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Isolation and Identification of Fungi Pathogen from Rotten White Yam Sold in Mubi Metropolis Adamawa State - Nigeria

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Abstract

This study was aimed to isolate Fungi from Rotten White Yam sold in Mubi Market, Adamawa State, Nigeria. The rotten samples of white yam were cut into pieces each with a sterile razor blade and cultured on potatoes dextrose agar (PDA). It was later incubated at room temperature for 1-5 days after which the fungal growths were observed. A total of thirty (30) white yam fungal isolates were obtained from the samples, fungi isolated and the frequency of occurrence include aspergilus spp 63.3%, fusarium spp 10.0%, monilla spp 3.3%, penicillium spp 16.7% and mucor spp, 6.7% from the three market. These species of Fungi were associated with contamination of white yam. Based on these findings, it was observed that perishable food such as white yam are susceptible to spoilage by fungi. This may due to spores of these organisms which were easily transmitted via the air, leading to spoilage of these vegetables.

Keywords: Isolation, Fungi, Rotten White Yam, Spoilage, Harvesting, Transportation.

Introduction

Yam, is a family member of Diocoraceae and genus Dioscorea, is a tuber crop with significant economic and cultural value in tropical nations (Yan Wang *et al.*, 2021). There are numerous yam cultivars grown in Africa, including Dioscorea (white yam), D. cayanensis (yellow yam), D. alata (water yam), and D. dumentorum (cluster yam), with the first two cultivars being the most well-known (Alamu, *et al;* 2016). Cluster yam or bitter yam are common names for the cultivar Dioscorea dumentorum Pax. Although it is grown in the south-west and south-east of Nigeria, the yam is underutilized in that nation (Anwadike,2016). The widespread perception among consumers and farmers that bitter yam has an unpleasant taste and is expensive is likely a contributing factor to its under-utilization.

A vital number of plant pathogens affect a wide range of woody and herbaceous plants found in the genus Colletotrichum. The plant's leaves, petioles, stems, veins, and even tubers are all impacted by the genus Colletotrichum, which also causes dieback, premature abscission, and the final death of the entire plant (Akombo, 2016). According to Nkese Ime Okon *et al.* (2022), the disease typically has an important impact on infected plants, turning an area of recent healthy yam plants from green to black in some weeks. The fungi Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromine, and Colletotrichum are also frequently linked to yam rots, include *Aspergillus flavus, Aspergillus niger,*

Botryodiplodia theobromae, Colletotrichum spp, Fusarium oxysporum, Penicillium chrysogenum, Pennicillium digitatum (FAO, 2013*). Colletotrichum* species are occasionally identified in yam tubers (Abdollahzadeh, 2013).

A number of issues, including those brought on by the genus Colletotrichum, which results in a yield loss of roughly 90%, limit the production of yams (Egesi, 2009). Based on perceived scientific and commercial importance, the genus Colletotrichum was recently ranked as the ninth most significant group of plant pathogenic fungi in the world (Asiedu, 2010). Fungus control techniques come in many different forms. Although chemical management of soil-borne diseases offers some degree of control, it also has negative environmental implications by disrupting helpful soil microorganisms (Ogunleye, 2014). Therefore, in recent years, the hunt for these biological agents has increased. Biological control of plant pathogens has thus been suggested as a potential management technique. According to Okeke et al. (2015), trichoderma is the most widely utilized fungal biological control agent and has a long history of being an efficient antagonist against plant pathogenic fungi (Okeke, *et al*., 2015).

This work is aim at use of indigenous control methods that are environmentally friendly, cost effective and could easily be practiced on yam farms. Therefore, this study is conducted to assess the effectiveness of isolate and identify fungi associated with the rotted white yam sold in Mubi market.

