*Dioscorea rotundata* Poir) and cocoyam (*Xanthosoma sagittifolium* (L) Scott), was investigated in this study. The percentage occurrence of fungi pathogen on diseased yam across markets in Awka showed that *Rhizopus stolonifer* and *Fusarium oxysporum* had 100% occurrence in all the markets surveyed in this study, namely Eke Awka, First market, Nkwo Amaenyi and Orie Amansea. This is followed by *Aspergillus flavus* and *Aspergillus niger* with 75% percentage occurrence across board while *Aspergillus terreus* had only 50% percentage occurrence on yam samples collected from the four markets. This observation is in tandem with Okigbo *et al*. (2015) and Anuagasi *et al*. (2017) in their study on the fungi pathogen responsible for yam rot in Awka.

Cross-infection of fungi pathogens isolated from diseased yam samples inoculated on a fresh cocoyam sample showed that *Rhizopus stolonifer* and *Aspergillus flavus* had almost similar pathogenicity from Day 3 to the 6th day, although *Rhizopus stolonifer* had a higher pathogenicity (4.88±0.00^{a}) on the fresh yam sample on day 6. Thus, this finding is novel and has no precedence, and therefore cannot be compared with earlier works.

Cross-infection of fungi pathogens isolated from diseased cocoyam samples inoculated on fresh yam samples showed that *Phytophtora parasitica* had a high and consistent increase in pathogenicity on the fresh yam with the highest being (5.40+-00^{a}) on Day 6; this observation is a novel one and could not be compared with earlier findings.

Proximate composition of healthy and diseased yam and cocoyam was evaluated (Table 7). The percentage proximate composition assay showed that the fresh yam and cocoyam samples had more carbohydrate content (78.50+-0.30^{a}) and (69.21+-0.26^{b}) respectively, as against the very low carbohydrate content in the decaying yam and cocoyam samples (13.80+-0.22^{b} and 10.62+-0.02^{b}) respectively. This observation was the same for the protein contents, fat, fiber as well as ash content, with the healthy yam and cocoyam samples having higher values for this proximate content while the diseased yam and cocoyam had lower values. This low result in diseased yam and cocoyam could be attributed to the deterioration brought about by fungi pathogens on the tissues of these plants; this deterioration reduces the quality and nutritional value of these plants, thereby making them inedible. However, the proximate composition observed in this study is unprecedented as most of the findings were focused on qualitative and quantitative phytochemical constituents of yam and cocoyam, as reported by Okigbo *et al*. (2015), although their report when compared to the findings of this study showed that there was reduction in phytochemical quantity and quality of yam and cocoyam affected by fungi pathogens.

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CONCLUSION

The post harvest rot of white yam and cocoyam poses an obvious challenge which requires urgent attention considering their economic importance in Nigeria. The results obtained in this study obviously show that cross-infection of fungi pathogens isolated from diseased yam and cocoyam samples could induce rot on healthy yam samples or cocoyam samples; thus, cross-infection was ascertained to be possible in this study amongst these crops: yam and cocoyam. Since this is a new discovery, efforts should be channeled to study the cross-infection of other root and tuber crops.

It is recommended that further investigation should be carried out on the chemical nature of the active principles of yam and cocoyam besides their proximate content. Also, it is very important to adopt good storage patterns to prolong the storage of white yam and cocoyam after harvest. Furthermore, screening of plant materials and bio-fungicides with potential inhibitory effects against post harvest rot of yam and cocoyam should be evaluated. The findings of this study will be relevant to farmers who suffer huge losses as a result of fungi attacks on their stored produce. It will help them understand the concept of cross-infection and further plan the storage of their produce properly. This research will also serve as a basis for further research into disease infection and cross-infection in crops.

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