Field Survey of Symptoms and Isolation of Fungi Associated with Post-harvest Rots of White Yam (Dioscorea Rotundata Poir.)

Ezeibekwe IO, Umeoka N* and Izuka CM

Department of Plant Science and Biotechnology, Faculty of Science, Imo State University Owerri, Imo State, Nigeria

Abstract

Investigations were carried out on field survey of symptoms and isolation of fungi associated with the Post-harvest rots of white yam (Dioscorea rotundata Poir.) at Orlu, Imo State. The results of disease incidence and severity showed that dry rot had the highest percentage incidence of 67.5%, followed by wet rot (47.5%) and soft rot 45.0%. Anthracnose recorded 37.5% and powdery mildew recorded 42.5%. The severity result also followed the same trend, with dry rot having the highest percentage of 26.8%, soft rot 23.7%, wet rot 23.2%, anthracnose 16.3% and powdery mildew recorded 15.1%. The fungi were isolated and identified as Trichoderma viride (Pers.), Pythium aphanidermatum (Edson), Aspergillus fimigatus (Fresenius), Penicillium expansum (Link.), Geotrichum candidum (Link.), Fusarium oxysporum (Link.), Botryodioidia theobromae (Sac.) and Aspergillus niger (van Thieghem). Fungal organisms occurred consistently with soft rot, dry rot, wet rot and anthracnose of D. rotundata with A. fimigatus (Fresenius) occurring more frequently, 45.00%, followed by T. viride (Pers.) 20.00%, P. aphanidermatum (Edson) recorded 15.00%, P. expansum (Link.) and G. candidum (Link.) have 10.00% each.

Keywords: Symptoms; Isolation; Fungi; Post-harvest rots; D. rotundata

Introduction

Yams (Dioscorea spp.) are among the most important staple foods in the world, especially in some parts of the tropics and subtropics. The role played by yam in the food economy in most West African countries cannot be over-emphasized. It is one of the most important dietary sources of energy produced within the tropics [1]. Nigeria produces yams for local consumption and the export market. The country is a leading exporter of yam in the world (about 12,000 tons annually). Water yams (D. alata) are consumed when white yam becomes scarce or expensive [2]. One of the most pressing problems facing the countries of the third world is food scarcity. It is reported that nearly 1 billion people are challenged by severe hunger in these nations of which 10% die from hunger-related complications. A substantial part of this problem from hunger stems from inadequate agricultural storage and produce preservation from microbes-induced spoilages [3]. According to Arya [4], of all losses caused by plant diseases, those that occur after harvest are the costliest. Cassava, yam and sweet potato are important sources of food in the tropics. Others are cocoyam, rice, maize, wheat, sorghum, millet and various fruits, legumes and vegetables.

Yam tubers and plants are prone to several diseases. These diseases not only them unappealing but also reduce the quantity of yam produced. Viruses, bacteria, fungi, nematodes and many other factors are these diseases. Specifically, fungal infections have constituted a very limiting factor all over the world and to other tuber crops. A good number of pathogens are soil inhabiting, some gain their entrance into the plant through invasion of the roots causing harm to the plant [3]. Most rots of yam tubers are caused by pathogenic fungi such as Aspergillus flavus, Aspergillus niger, Botryodioidia theobromae, Fusarium oxysporum, Fusarium solani, Penicillium chrysogenum, Rhizoctonia spp., Penicillium oxalicum, Trichoderma viride and Rhizopus nodosus etc., [5]. The field diseases are those diseases that cause economic damage to yam in the field from the seedling stage to the point of harvest. Anthracnose disease of yam has a considerable impact on yam production world-wide [6]. This is caused mostly by the fungus Colletotrichum gloeosporioides [7]. IITA [8] reported that Glomerella cingulata (isolate number IMI W3725) was the yam anthracnose inducing pathogen in Southwestern Nigeria. G. cingulata is the perfect state of C. gloeosporioides, the form that is usually found causing field anthracnose disease.

On susceptible yam cultivars, symptoms appeared at first as small dark brown or black lesion on the leaves, petioles and stems. The lesion is often surrounded by a chlorotic halo enlarged and coalesced, resulting in extensive necrosis of the leaves and die-back of the stem [9]. The withered leaves and stem die-back gave the plant a scorched appearance hence the name ‘scorch’ disease [8]. Previous work of Amusa [9] indicated that yam anthracnose is a disease complex, which has however been associated with the activities of Colletotrichum gloeosporioides, Curvularia pallescens, Curvularia eragrostides, Pestalotia spp., and Rhizoctonia solani. PANS [10] have reported that Pestalotia spp. mainly affects D. esculenta but appear to act as a secondary invader after infection by C. gloeosporioides on D. alata and D. cayenensis. There is need to carryout field survey of symptoms and isolation of fungi associated with the Post-harvest rots of white yam (D. rotundata poir.) to expand the frontiers of knowledge and hence advice the farmers accordingly. Considering the rate of tuber rots of yam and food scarcity as a result of damage caused by microorganisms especially those caused by fused, there is urgent need to determine the causative organisms and disease severity so as to offer a lasting solution and hence reduce food scarcity. The aim of the present study is to investigate the disease incidence and severity of tuber rots of D. rotundata at Orie-okporo market, Orlu L.G.A, Imo State. To isolate, identify and establish the pathogenicity of fungal organisms associated with tuber rots of D. rotundata.