Materials and Method

Study Area

Mubi is located in the Northern part of Adamawa state between latitudes 9° 26" and 10° 10" N and longitude 13° 10" and 13° 10" E. It is bordered by the Mountain ranges of the Mandara in the republic of Cameroon to the East, Michika Local Government area to the North, Hong to the South and Askira-Uba to, the West and occupies a landmass of about 506,440 square kilometers (Nwagboso et al; 2012, Amina *et al;* 2023). The climate of the area is characterized by a typical wet and dry season. The dry season span for 5 months (November to March), while the wet season lasts between April and October each year. The annual rainfall ranges from l,700-1,050 mm (Adebayo, 2014). Mubi North Local Government is located in the North-Eastern part of Adamawa state. The geographical location is between latitude 90° 33 95° North of the equator and between Longitude 3° 0914019 East. The local Government Area is bounded with Cameroon republic to the East Mubi South Headquarters is located in central Mubi town district the population size as revealed by the 2006 census the local government population 156,393.00 the major tribes in the local government area are Fali,Duge, Njanye, Margi, Higgi, Fulani and Hausa, the people are endowed with rich traditional culture (Amina *et al;* 2023). The vegetation in the Local Government is Sudan Savannah; this maintains an annual rainfall ranging from 700-900m and rainy season lasts for about 5-6 months in the local government area the farming activities in the local government are food crops and cash crops. A food crop comprises of cereals, legumes and root crops while cash crops are mainly rice, groundnut, millet and sugarcane. Mubi south

town was created in 1996 by the General Sani Abacha regime, Mubi south is located in North Eastern part of Adamawa state. The geographical location is located between latitude 900 33, 900 45 North of the Equator and between 3° 09-14°-18 East.

Study Design

The study was conducted using surveyed design and experimental design. The surveyed designs including Burkonu Market, Mubi main Market and Kuturu market where white yam are sold.

Materials/Reagents

Conical flask, petri-dish, measuring cylinder, microscope, anti-biotic, ethanol, weighing balance, potato dextrose agar (PDA), distilled water and 30 yam samples.

Samples Collection

Ten different white yam samples were purchased weekly from 3 selected markets in Mubi metropolis namely; Burkono Market, Mubi main market and Kuturu. A total of 30 samples of white yam was collected respectively. The samples were aseptically collected using sterile polythene bags and disposable sterile hand gloves and transported to microbiology laboratory of federal polytechnic, Mubi for analyses.

Materials Sterilization

All the glass wares will be properly washed, dried and sterilized in the Autoclave at 121°C for 15 minutes, the entire working surfaces was also disinfected with ethanol to reduce contamination.

Sample Processing

One gram of each rotted white yam was carefully cut with the aid of a sterile scalpel and enriched in sterile soba-roud dextrose broth for twenty-four hours.

Isolation of Fungi

Isolation of the fungi was carried out as described by (Amusa et al, 2002). Segment (2g) of tissues from the rotted white yam was cut with sterile scalpel and placed on potato dextrose agar containing streptomycin (to prevent the growth of bacteria) in Petri dishes and incubated at room temperature for 5days. Pure cultures were obtained from the isolation.

Identification of Fungi

The fungal was identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation using the slide culture technique and microscopic examination.

Slide Culture Technique

The technique was also adopted for the identification of the isolated fungi using lactophenol stain.1g of pure culture was spread on a clean microscopic slide.The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified.

Microscopic Examination

Slides of the mycelium observed from different isolates were prepared as follows: A few drops of lacto phenol cotton blue solution was placed at the centre of clean grease free slides. A small portion of the unidentified fungal isolates were picked with sterile wire loop. The portions were placed in the lacto phenol cotton blue droplet on the slides and emulsify out with a sterile wire loop. Cover slips were placed at the centre of the slides for viewing and examining the structure of the mycelia, spore structure and fruiting bodies were identified with the help of Standard Color Atlas of Diagnostic Microbiology by (Louis *et al*., 1997).

Pathogenicity Test of Isolated

A pathogenicity test was conducted to proof Koch postulate (Morsy *et al*., 2009). All fresh samples were separately washed in 10% (v/v) sodium hypochlorite solution and rinsed in three changes of running tap water and allowed to dry. A ruler was used to mark a (2mm) diameter circle on each sample; a sterilized needle was used to streak fungal hyphae on marked portions. Controls were inoculated with sterile distilled water. Materials were placed on the laboratory bench. Sterilized forceps was used to remove portions from the diseased areas on the 4^{th} day and placed on freshly prepared potato dextrose agar plates and incubated at 25.7 ±2°c for 3 days. Fungal growth that appeared was recorded.

Data Analysis

Data was analyzed and identified fungi associated with the spoilage of white yam among different species were compared using simple percentages.

Results

Table 1: to c shows the different characteristics fungi observation of macroscopic morphological appearance such as Pigmentation colour, Growth nature and conidial shape.

