Journal of Food Processing & Technology

Ezeibekwe et al., J Food Process Technol 2016, 7:12
DOI: 10.4172/2157-7110.1000642

Research Article Open Access

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

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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 fumigatus* (Fresenius), *Penicillium expansum* (Link.), *Geotrichum candidum* (Link.), *Fusarium oxysporum* (Link.), *Botryodiplodia 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. fumigatus* (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, Botryodiplodia 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 *Glomerela 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*.

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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

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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].

$$Disease\ incidence = \frac{(Number\ of\ diseased\ yam\ tubers)}{(Total\ number\ of\ yam\ tubers\ sampled)} \times \frac{100\%}{1}$$

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:

$$Severity = \frac{(Sum\ of\ numerical\ ratings)}{(Total\ number\ of\ observed\ yam\ tubers)} \times \frac{100\%}{Maximum\ diseased\ class\ (5)}$$

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 sterile dispensed into sterile Petri-dishes. Slants were then prepared by dispensing the dissolved media into the McCartney bottles before autoclaving and then the bottles were slanted to cool. Sterility test was performed to the media by incubating them uninoculated for 24 hours at 37°C as described by Cheesbrough [12]. Only media that passed the sterility test were used.

Isolation of fungal species from rotten yam tubers

Pieces of diseased tissues cut from the periphery of rotten yam tubers 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]:

$$Percentage\ occurance = \frac{Total\ number\ of\ fungal\ occurence}{Total\ number\ of\ plates} \times \frac{100}{1}$$

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 Orlu, Imo State was as shown in Tables 1 and 2. Dry rot occurred

Day of Observation	Soft rot	Wet rot	Dry rot	Anthracnose	Powdery mildew
1st Day	15.0	17.5	25.0	15.0	12.5
2nd Day	17.5	15.0	20.0	10.0	15.0
3rd Day	12.5	15.0	22.5	12.5	15.0
Total % disease incidence	45.0%	47.5%	67.5%	37.5%	42.5%

Table 1: Incidence of post-harvest rots of *D. rotundata* tubers in Orlu, Imo State.

Day of Observation	Soft rot	Wet rot	Dry rot	Anthracnose	Powdery mildew
1st Day	10.0	8.8	8.1	7.5	6.3
2nd Day	5.6	6.9	8.1	5.0	5.0
3rd Day	8.1	7.5	10.6	3.8	3.8
Total % disease severity	23.7%	23.2%	26.8%	16.3%	15.1%

Table 2: Severity of post-harvest rots of *D. rotundata* tubers in Orlu, Imo State.

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 microscocopic characterization (Figures 1-5, Tables

Figure 1: Plate of Yam tuber infected with soft rot disease (Scale: 0.29).

Figure 2: Plate of Yam tuber infected with wet rot disease (Scale: 0.29).

Figure 3: Plate of Yam tuber infected with dry rot disease (Scale: 0.29).

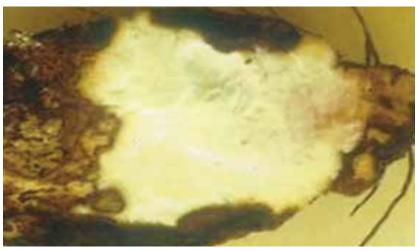

Figure 4: Plate of Yam tuber infected with anthracnose disease (Scale: 0.29).

Figure 5: Plate of Yam tuber infected with powdery mildew disease (Scale: 0.29).

Isolates	Morphological Features	Microscopic Features	Remark/Inference
A	White fluff colony on PDA and then became more compact and wooly. 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 conidiopores that had secondary branches known as metulae. On the metulae, arranged in whorls, were flask-shaped sterigmata 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. The conidiophore was enlarged at the tip, forming a rounded vesicle that was completely covered with flask-shaped stigmata that produce chains of round, conidia.	Isolate identified as *Aspergillus fumigatus* (Fresenius)
D	Colony on PDA appears light brown and beneath is mushy with earthly musty odour.	Conidia phialospores; phialides upright, brushlike conidiophores arising from the mycelium singly or less often in synnemata, 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.)

F	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.	Phialides are cylindrical, with a component of a complex branching system. Macroconidia were produced from phialides on branched conidiophores. Macroconidia have a distinct basal foot cell and pointed distal ends. They tend to accumulate in rafts. Microconidia were formed on short simple conidiophores.	Isolate identified as *Fusarium oxysporum* (Mart.)
G	Black, ostiolate, erumpent, stromatic, confluent growth on PDA.	A compact mass of hyphae on which or in which conidia or fruit bodies are borne. Simple conidiosphores, conidia dark ovoid to elongate.	Isolate identified as *Botryodiplodia theobromae* **(**Sac.**)**
H	Initially white, quickly becoming black with conidial production. Reverse was pale yellow and growth in the PDA.	Hyphae were septate and hyaline. Conidia head were radiate initially, splitted into columns at maturity.	Isolate identified as *Aspergillus niger* (van Tieghem)

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

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

1 and 2), of fungal isolates identified as the causative organisms of the *Dioscoria rotundata* rot implicated *Trichoderma viride* (Pers.), *Pythium aphanidermatum* (Edson), *Aspergillus fumigatus* (Fresenius), *Penicillium expansum* (Link.), *Geotrichum candidum* (Link.), *Fusarium oxysporum* (Link.), *Botryodiplodia theobromae* (Sac.) and *Aspergillus niger* (van Thieghem). Fungal organism occurred consistently associated with soft rot, dry rot, wet rot and anthracnose of *D. rotundata* (Poir) tuber with *A. fumigatus* (Fresenius) occurring often in various plates 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 (Tables 3 and 4).

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-okporo market, in Isiala-okporo 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 and (26.8%, 23.7%, 23.2%). These findings are in agreement with the findings of Amusa and Baiyewa [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. theobromea* and *A. niger* were the major casual organisms that bring about the Post-harvest loses 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.

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