*Corresponding author: Umeoka N, Department of Plant Science and Biotechnology, Faculty of Science, Imo State University Owerri, Imo State, Nigeria, Tel: 083431321; E-mail: izukachukwuemeka@gmail.com

Received November 17, 2016; Accepted December 07, 2016; Published December 13, 2016

Citation: Ezeibekwe IO, Umeoka N, Izuka CM (2016) Field Survey of Symptoms and Isolation of Fungi Associated with Post-harvest Rots of White Yam (Dioscorea Rotundata Poir.). J Food Process Technol 7: 642. doi: 10.4172/2157-7110.1000642

Copyright: © 2016 Ezeibekwe IO, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Materials and Methods

Location of study

Samples were collected with clean polyethylene bags, labeled and taken to the laboratory at the Department of Plant Science and Biotechnology in Imo State University, Owerri were the practicals took place.

Sample collection

Yam tubers with symptoms of rot were obtained from Orie-okporo daily market in Isiala-okporo Autonomous Community (where many villages sell their products), Orlu L.G.A Imo State.

Disease survey

A survey of post-harvest rots of yam was carried out by obtaining 40 tubers of yam in each visit from Orie-okporo market in Isiala-okporo Autonomous Community, Orlu L.G.A Imo State on three (3) different days. Visual inspections of the yams were carried out by separating diseased yam tubers from apparently healthy yam tubers. The diseased yam tubers were further separated into groups based on symptoms. The field disease symptoms were observed and recorded. Disease incidence was calculated for each symptom by using the formula described by Ezeibekwe et al. [11].

\[
\text{Disease incidence} = \frac{(\text{Number of diseased yam tubers})}{(\text{Total number of yam tubers sampled})} \times 100\%
\]

The severity of rot infection was assessed by using the following scale: -

0 = Healthy
1 = 1% to 25% slight infected.
2 = 26% to 50% moderate infected.
3 = 51% to 75% extensive infected.
4 = 76% to 100% completely rotted.

A percentage rot score per sample of the yam tubers was derived from the total rot scores as follows:

\[
\text{Severity} = \frac{(\text{Sum of numerical ratings})}{(\text{Total number of observed yam tubers})} \times \frac{1}{(\text{Maximum diseased class})}\times 100\%
\]

Medium preparation

The medium, Potato Dextrose Agar (PDA) which is a semi-synthetic medium was used for the experiment, and it was prepared following the manufacturer’s directive. Thirty-nine grams (39 g) of the PDA media was dissolved in 1 litre of sterile distilled water and sterilized by autoclaving at 121°C and 15 psl for 15 minutes as instructed by the manufacturer. 0.5 gram of penicillin, an antibiotic was added to the autoclaved medium so as to inhibit any bacterial growth, and then it was shaken properly. It was allowed to cool to about 45°C and then sterilized with a sterilized knife were surface-sterilized in 5% sodium hypochlorite solution for 5 minutes. The surface sterilized diseased tissues were washed three times using sterile distilled water. The tissues were allowed to dry in a sterile Lamina flow chamber. The dried disease tissues were plated on a Potato Dextrose Agar (PDA) medium. Four to five days after incubation, mycelia that grew from the plated yam tissues were sub-cultured onto fresh PDA. Further sub-culturing was carried out until pure cultures of single specie isolates were obtained. From these pure cultures, inocula of the different fungal specie isolates were obtained for the pathogenicity tests. The percentage occurrence of the organisms isolated from Post-harvest rot of D. rotundata (white yam) tuber was recorded and calculated using the formula [13]:

\[
\text{Percentage occurrence} = \frac{\text{Total number of fungal occurrence}}{\text{Total number of plates}} \times 100\%
\]

Identification of fungal isolates

Characteristics of fungal isolates from rotten yam tubers such as pigment production, pH, colony texture, spore or conidia-producing structures and spore shapes were observed and documented. The characteristics were observed from fungal tissues grown on PDA for one week or more, depending on the fungal species. Spore and mycelium characteristics were studied using the compound microscope. The microscopic examination was carried out by Lactophenol Cotton Blue (LPCB) wet mount [14] preparation which is the most widely used method of staining and observing fungi. The preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls. LPCB mount was carried out to observe the structure of fungi. These characteristics were used in identifying the fungal organisms to the species level, following standards described by Barnett and Hunter [15].