Table 2: Shows the different species of fungi such as *aspergillus spp, penicillium spp, fusarium spp, mucor spp, and monilla spp* as observed under microscope present in Main market Mubi.

Table 3: Shows the different species of Fungi such as *Oxysporum spp*, *Flavus spp*, *Citrinun spp*, and *Fumigate spp* as observed under the microscope in Borkonu Market.

Table 4: Shows the different species of Fungi such as *Fumigates spp, Niger spp, Citrinum spp, Gramimearum spp, Circinelloides spp* as observed under the microscope in Kuturu Market.

Table 5: shows the percentage and frequency of 30 samples of yam investigated; *aspergilus spp* was encountered in 19 samples which constitute a prevalence of 63.3%. The least detected spoilage fungus was *monilla spp* that a prevalence of 3.3%.

Table 6: shows the result of pathogenicity, the white yam induced rot in the healthy-looking yam tubers after 14 days of inoculation, symptoms of infection was seen on the inoculated yam tubers. The yam tuber that was not inoculated with the test fungi used as control experiment, did not show any sign of rot indicating absence of reproductive porpagules in the bored yam tissues.

Table 1: Morphology Appearance of the Fungal Isolate.

Sample of Main market	Pigmentation colour	Growth nature	Conidial shape
Yam 1	White	Wooly	Irregular
Yam 2	White with black spores	Wooly	Irregular
Yam 3	White with black spores	Wooly	Irregular
Yam 4	Black	Wooly	Irregular
Yam 5	Black	Powdery	Irregular
Yam 6	White with black spores	Wooly	Irregular
Yam 7	White with black spores	Wooly	Irregular
Yam 8	White	Wooly	Irregular
Yam 9	White	Wooly	Oval
Yam 10	White	Wooly	Irregular

Table 2: Microscopy of Identified fungi

Sample of Main market	Genus	Species
Yam 1	*Aspergillus*	*niger*
Yam 2	*Aspergillus*	*Fumigates*
Yam 3	*Aspergillus*	*Niger*
Yam 4	*Aspergillus*	*Fumigates*
Yam 5	*Aspergillus*	*Fumigates*
Yam 6	*Penicilliun*	*Citrinum*
Yam 7	*Aspergillus*	*Fumigates*
Yam 8	*Mucor*	*Circinelloids*
Yam 9	*Penicilliun*	*Roquetorti*
Yam 10	*Monilla*	*spp.*

Table 3: Microscopy of identify fungi

Sample of Borkono Market	Genus	Species
Yam 1	*fusarium*	*oxysporum*
Yam 2	*Aspergillus*	*Flavus*
Yam 3	*Penicilliun*	*citrinum*
Yam 4	*Aspergillus*	*Fumigates*
Yam 5	*Aspergillus*	*Fumigates*
Yam 6	*fusarium*	*Oxysporum*
Yam 7	*Aspergillus*	*Fumigates*
Yam 8	*Aspergillus*	*Fumigates*
Yam 9	*Aspergillus*	*Fumigates*
Yam 10	*Aspergillus*	*Fumigates*

Table 4: Microscopy of identify fungi

Sample of Kuturu Market	Genus	Species
Yam 1	*Aspergillus*	*Fumigates*
Yam 2	*Aspergillus*	*Niger*
Yam 3	*Penicilliun*	*citrinum*
Yam 4	*Aspergillus*	*Niger*
Yam 5	*Penicilliun*	*Citrinum*
Yam 6	*fusarium*	*Gramimearum*
Yam 7	*mucor*	*circinelloides*
Yam 8	*Aspergillus*	*Niger*
Yam 9	*Aspergillus*	*Niger*
Yam 10	*Aspergillus*	*Niger*

Table 5: Shows the number of species of fungi isolated from the sample obtained from the three markets in frequency

Fungi	Frequency	Percentage %
Aspergillus spp	19	63.3
Fusarium spp	3	10.0
Penicillium spp	5	16.7
Mucor spp	2	6.7
Monilla spp	1	3.3
Total	**30**	**100**