Statistical analysis

The set up were arranged in Complete Randomized Design (CRD). The data collected were subjected to statistical analysis of variance (ANOVA) using SPSS 20.0 version (Statistical Package for the Social Sciences) (Dr. Mbagwu) to determine the means as expressed in Tables 1 and 2.

Results

Incidence and severity

The incidence and severity of surveyed Post-harvest rots of D. rotundata tubers obtained from Orie-okporo market, in Isiala-okporo Autonomous Community, Orlu, Imo State was as shown in Tables 1 and 2. Dry rot occurred...
most in stored tubers with the percentage incidence of 67.5%. This was followed by wet rot (47.5%), soft rot have (45.0%), anthracnose (37.5%) and powdery mildew (42.5%). The severity results also followed the same trend as shown in Table 2 with dry rot being most severe (26.8%) and soft rot 23.7%, wet rot 23.2%, anthracnose 16.3% and powdery mildew 15.1% (Figures 1-5).

Identified of fungal isolates

Macro and microscopical characterization (Figures 1-5, Tables 3-6)

| Isolates | Morphological Features | Microscopic Features | Remark/Inference |
|----------|------------------------|----------------------|------------------|
| A        | White fluff colony on PDA and then became more compact and woolly. Later produced green patches due to formation of conidia. Reverse was light orange. | Septate hyphae; conidiophores were short, often branched, and flask-shaped at end. Conidia were rounding, single-celled, and clustered together at the end of each conidiophore. | Isolate identified as Trichoderma viride (Pers.) |
| B        | Colony surface on PDA was white at first, and then became very powdery, blueish green, with a white border and reverse was white. | Septate hyphae with branched or unbranched conidiophores that had secondary branches known as metulae. On the metulae, arranged in whorls, were flask-shaped stigmata that bore unbranched chains of round conidia. The entire structure formed the brush appearance. | Isolate identified as Pythium aphanidermatum (Edson) |
| C        | Colony surface on PDA at first appeared white, then shade of black. Texture velvety and reverse was goldish. | Septate hyphae; unbranched conidiophore arising from a specialized foot cell. | Isolate identified as Aspergillus fumigatus (Fresenius) |
| D        | Colony on PDA appears light brown and beneath is mushy with earthy musty odour. | Conidia phialospores; phialides upright, brushlike conidiophores arising from the mycelium singly or less often in synnema, branched near the apex, penicillate ending in a group of phialides, conidia hyaline, 1-celled, globose in basipetal chains. | Isolate identified as Penicillium expansum (Link.) |
| E        | Colonies on PDA were white, moist, yeast-like and easily picked up at the early stage. Later submerged hyphae were seen at the periphery, giving the appearance of ground glass. | Coarse hyphae that segment into rectangular arthrospores varying in size and roundness of their ends. | Isolate identified as Geotrichum candidum (Link.) |
Rapid growth on PDA at 25°C, produce woolly to cottony, flat spreading colonies. From the front, the color of the colony was white. From the reverse, it was dark purple.

**Table 3:** Identification of organisms using morphological and microscopic features of fungi.

| S/N | Fungi Isolates       | No. of Occurrence | Occurrence (%) |
|-----|----------------------|-------------------|----------------|
| 1   | *T. viride* (Pers.)  | 4                 | 20             |
| 2   | *P. aphanidermatum* (Edson) | 3           | 15             |
| 3   | *A. fumigatus* (Fresenius) | 9           | 45             |
| 4   | *P. expansum* (Link.) | 2                 | 10             |
| 5   | *G. candidum* (Link.) | 2                 | 10             |

| Initialy white, quickly becoming black with conidial production. Reverse was pale yellow and growth in the PDA.

**Table 4:** Incidence of pathogen rotted tuber of *D. rotundata*.

| S/N | Fungi Isolates       | No. of Occurrence | Occurrence (%) |
|-----|----------------------|-------------------|----------------|
| 1   | *T. viride* (Pers.)  | 4                 | 20             |
| 2   | *P. aphanidermatum* (Edson) | 3           | 15             |
| 3   | *A. fumigatus* (Fresenius) | 9           | 45             |
| 4   | *P. expansum* (Link.) | 2                 | 10             |
| 5   | *G. candidum* (Link.) | 2                 | 10             |

Discussion

The results on disease incidence and severity showed that there existed a high rate of post harvest rot of *D. rotundata* tuber obtained from Orie-okporko market, in Isiala-okporko Orlu, Imo State, with dry, soft and wet rot having the highest percentage value of disease incidence (67.5%, 45.0%, 47.5%) and severity (26.8%, 23.7%, 23.2%). These findings are in agreement with the findings of Amusa and Baiyewu [16] who reported that soft rot disease is the most serious disease of yam tubers and it can also be known as wet breakdown. The dry rot is considered as the most devastating of all the storage diseases of yam. Dry rot alone causes a marked reduction in the quantity, marketable value and edible portions of tubers and those reductions are more severe in stored yams. The results of this study have shown that *T. viride, P. aphanidermatum, A. fumigatus, P. expansum, G. candidum, F. oxysporum, B. theobromae* and *A. niger* were the major casual organisms that bring about the Post-harvest losses of *D. rotundata* tuber rot in Orlu, Imo State [5,11,13,17-20].

**Conclusion**

There are eight (8) fungi namely *T. viride, P. aphanidermatum, A. fumigatus, P. expansum, G. candidum, F. oxysporum, B. theobromae* and *A. niger* as casual agents of *D. rotundata* tuber rot at Orlu, Imo State. *A. fumigatus* recorded highest incidence in the disease occurrence while *T. viride* was most severe. Careful handling of the crops is recommended from harvest, through packaging, transportation and storage to minimize rot.

**References**

1. Okigbo RN, Ogbonnaya UO (2006) Antifungal effects of two tropical plant extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam rot. Africa J Biotechnol 5: 727-731.
2. FAO (2005) FAO Annual Report. Food and Agriculture Organization production year book, FAO, Rome.
3. Kana HA, Aiyu IA, Chammang HB (2012) Review on neglected and underutilized root and tuber crops as food security in achieving the millennium development goals in Nigeria. J Agri Vet Sci 4: 27-33.
4. Aria A (2010) Recent advances in the management of plant pathogens: Botanicals in the fungal pest management. Management of fungal plant pathogens, CAB International, UK.
5. Aaidoo KA (2007) Identification of yam tuber rot fungi from storage systems at the Kumasi Central market. Imo state University, Nigeria.
6. Simon PW (1993) Plant pigments for colour and nutrition. Horti Sci 32: 12-13.
7. Nwankiti OA, Arere OB (1980) Diseases of yam in Nigeria. Pest articles and news summaries (PANS) 24: 468.
8. IITA (1993) Crops improvement division: Tuber root improvement program archival reports (1989-1993). Part III yam, Ibadan, Nigeria.
9. Amusa NA (1997) Fungi associated with anthracnose symptoms of yam (*Dioscorea spp*) in South-west Nigeria and their roles. Crop Res 13: 177-183.
10. PANS (1984) Pest control in tropical root crops: Pest articles and news summaries, Center for Overseas Pest Research, London 4: 147-162.
11. Ezeibeke IO, Onwu Martin O, Anyaegbu PO (2008) Post-harvest rot diseases of fruits of *Asimina triloba* (*Carica papaya*) in some parts of Imo. Int Sci Res J 1: 107-112.
12. Cheesbrough M (2004) District laboratory practice in tropical district laboratory practice in tropical countries (Part-2). Cambridge University Press, Cambridge, UK.
13. Ezeibeke IO, Opara MI, Mbogwu FN (2009) Antifungal effect of aloe vera gel on fungal organisms associated with Yam (*Dioscorea Rotundata Poir*) rot. J Mol Genetic 11: 1-17.
14. Thomas PA, Kuriakose T, Kirupashanker MP, Maharajan VS (1991) Use of lactophenol cotton blue mounts of corneal scrapings as an aid to the diagnosis of mycotic keratitis. Diagnostic Microbiol 14: 219-224.
15. Barnett HL, Hunter BB (1998) Illustrated genera of imperfect fungi (4th edn). Burgess Publications Company, UK.
16. Amusa F, Baiyewu J (2003) Fungal toxic activity of extract of *Azadirachta indica* and *Senna alata*. Agris Res 11: 211-225.
17. Okigbo RN, Ikedugwu FEO (2001) Evaluation of water losses in different regions of yam (*Dioscorea spp.*) tuber in storage. Nigeria J Experiment Appl Biol 3: 320.
18. Okwu OA (2004) Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria. J Sustain Agri Environ 6: 30-34.
19. Nwofor MI, Fajola AO (1984) Cultural studies on *Botryodiplodia theobromae* and *Sclerotium rolfsii* causing storage rots of cocoyam (*Colocasia esculenta*). Fitopatologia Brasileia 11: 443-454.
20. Onuoha CI, Nwagbara EC (2011) Comparative studies of rot fungi of plantain and banana of different post-harvest ages. Global Res J Sci 1: 54-57.