Table 6: Physiological change of tuber during pathogenic testing

Sample Yam	Final weight of infected tuber	Rot category	Observed symptom of spoilage	(Wt loss) Weight of rotten tuber portion	Pathogenicity
1	950g	Dry rots	White/brownish powdery colour	250g (0.26)	+
2	1.50kg	Dry rots	Brownish soft colour	479g (319.3)	+
3	1.50kg	Dry rots	Black Brownish round the spoiled portion	33k (22)	+
4	1.250kg	Dry rots	Black Brownish round the spoiled portion	163g (130.4)	+
5 control	1.150kg	Nothing	White no damage	1.150kg (1)	-

Discussion

White yam is essential farm produce that can get infected at the point of harvest, during handling, processing, and storage or sale. This has been causing serious economic impact on farmers, consumers and the society at large. Environmental conditions such as temperature, humidity, acidic pH and other factors also, contribute to spoilage of white yam as reported by Nkese Ime Okon *et al.* (2022).

Mubi and indeed Adamawa state has a climate with high temperature and humidity that supports the growth and proliferation of these fungal species. The findings of this study showed that several fungi at different frequency of occurrences were found to be associated with white yam commonly sold at mubi main market, borkono market, kuturu market. The most commonly encountered fungi associated with white yam contamination were; *aspergilus spp* 63.3%, *fusarium spp* 10.0%, *monilla spp* 3.3%, *penicillium spp* 16.7% and *mucor spp,* 6.7%. This is closely linked to agreement with the findings of Fowowe. B and. Shuaibu M. I (2014). Isolation of *aspergilus spp and penicillium spp*. from fungi associated with contamination of white yam sold in Mubi metropolis. Some of the fungi isolated from this

research have been reported to produce secondary metabolites which are potentially harmful to humans and other animals (Baiyewu *et al.*, 2007).

Conclusion

Isolation and identification of fungi pathogen from rotten white yam sold in Mubi metropolis was successfully carried out. White yam sold in Mubi markets were found to be contaminated with different species of fungi which are accountable for the spoilage of these white yam Findings from this research revealed that white yam had the highest contamination from *aspergilus spp*, *and least is monilla spp* respectively.

Recommendations

From this study, the following recommendations were made:

- Appropriate control measures should be employed during the harvesting, transportation, handling and processing of white yam or any other perishable food items because of their susceptibility to spoilage by microorganisms particularly fungi.
- The white yam sellers should collaborate with Government to ensure provision of good storage facilities in the market in addition to vendor education through sensitization, focused group discussion and workshops among others
- Further research should be carried out on other species of microorganisms such as bacteria, viruses and protozoa.

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Conflict of Interest

The authors declare no conflict of interest.

References

Abdollahzadeh J, Goltapeh EM, Rouhani H (2003) Evaluation of Antagonistic Effect of *Trichoderma* species in Biological Control of Causal Agents of Crown and Root Rot of Sunflower *(Sclerotinia minor) in vitro*. Agricultural Sciences Tabriz 13(2): 13-23.

Akombo RA (2016) Studies on the Antimicrobial Potency of Five Crude Plant Extracts and Chemical Fungicide in *in vitro* Control of *Aspergillus flavus,* Causal Agent of White Yam (*Dioscorea rotundata*) Tuber Rot. *Journal of Plant Sciences and Agricultural Research 1(1): 1-8.*

Alamu E Oladeji, Maziya-Dixon Bussie, Ferede-Menkir Roman, Popoola Ibironke, Asiedu Robert, Gondwe Therese (2016). Characterization and Classification of the Provitamin A carotenoids of Deep Yellow-fleshed Bitter Yam (Dioscorea dumetorum) Varieties. *Journal of Food and Nutritional Research*,Vol.4 (10).640 -645

Amaeze NJ, Ezeh PA, Dan-kishiya AS (2013) Evaluation of Garlic (*Allium Sativum*) and uziza (*Piper Guineense*) on the Control of Tuber Rot Fungi of Potato and carrot. *American Journal of Research Communication 1(9): 167-174*

Amina I. O, Bake I. D. I and Omar A.W.H (2023). Effects of Different Rates of NPK 15:15:15 Fertilizer on Growth and Yield of Okra *(Abelmoschus Esculentus (L.) Moench)* in Mubi North Local Government Area of Adamawa State,Nigeria. African Journal of Agricultural Science and Food Research. Vol 10. (1) 1 - 13

Anwadike, B.C. (2009). Fungal Rot of White Yam (*Dioscrea rotundata*) sold in Warri Markets. *Warri Journal of Pure and Applied Science*, 10: 53 -59

Anwadike, B. C (2016) fungal decay of the yam cultivar (dioscorea dumentorum pax) sold in open markets in Nsukka, Nigeria. *Global Scientific Journals*. Vol. 7 (5), 1054 - 1068.

Asiedu, R. and Alieu, S. (2010). Crops that feed the World 1.Yams. *Food Security*. 2(4): 305- 315.

Egesi CN, Onyeka TJ, Asiedu R (2009) Environmental stability of resistance to anthracnose and virus diseases of water yam (*Dioscorea alata*). African Journal of Agricultural Research 4(2): 113-118.

FAO (2001). Food and Agriculture organization of the United Nations: Production 1995) Journal Rome, Italy, vol. 50.

Fowowe. B and. Shuaibu M. I (2014). Post Harvest Handling and Transportation of Pawpaw (Carica Papaya) in Ekiti State, Southwestern Nigeria. Advances in Agriculture, Sciences and Engineering Research. Vol. 2 (10). 385 – 387.

Jova, M.C., Kosky, R.G., Perez, M.B. and Vega, V. M (2005). Production of yam microtubers using a temporary immersion system. *Plant Cell, Tissue, Organ Culture*, 83: 103 -107

Louis E. Grivetti, Robert H. Glew, Dorothy,J. Vanderjagt, Cassius Lockett, Garrett C. Smith, Andrze A, Mark Millson (1997). Amino acid, Fatty acid, and Mineral Composition of 24- Indigenous Plants of Burkina Faso. Journal of Food Composition and Analysis. Vol. 10 (3) 205 – 217.

Markson AA, Amadioha AC, Omosun G, Madunagu BE, Udo SE, Umana EJ (2012) Control of Botryodiplodia theobromae causing Tissue Rot of White Yam (*Dioscorea rotundata Poir*). *Scholarly Journal of Agricultural Science 2(1): 1-7*.

Medoua, C.N., Muna, I., Agbor-Egbe and Mbofong, C.M. (2005).Study on the hard to cook property of stored yam tubers (*Dioscorea dumentorum*) and some determining Biochemical factors. *Food Res. Int.* 38: 143 -149.

Mohammed Isa Shuaibu and Babajide Fowowe. (2014). Is foreign Direct Investment Good for the Poor: New Evidence from African Countries. Springer Journal of Econ Change Restruct. Vol. 47. 321 - 339

Morsy E,M, Abdel-Kawi K.A.and Khalil M.N.A (2009). Efficiency of Trichoderma viride and Bacillus subtilis as biocontrol agents against Fusarium solani on tomato plants. Egyptian Journal of Agricultural Vol. 37 (1): 47 - 57

Musieba, F.S., Ekoh, R.K., Mibey, S.S. Wanjiku and Moraa, K. (2009). Proximate Composition Amino Acids and Vitamins Profile of *Pleurotu scitrimpleatus* Singer. An indigenous mushroom in Kenya. *Am. J. Food Technpl.,*81: 200 -206.

Ogunleye AO, Ayansola OT (2014) Studies of Some Isolated Rot-Causing Mycoflora of Yams *(Dioscorea Spp.). American Journal of Microbiology and Biotechnology 1(1): 9-20.*

Okeke, Okigbo NR, Enweremadu CE, Agu CK, Irondi RC, Okeke BC, et al. (2015) Control of White Yam (*Dioscorea rotundata*) Rot Pathogen using Peel Extract of Water Yam (*Dioscorea alata*). Advances in Applied Science Research 6(10): 7-13

Nkese Ime Okon, Aniedi-Abasi Akpan Markson, Ekeng Ita Okon, Effiom Eyo Ita, Edak Aniedi Uyoh, Ene-Obong Effiom Ene-Obong, Valentine Otang Ntui (2022). Characterization of some fungal pathogens causing anthracnose disease on yam in Cross River State, Nigeria. Journal PLOS ONE pp.1 - 19

Nwagboso N.K Uyanga J. A, Amadi D.C.A and D.F. Jatau (2012) Desertification and Population Dynamics in Nigeria. IOSR Journal Of Environmental Science, Toxicology and Food Technology Vol. 1, (5). 1 – 8.